Evaluation of Two Lipid-Based Edible Coatings For Their Ability to Preserve Post Harvest Quality of Green Bell Peppers

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Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
in
Human Nutrition, Foods, and Exercise

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August 29, 1997
Blacksburg, Virginia

Keywords: Edible Coatings, Ascorbic Acid, Post Harvest, Bell Peppers

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(ABSTRACT)

Two lipid-based edible coatings, Apex B (AC Humko, Memphis, TN) and Durafresh (Pacrite, Ecoscience Produce Systems, Orlando, Fl) were evaluated for their ability to preserve post harvest quality changes in green bell peppers (C*apsicum annum L.* cv. King Arthur). Post harvest storage quality conditions tested included respiration rates, weight, color and texture changes, and stability of ascorbic acid (AA) and dehydroascorbic acid (DHA) content. Results indicated that no appreciable changes between days or treatment groups occurred in three of the parameters tested: weight, texture, and hue angle ($p>0.05$ for all parameters). Significant weekly changes were seen in respiration rates, dehydroascorbic acid content, and chromaticity values. Respiration rates and DHA were significantly higher during the last two weeks of the study ($p=0.0001$, $p=0.0001$ respectively). Chromaticity values were significantly lower, indicating a more faded color on the 14^{th} day of the study alone ($p=0.0097$). Initial AA levels were much lower than expected (average initial content=78.72mg/100g). Significant differences between coated and uncoated pepper groups were seen in AA and DHA levels. AA content was found to be significantly lower in coated peppers ($p=0.0279$), while DHA levels were significantly higher in coated groups ($p=0.0126$). Overall, coated groups differed little from uncoated counterparts, except in the area of vitamin content in which the coated peppers showed an increase in vitamin breakdown. Despite the results, modifications of lipid coatings are needed, such as creating bilayer and composite coatings that contain either polysaccharide or protein constituents to enhance coating effectiveness.

Acknowledgements

Dr. Frank Conforti, Chair

Dr. William Barbeau

Dr. Richard Marini

Carolyn Harris

Dr. Marvin Lentner

Susan Harris

Mom, Dad, Stephanie, and Tom

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Chapter 1: Introduction

Edible coatings, which are defined as thin layers of wax or other material applied to the surface of a food, have been used for over 800 years. Records dated as early as the 12th and 13th centuries showed that wax coatings were applied to citrus fruits in China (Hardenburg, 1967). Such coatings decreased the availability of oxygen to the fruit and therefore induced fermentation. In the United States, wax coatings have been used commercially since the 1930's, when oranges were coated with melted paraffin waxes (Kaplan, 1986). These early coatings were used to reduce post harvest water loss. Later, coatings were used to create the appearance of a glossy skin. More recently, coatings have been used to preserve attributes associated with fruit and vegetable quality, as well as increase shelf life (Kester and Fennema, 1986).

As the purpose for coating fruits and vegetables has changed so have the types of coatings used. First, when prevention of water loss was the goal, coatings were made primarily of paraffin wax. However, when added glossiness and shine became the desired characteristics, the coating materials changed to "solvent" types of coatings. These solvent coatings were made from petroleum solvents in which resins, plasticizers, and other film forming agents were added (Kaplan, 1986). In the 1950's carnauba waxes were introduced, however, due to the dull appearance they produced, such waxes were only used in combination with polyethylene and paraffin wax for long term storage of lemons. In the 1960's waxes made of resins and shellacs dissolved in water became popular for use on citrus commodities (Kaplan, 1986). More recently waxes and edible coatings made from proteins, polysaccharides, and various combinations of theses products, have been used on many other fruit and vegetable commodities as well as for other food applications including nuts, and meat products (Kester and Fennema, 1986). Such coatings have been used to reduce moisture loss and surface wounding, as well as to reduce a variety of diseases in apple varieties (Hardenburg, 1967; Kester and Fennema, 1986). Wax coatings retarded respiration, inhibited oxygen supply and increased carbon dioxide content within apple and pear fruit (Smock, 1935). Early research (Trout et al., 1942) showed that coatings

applied to apples decreased the diffusion of gases across the skin, increased internal carbon dioxide, reduced internal oxygen, reduced the rate of respiration, and delayed ripening changes.

The mechanism by which coatings preserve fruits and vegetables is by producing a modified atmosphere surrounding the product. This modified atmosphere can serve several purposes, including reducing oxygen availability and increasing the fruit or vegetable's internal carbon dioxide concentration (Smith et al., 1987). Modified atmospheres created by coatings are produced by the physical trapping of carbon dioxide gas within the fruit tissues during respiration. For example, coating bell peppers resulted in increased internal levels of carbon dioxide and decreased concentrations of oxygen (Lerdthanangkul and Krochta, 1996). The increased levels of carbon dioxide have been shown to lower respiration rates, and therefore delay senescence. In addition, coatings may have different levels of permeability to oxygen. Decreased oxygen permeability can also serve to reduce respiration and increase shelf life.

In addition to reducing respiration rates, coatings also act as hydrophobic barriers and therefore prevent water loss from transpiration. Such a feature is highly desirable for fruit and vegetable commodities. Water loss can lead to decreased turgor pressure which results in shriveling and wilting, both of which render produce not saleable (Kester and Fennema, 1986). Coatings successfully reduce weight loss in green peppers, zucchini and cucumbers (Habeebunnisa et al., 1963; Avena-Bustillos et al., 1994). Other quality improvements related to edible coatings include slower softening and texture changes, as well as increased color retention, all of which have been demonstrated on green bell peppers (Lerdthanangkul and Krochta, 1996; Habeebunnisa et al, 1963).

Chapter 2:Review of Literature

I. Physiological Processes Important to Post Harvest Fruit Quality

Fruits still attached to the plant are active tissues undergoing normal physiological processes. Upon harvest these physiological processes become disrupted and can cause detrimental changes in the quality of the product. Some of the naturally occurring physiological processes that are important to consider include: respiration and gas exchange, transpiration, and hormone production.

A. Respiration

Under ideal conditions, most plants, including their fruits, respire aerobically. Aerobic respiration in plants involves the break down of sugars and other energy sources made during photosynthesis. These energy sources are broken down by the well know metabolic pathways (glycolysis, the Kreb's cycle and oxidative phosphorylation) and are used to form adenosine triphosphate (ATP). During this normal respiratory process the plant and all of its tissues use oxygen from the atmosphere as a terminal electron acceptor in oxidative phosphorylation, and release the aerobic by-product carbon dioxide. Although anaerobic metabolism rarely occurs, plants may also produce ATP through fermentation. This process however is hard for plants to continue due to the formation of harmful metabolic waste products (Kader, 1987).

A.1. Respiration of Harvested Commodities

Harvested commodities continue to respire aerobically. However, the act of harvesting a product does create some disturbances in the normal respiration patterns. One of the main changes in the respiration of a harvested fruit is the alteration of the fruits' internal atmosphere. Normally, prior to harvest, the external tissues of a fruit are exposed to atmospheric concentrations of oxygen,

nitrogen, and carbon dioxide. However, the internal atmosphere, which may be as small as gas filled spaces between dense tissue, as in an apple, or a large open cavity as in a pepper, is vastly different. The internal atmosphere does contain oxygen and nitrogen, however in addition to these two gases, the respiration product, carbon dioxide, is also present. Internal carbon dioxide is usually present at low concentrations of 3% to 6%; however, these concentrations may rise to 20-30%. These high levels of carbon dioxide retard aerobic respiration because it replaces some of the oxygen otherwise present and available to tissues (Phan, 1987).

When a fruit is picked the protective outer cellular layer, known as the cuticle barrier, is disrupted and the gases once confined are now free to escape. During this escape a large influx of oxygen from the outside environment occurs in addition to an outflow of carbon dioxide. In the new environment containing higher oxygen and lower carbon dioxide concentrations, the respiration rates of the internal cells are no longer suppressed and respiration increases. The rapid respiratory rise depletes the metabolites used in the respiratory processes, and along with that depletion an increase in all oxidative processes occurs, which in turn will serve to hasten the fruits' ripening and eventual senescence (Phan, 1987).

In addition to the disruption of the oxygen and carbon dioxide balance due to the harvest-induced tissue damage, other gases, namely the hormone ethylene, are also liberated. Ethylene is responsible for inducting certain enzymes, including chlorophyllase and peroxidase, which once activated can alter certain fruit components, including degradation of chlorophyll and formation of isocoumarin, a bitter compound found in carrots. Ethylene concentrations will plummet for a short time immediately following harvest, however, after the fruit has sealed the wound produced by harvest, ethylene concentrations within the tissues will return to the pre-harvest state. This return to the higher levels has been thought to hasten the ripening of fruits which show a post harvest increase in respiration rate, known as the respiratory climacteric (Burton, 1982a). The ethylene concentration prior to harvest does not hasten respiration because of the inhibitory effect of the high carbon dioxide levels in the pre-harvest fruit (Burg and Burg, 1965).

A.2. Factors Altering Respiration

Pre-harvest and post harvest aerobic respiration in fruits can be altered by many external factors such as temperature, oxygen and carbon dioxide concentrations, ethylene concentration, and other stresses to the plant.

A.2.a. Temperature

Temperature greatly influences the rate of respiration of fruits and vegetables, and not surprisingly plays an important role in maintaining post harvest quality of fresh vegetables and fruits. According to Van't Hoff's rule the velocity of a biological reaction may double or even triple for every 10 degree Celsius increase (Kader, 1987). This 10° C interval has been called the temperature coefficient, or Q10. This temperature coefficient can be calculated by determining the respiration rate of a given fruit at two different temperatures, and then applying the following equation:

$Q10 = [(R1/R2)] \times [10/(T2-T1)]$

where R1 = rate of respiration at temperature T1

R2 = rate of respiration at temperature T2

T1 and T2 = temperatures in degrees Celsius

Therefore respiration rates can increase with increasing temperature. However, the rate of respiration increase compared with the increase in temperature is not linear. In fact, although respiration itself continuously increases, the rate of change between different temperature ranges actually decreases until about 40°C. At this point the respiration rate actually decreases because the plant is near its thermal death point. The thermal death point is the point at which enzymatic denaturation and interference with metabolism occur (Kader, 1987).

Lower temperatures can also negatively influence respiration. As the temperature surrounding a commodity is lowered, the respiration rates are slowed. Some vegetables and fruits exhibit sensitivity to lower temperatures and may endure chilling injury. Chilling injury refers to the damage that occurs when cold sensitive fruits are exposed to non-freezing temperatures below their cold threshold level (Lyons and Breidenbach, 1987). At the lower temperatures, altered glycolysis and oxidative phosphorylation may cause higher respiration rates that would not occur at warmer temperatures. Vegetables susceptible to chilling injury also display abnormally high respiration rates upon transfer from the chilling temperature to a higher temperature (Eaks, 1956). This elevated respiration rate can cause damage to tissues (Wang, 1982).

A.2.b. Atmospheric Gas Composition

Gas composition of the atmosphere surrounding a fruit also affects the respiration rate. Obviously respiration requires the presence of oxygen. The normal atmospheric concentration of oxygen, 20%, is optimal for respiratory processes. However, when this level drops, the respiration rate of respiration may be slowed (Greulach, 1973a). A minimum of 1-3% of oxygen is required to maintain some level of aerobic metabolism. When the concentration of oxygen falls below this level decarboxylation reactions in the aerobic metabolic pathway are inhibited, and the plant switches to the anaerobic pathway of fermentation. Few plants and plant tissues can survive for a long period utilizing this pathway due to the accumulation of ethanol and acetylaldehyde which are toxic to the plant's cells, thus senescence is hastened (Kader, 1987).

Carbon dioxide levels in the atmosphere also affect respiration rates. High concentrations of this gas have an inhibitory effect on the respiration rate. The mechanism for reduction of respiration occurs via feedback inhibition, whereby, carbon dioxide being an end product of aerobic metabolism serves to inhibit respiration when the concentration is kept high. High levels of carbon dioxide inhibit the decarboxylation reactions of normal respiration, and thus slow down the Kreb's

cycle. However, if the concentration of carbon dioxide is too high (over 20%) anaerobic respiration will ensue and result in damaged plant tissue (Kader, 1987).

A.2.c. Ethylene Production

The hormone ethylene is a normal physiological product of fruit. When a fruit is exposed to this hormone, respiration rates have been shown to rise. Upon exposure to the hormone, climacteric fruits have increased respiration rates, which eventually decreases the time it takes for them to reach their climacteric respiration peak (Kader, 1987).

A.2.d. Physical Stresses

Bruising or wounding a fruit can increase the respiration rate proportionally to the extent of the damage. Disease of the plant tissue also increases respiration rate. Water stress, which occurs in low humidity situations, can increase respiration rate, but when the plant loses more than 5% of its water the respiration rate is reduced and wilting may occur (Kader, 1987).

A.2.e. Age of the Commodity

Respiration rates are influenced by plantage cultivar and environmental conditions (Kader, 1987). The stage of development a fruit has reached at harvest influences its respiration rate. Generally, respiration rates are highest in plants that are in a stage of development and growth, and as the plants mature their respiration rates tend to decline. For example, fruits and vegetables picked during their active growth periods, like leafy or floral vegetables, have a high respiration rate. Usually, in these types of vegetables, the rate of respiration rapidly decreases during post harvest due to the depletion of respiratory substrates. Some fruit-vegetables do not show this decline in respiration rate. These fruit types actually have increased post harvest respiration, and are termed "climacteric" fruits. Climacteric fruits exhibit increased respiration that reaches a maximum rate

after harvest. Such climacteric fruits include tomatoes, and several types of melons. Examples of non-climacteric fruits include peppers, green beans, and eggplant. Climacteric fruits also have increased metabolic activity that parallels their increased respiration rate. Climacteric fruits will ripen during this post harvest increased metabolic period (Kader, 1987).

A.2.f. Surface Area to Volume Ratio

The surface to volume ratio of a fruit can also affect the respiration rate. A greater surface area allows for a higher amount of gas exchange to occur and thus enhances respiration (Phan et al., 1975). In addition, the presence of any natural coating will also alter respiration. Vegetables or fruits with natural coatings, including natural waxes, have lower respiration rates than fruits without such protective barriers (Phan et al., 1975).

B. Transpiration of Plant Products

Transpiration is the movement of water through the cellular tissue of a plant, and eventual evaporation of this water from plant surfaces. This movement of water is driven by the gradient existing between the tissue of the plant and the surrounding air (Ben-Yehoshua, 1987). For leaves this gradient is created in the following manner. Water evaporates from the walls of internally located mesophyll cells, through the peripherally located epidermal cells, into the surrounding air. The evaporation from the plant's surfaces causes more water molecules to diffuse from the cytoplasm of the mesophyll cells onto their surface, where the water can again be transferred to epidermal cells and evaporate. As this diffusion takes place, water from the xylem in leaf veins moves into the mesophyll cells to replace the water that has evaporated. This water moving out of the leaf veins is replaced by water from the xylem cells leading into the veins from the stem. Lastly, water moves into the root xylem from the soil. This act of water moving through the plant tissue continues until the soil is so dry that the gradient between the plant tissue and surrounding air does not exist (Starr and Taggart, 1989).

Transpiration serves two purposes: first transpiration contributes to the lowering of the surface temperature of the plant's tissues. This lowering of temperature occurs when the water within the plant's cells passes into the gaseous phase. The evaporation of water requires energy, which is thus removed in the form of heat from the plant's surface. The second function of transpiration relevant to post harvest, is the maintenance of turgidity of the plant's tissues and fruits (Phan, 1987). Turgidity is maintained by various different types of structures specific to each plant.

B.1. Plant Structures For Prevention of Moisture Loss

As much as 90% of the water moving into a plant can be lost through transpiration. Plants have therefore developed specialized tissue structures for preventing moisture loss. Depending upon the commodity, some tissues are more effective at reducing loss than others. For example, parts of plants normally exposed to air, like fruit or flower vegetables, have developed structures to slow moisture loss. However, vegetables normally found underground, such as root or tuber vegetables, do not have extensive protective structures against water loss (van den Berg, 1987).

B.1.a. Cuticle

Surface cells of plants, including fruit, are equipped with a cuticle and epicuticular wax. The cuticular layer functions as a hydrophobic barrier, providing resistance to water and gas exchange. The cuticle consists of layers of wax and other lipids secreted by the epidermal cells. These layers are made up of cellulose, polyuronic acids, proteins and phenolic compounds all combined with waxes and arranged in a matrix on the plants surface (Lauchli, 1976). These waxes vary greatly between different commodities, for example a lime may have very little cuticle wax where as fruits like grapes or plums actually have "blooms" of epicuticular wax.

B.1.b. Stomata

Stomata, which are openings in plant tissue surfaces, are the primary sites of transpiration as well as carbon dioxide uptake. Stomata help regulate both water loss and carbon dioxide intake by utilizing two cells on either side of the stomata opening. These cells, termed guard cells, open or close depending upon how much water and carbon dioxide they contain. When the plant begins photosynthesis it uses carbon dioxide from the air to create starch and sucrose. The use of carbon dioxide in this process lowers the amount of the gas in the guard cells, and causes potassium ions to be pumped in from surrounding epidermal cells. The increase in potassium ions causes water molecules to move into the cells via osmosis. This influx of water causes an increase in turgor pressure and results in the stomata opening. The photosynthetic process keeps carbon dioxide low, and the plant loses water through open stomata. The continual act of transpiration causes the carbon dioxide to continually move into the plant tissue for photosynthesis during the day. However at night, when photosynthesis stops, carbon dioxide is not consumed, but is now released as a by-product of aerobic respiration. This release of carbon dioxide results in its accumulation in the plant tissues. Potassium then moves out of the guard cells, taking water with it osmotically. The loss of water results in decreased turgor pressure in the guard cells, which cause the stomata to close and allows water to be conserved by way of reduced transpiration (Starr and Taggart, 1989).

B.1.c. Lenticles

Gas exchange in fruits may also occur through the lenticles which arise in some fruit after the stomata stop functioning early in development. These structures can become filled with suberin, or cutin. Suberin is a complex fatty substance which is the basis of cork. Cutin is an insoluble mixture containing waxes, fatty acids, soaps and resinous material. When this happens gas exchange and moisture loss may be prevented, however in other types of fruit the lenticles may remain open and do not function to prevent moisture and gas loss (Kolattukudy, 1980).

B.1.d. Periderm

The periderm is an outer tissue made up of suberin-containing layers of epidermal cells. This makes this layer impermeable to water, and permeable to gases only through lenticles which have replaced the original stomata. An example of a vegetable with a periderm is a potato tuber (Ben-Yehoshua, 1987).

B.2. Post Harvest Changes Related to Alteration of Transpiration

B.2.a. Disruption of the Water Supply

As previously stated, water is continuously flowing through plant tissues by the process of transpiration. The evaporation process occurring at leaf surfaces serves to create a negative pressure force which causes water to be pulled through the tissues of the plant. When a fruit is removed from the plant, the replenishing water source, the soil, is cut off and turgor is altered. The speed at which damage from loss of turgidity occurs depends on the characteristics of the commodity, including its rate of respiration, size, and state of maturity.

B.2.b. Respiration

Respiration produces water and heat, both of which directly affect transpiration. The metabolic water produced through respiration remains within the fruits' tissue, however, the carbon dioxide lost to the air through open stomata can result in weight loss of harvested fruits. Heat generated during increased respiration after harvest may also contribute to weight loss of a fruit. The heat lost to the environment contributes to increased evaporation of water. Respiratory heat increases temperature of the fruit or vegetable and thereby increases the rate of evaporation.

B.2.c. Air currents

Air currents also affect rates of transpiration. Normally, in still air, the relative humidity near the surface of a fruit is higher than the ambient air. This high humidity results in very little evaporative losses in the fruit. However, in leaves, when air currents reach a speed of 84 cm/second or greater this protective high humidity area is disturbed (Nobel, 1974), the relative humidity normally in the boundary layer around the fruit disappears, the humidity declines and transpiration losses to the atmosphere increase.

B.2.d. Fruit Size

Fruit size, or more specifically the surface to volume ratio of a fruit, has a dramatic effect on transpiration rates. The lower surface to volume ratio of larger fruits allows less moisture loss per unit weight. For example, greater water loss occurred in longer more narrow carrots as compared to their thicker counter parts, and small apples lost more water than larger fruits (Phan et al., 1975; Sastry et al., 1978).

B.2.e. Stage of Maturity

Water losses from transpiration may also be affected by the stage of fruit maturity. In general, climacteric fruits have increased transpiration at very early (pre-climacteric) stages. Increased transpiration also occurs at the beginning of the climacteric phase. Other fruits may have different patterns. For example, apples have higher transpiration rates early in the season, probably due to their thinner skin at this stage, whereas more mature fruits, which have developed a thicker skin, can reduce transpiration more successfully (Phan et al., 1975).

C. Plant Hormone Production

Plant hormones, also known as phytohormones, play an important role in the growth and

development as well as the eventual senescence of plant tissues. Plant hormones can be divided into two types, those that promote growth and development and those that inhibit growth and enhance senescence. Growth promoting hormones include auxins, gibberellins, and cytokinins. Growth and development inhibitors include abscisic acid and ethylene. Auxin is the hormone responsible primarily for cell elongation, cell enlargement and cell differentiation. Gibberellins and cytokinins also function in growth and development. Abscisic acid is involved in the induction of dormancy, inhibition of growth, and the promotion of abscission and senescence. Ethylene is also involved in the promotion of senescence, and is considered in further detail in the following section (Greulch, 1973b).

Ethylene directly and indirectly regulates metabolism. Increased levels of ethylene increase respiratory activity, increase enzymatic activity, decrease cell compartmentalization, alter auxin transport and metabolism (Pratt and Goeschl, 1969). Despite all of these actions the actual mechanisms of ethylene's actions to promote senescence are unknown.

Ethylene promotes many changes in fruits and vegetables, such as loss of green color. For example, green color loss in green peppers declined with reduced ethylene exposure (Saltveit, 1977). Ethylene plays a role in abscission, which is the natural separation of the fruit from the mother plant (Reid, 1985), and induction of enzymes like polyphenol oxidase, peroxidase and pectinase, which all are related to post harvest changes. For example, softening of watermelon due to ethylene exposure was demonstrated by Risse and Hatton (1982). Ethylene also promotes loss of acidity, the conversion of starch into sugar, and formation of aroma compounds in climacteric fruit (Pratt and Goeschl, 1969). Ethylene was associated with increased rates of respiration in fruits and vegetables. For example, Sarkar and Phan (1979) found that exposure to ethylene increased respiration rates of carrots.

II. Post Harvest Quality Changes

Many quality changes in produce can be observed after harvest. Such changes include reduced sensory appeal, including texture, flavor and color changes. Other quality changes involve deterioration of nutrients, and altered storage capabilities.

A. Sensory Quality Changes

A.1. Color

Fruits and vegetables contain 5 major pigments, including chlorophylls, carotenoids, anthocyannins, anthoxanthins, and betalains. The following section describes some of the factors that alter these pigments.

A.1.a. Chlorophyll

Chlorophyll pigments are found in clusters called photosystems located in the thylakoid membrane contained within the chloroplast. These pigments give a green color to fruits and vegetables. Changes in chlorophyll content are probably the most dramatic post harvest color alteration. The loss of chlorophyll is influenced by light, temperature, and humidity, although the influence of these factors is different for different vegetable tissue types. For example, light can enhance degradation of chlorophyll in tomatoes, while light enhances chlorophyll production in potatoes (Martens and Baardseth, 1987).

Chlorophyllase catalyzes the cleavage of phytol from chlorophyll to from chlorophyllide as well as removes the phytol group from pheophytin (derivative of chlorophyll) to form pheophorbide (Whitaker, 1996). This enzyme is activated after harvest by the increased heat produced in post harvest commodities. The activity of chlorophyllase also depends upon external factors. For example, chlorophyllase production is enhanced when ethylene is present, thus higher levels of ethylene will be associated with lower chlorophyll content, and hence, decrease green color

(Martens and Baardseth, 1987).

Green pigment may also be lost through photodegradation, which occurs when chlorophyll molecules are bleached by light and oxygen. This process may occur during senescence. Normally, lipid membranes and other pigment molecules like carotenoids protect the chlorophyll pigments. However deterioration of these membranes during senescence makes the chlorophyll molecules vulnerable to degradation (Llewellyn et al., 1990a; Llewellyn et al., 1990b).

A.1.b. Carotenoids

Carotenoids are pigments with colors ranging from yellow to orange. Carotenoids are divided into two groups based on structure: the oxygenated carotenoids, or xanthophylls, and the hydrocarbon carotenes (von Elbe and Schwartz, 1996). The carotenoid content of plants can be affected by many different factors, such as the stage of maturation, growing climate, whether the plants were fertilized or treated with pesticides, and soil type (von Elbe and Schwartz, 1996).

Carotenoids may also be affected by exposure to oxygen. Oxygen damages carotenoids by attacking their numerous double bonds. Normally carotenoids are located within membrane bound chromoplasts which help prevent their exposure to oxygen. However, if tissue becomes damaged carotenoids may come in contact with oxygen.

Exposure to light also initiates reactions that may decrease carotenoid pigments. Again, because of their repeated double bond structures carotenoids are susceptible to free radical attack. Free radicals may be present in tissues as by-products of aerobic respiration, as a result of the effects of light on the tissue, or as a result of other normally occurring processes. Normally, carotenoids can serve to quench free radicals and serve to protect other tissues. However, if their protection capabilities are overwhelmed the carotenoids may be irreversibly damaged. Damage may result in loss of orange or yellow pigment in a fruit or vegetable. Free radical attack may be enhanced with

increased light exposure as well as hormonal production of ethylene during storage. These substances can stimulate free oxidative changes in the pigments (Gregory, 1996; Burton, 1982b).

Enzymes may also degrade carotenoid pigments. The enzyme lipoxygenase indirectly increases oxidative damage to carotenoids. The mechanism by which lipoxygenase works is first by its degradation of polyunsaturated fatty acids into peroxides. Peroxides in turn attack the double bonds of carotenoids and cause color loss (Whitaker, 1996).

A.1.c. Anthocyanins

Red-blue pigments of fruits and vegetables are known as anthocyanins. These pigments are part of a subgroup of phenolic compounds found in fruits and vegetables, called flavonoids. Anthocyanins are unstable compounds which readily change color and are degraded under a number of conditions including changes in pH, temperature, oxygen concentrations, and the presence of enzymes. Because of the structure of anthocyanins, which consist of many double bonds, they too, like carotenoids, are susceptible to oxidative damage (von Elbe and Schwartz, 1996).

Enzymes may also affect anthocyanin pigments. Collectively these enzymes are termed anthocyanases. The anthocyanases are composed of two subgroups of enzymes: polyphenol oxidases, and glycosylases. The glycosylases hydrolyze glycosidic linkages. This reaction makes the anthocyanins less soluble which results in their change to a colorless pigment. The enzyme polyphenol oxidase, along with a co-substrate, oxygen, works indirectly to degrade anthocyanins. This mechanism works by enzymatically converting phenolic compounds into quinones. The quinones then react with the anthocyanins and from oxidized pigment products (Markakis, 1982).

A.1.d. Betalains

Betalains are purplish red pigments similar in color, but not structure, to anthocyanins. Like other pigments previously mentioned, betalains are subject to degradation by light and oxygen. Oxidative degradation of betalains occurs via attack by molecular oxygen, and not electron deficient free radical species, as seen with other pigments. The presence of light, too, accelerates the amount of degradation accomplished by oxygen (von Elbe and Schwartz, 1996).

A.1.e. Browning by Polyphenol Oxidase

The enzyme polyphenol oxidase, which is present in high quantities in certain fruits and vegetables, including apples and pears, causes a degradative change in color known as enzymatic browning. This enzyme reacts with its substrate, phenolic compounds, in conjunction with its co-substrate, oxygen, to produce quinones. These quinones undergo further oxidation and then polymerize to form brown pigments known as melanins. Because the enzyme requires oxygen, exposure to air via tissue injury will cause this reaction to occur. Many methods for prevention of enzymatic browning exist, some of which eliminate the co-substrate oxygen, while others denature the enzyme (Whitaker, 1996).

A.2. Flavor

Many combinations of compounds in foods give rise to their characteristic flavors. Volatile flavor components may exist in intact tissues, they may be formed enzymatically when cells rupture, or may be produced by microorganisms. Since a large number of compounds are present in vegetables and fruits it is usually very hard to distinguish which compounds are responsible for providing the characteristic flavors. Some of the compounds in fruits and vegetables that have been identified are reviewed in the following sections.

A.2.a. Flavors From Sulfur Containing Vegetables

Sulfur compounds give rise to characteristic flavors of garlic, onions, cabbage and turnips. The production of these flavors is the result of tissue damage, which results in release of specific enzymes contained within the tissues. Examples of these enzymes include allinase in onions and garlic, and glucosinolases in cabbage and brussel sprouts. These enzymes once released are free to react with precursor flavor compounds and convert them into the characteristic volatile flavor (Lindsay, 1996).

A.2.b. Methoxy Alkyl Pyraxine Derived Flavors

Green vegetables are sometimes described as having an "earthy-green" type of flavor and aroma. This flavor has been attributed to methoxy alkyl pyraxine. An example of a vegetable expressing this flavor compound in high amounts is the green bell pepper (Lindsay, 1996).

A.2.c. Fatty Acid Derived Flavors

Volatile compounds may also be derived from fatty acids. First, lipoxygenase, an enzyme that reacts with polyunsaturated fatty acids, produces break down products of these fatty acids which have particular flavors. In addition, beta oxidation of long chain fatty acids produces medium chain fatty acids which are responsible for flavors associated with the ripening of fruit. Other compounds associated with ripening fruit include volatiles from branched chain amino acids (Lindsay, 1996).

A.2.d. Citrus Flavors

Citrus flavors are attributed to several compounds including terepenes, aldehydes, esters, alcohols, and other volatile compounds (Shaw, 1991). Terpenoids are compounds that are synthesized in the fruit's tissue, and also occur in raw carrots.

A.3. Texture

Texture in fruits and vegetables is imparted by components of plant tissue and its cell walls. The cellular walls are made up of cellulose fibers which are held together by a cement like substance called pectin. The cell wall surrounds parenchyma cells, which are the edible portions of the vegetable. These cells take up water, which generates a hydrostatic pressure, giving rise to the crisp texture of vegetable and fruit products. After harvest several factors affect the texture of fruit and vegetable products. First, turgor pressure, and hence crisp texture is altered (Van Buren, 1979). Turgor pressure change results from decreased transpiration and respiration. Because additional water can not move into the plant cells, and water still is being continually lost from the plant's surface, wilting occurs.

Softening of fruits and vegetables is brought about by enzymatic dehydration of the pectin holding adjacent cells together. The ripening of fruit proceeds as follows. Pectin in an underripe fruit is in the form of protopectin. The enzyme protopectinase changes the protopectin into pectin, which is the substance found in ripe fruit. As the fruit begins to senescence and proceed to an overripe stage, the pectin is being changed into pectic acid by the enzyme pectinase. Pectic acid imparts the characteristic mushy texture to overripe fruit (Whitaker, 1996).

Microorganisms, particularly those that are pathogenic to plants, also produce a pectin altering enzyme known as pectin lyase. This enzyme splits the glycosidic bonds between pectin and pectic acid, which leads to degradation of texture (Whitaker, 1996).

B. Post Harvest Vitamin Changes

Fruits and vegetables are excellent sources of many needed vitamins. The two major vitamins found in fruits and vegetables are vitamins A and C. The role vitamins play in active plant tissue varies, however most vitamins function in plant metabolism. Although frequently overlooked as a

standard of quality in harvested commodities, vitamins are frequently vulnerable to depletion in harvested vegetables.

B.1. Ascorbic Acid

In plant tissue various forms of the same vitamin may exist. Ascorbic acid is an example of such a vitamin. Ascorbic acid can exist as reduced ascorbic acid, monodehydro ascorbic acid (an unstable intermediate), dehydroascorbic acid, and diketogulonic acid. Ascorbic acid in the reduced form can be reversibly oxidized to the dehydroascorbic acid form during the protective action against oxidative damage. However, should oxidative damage continue, dehydroascorbic acid can become irreversibly oxidized to 2,3 diketogulonic acid, a form which retains no ascorbic acid properties (Watada, 1987).

Several factors can enhance the conversion of ascorbate to 2,3 diketogulonic acid, including alkaline pH, higher oxygen concentrations, the presence of metal catalysts, heat, light, and low water activity. In addition to oxidative damage, enzymes also may function indirectly to lower vitamin C content. For example, ascorbic acid oxidase, an enzyme found in squash, can oxidize vitamin C. Lipoxygenase activity can also indirectly affect vitamin C content, by generating free radicals from the oxidation of polyunsaturated fatty acids which in turn can react with, and damage vitamin C (Gregory, 1996).

B.2. Beta Carotene

Carotenoids, which are known as vitamin A precursors, are also abundant in plant tissue. Beta carotene is the most well known carotenoid. These vitamins scavenge free radicals derived from aerobic metabolism in chloroplasts. In addition, as described earlier, carotenoids provide yellow orange pigments to plant tissue. Damage and loss of carotenoids, which result in lowered amount of vitamin A precursors, occur by the same mechanisms described earlier for loss of carotenoids

resulting in color loss. These include loss of carotenoid function due to oxidative damage, light, and lipoxygenase enzyme activity.

In addition to oxidative damage and enzyme degradation, the vitamin contents of a crop can be affected by many other variables. Such variables can be categorized as either horticultural practices or storage practices. Horticultural practices that may affect vitamin content include the amount of water the plant receives, the type of soil it grows in, the actual variety of the fruit or vegetable, and the stage of maturity of the commodity upon harvest. Storage conditions that may affect vitamin content include temperature, humidity and atmospheric gas composition (Gregory, 1996).

As storage temperature increases, the rate of vitamin loss increases. Again, according to Van't Hoff's law, for every 10° C increase in temperature the rate of a biological reaction, including vitamin degradation, can double, or even triple. However, this rule is usually only applied to early losses in nutrients, since the rate of loss declines as the amount of vitamin loss reaches the maximum (Watada, 1987).

Relative humidity is also involved in vitamin changes post harvest. The role relative humidity plays in vitamin loss is associated with the rate of wilting, and thus vegetables that lose water quicker, will be more adversely affected. Therefore, vitamin loss parallels moisture loss. Such problems can be prevented by maintaining a high humidity level in storage areas.

Atmospheric conditions affect nutrients, however, the effect depends on the type of fruit, as well as the storage temperature. For example, low oxygen atmospheric storage conditions inhibit ascorbic acid breakdown in green peppers (Bangerth, 1977).

III. Methods of Preservation of Fruits and Vegetables Post Harvest

Many methods have been used for lengthening the post harvest life of fruit and vegetable commodities by altering the physiological processes leading to eventual senescence and decay of fruit and vegetable products. Such alterations include the use of refrigeration, external alteration of storage atmospheric conditions, and use of packaging and coatings.

A. Refrigeration

Refrigeration, or lowering of the storage temperature, can serve to slow metabolic reactions including respiration and transpiration, as well as slow enzymatic activity which may deteriorate the produce. The temperature at which a commodity is stored is usually very specific to that particular product. If storage temperatures are too low, chilling injury may result. However, if temperatures are too high, metabolic processes can accelerate. In addition, a wide range of storage temperatures is also not advisable, because such conditions lead to rapid weight loss of produce (Salunkhe et al., 1991).

B. Alteration of Relative Humidity

Altering the relative humidity (RH) of the storage environment may also delay senescence. Perishable fruit and vegetable products should be maintained at RH levels of 90-95%. This high humidity level prevents moisture loss that may occur due to increased respiration and lowered transpiration. Water loss of up to 5-10% weight loss results in shriveling and a product not fit to be sold. However, humidity levels should not exceed 95% because growth of microorganisms may be enhanced (Salunkhe et al., 1991).

C. Controlled Atmospheric Storage

Controlled atmospheric (CA) storage refers to the removal or addition of gasses which creates a storage atmosphere vastly different from normal air storage conditions (Salunkhe et al., 1991).

Gases that may be altered include carbon dioxide, oxygen, carbon monoxide, nitrogen and ethylene. Usually, however, CA storage simply refers to lowered oxygen, and increased carbon dioxide and nitrogen conditions.

Benefits of controlled atmospheric storage include delayed senescence via retardation of physiological processes, including respiration, ethylene production, and textural changes, including softening. Fruit sensitivity to ethylene also decreases. In addition, control of chilling injury or other storage related problems, including decay by oxygen requiring pathogens is also accomplished under CA storage conditions (Salunkhe, et al., 1991).

Unfortunately, several problems are also associated with controlled atmospheric storage. Such problems include irregular ripening patterns of such fruit as bananas, pears, and tomatoes due to the lower oxygen concentration. Also, if oxygen concentrations are not adequate to maintain aerobic respiration, the anaerobic respiratory pathway of fermentation may begin. Fermentation by-products are associated with off flavors and aromas in fruits and vegetables. Some vegetables will also have increased susceptibility to decay because of the low oxygen and/or high carbon dioxide concentrations. Potatoes may be particularly adversely affected by CA storage. Such problems may include blackheart disease, stimulation of sprouting, and slowing of periderm formation (Salunkhe et al, 1991).

D. Use of Polymeric Films for Storage of Fresh Fruits and Vegetables

Films used to package fruits and vegetables have been used to produce conditions similar to controlled atmospheric storage. Films generate controlled atmospheric conditions by providing a barrier between the commodity and the outside atmosphere. A fruit or vegetable contained within the films will create the modified atmosphere itself. Utilization of existing oxygen results in low oxygen levels contained within the system, while carbon dioxide evolves and accumulates serving to slow physiological processes. The respiration rate is therefore equal to the rate that oxygen

can permeate the film and provide the commodity with the ability to aerobically respire. Films may be perforated to lower the carbon dioxide level, and prevent anaerobic respiration, however perforation does destroy the semipermeable nature of the film (Salunkhe et al, 1991).

E. Vacuum and Gas Flush Packaging

Vacuum storage and gas-flush packaging have also been used to help delay senescence of perishable commodities. Vacuum packaging, which involves the removal of all air components contained within the packaging material, reduces browning and maintains crisp textures of fruit and vegetable products. Products packaged this way also have higher nutritional values. Gas-flushed packaging involves removing excess atmospheric air from inside packaging materials and replacing it with either carbon dioxide or nitrogen. Such packaging methods have been used to preserve pre-cut salad ingredients and minimally processed fruits and vegetables. Again, with both of these methods, anaerobic respiration must be avoided due to the resulting development of off flavors and aromas associated with its by-products (Salunkhe et al, 1991).

F. Subatmospheric Storage

Subatmospheric storage, also known as low pressure or hypobaric storage, refers to the storage of produce at a given temperature, in a sealed container, at a constant subatmospheric pressure. This type of storage reduces the oxygen supply and therefore reduces the respiratory rate of the produce. In addition, ethylene and other gases evolved from the produce are removed, which results in inhibition of ripening and delay of senescence (Salunkhe et al, 1991).

G. Radurization

Radurization, which is the pasteurization of food by exposure to ionizing radiation also has implications in food preservation. Beneficial consequences of radurization include the elimination

of insect and microbial infestations, and delay in sprouting of bulb and tuber vegetables. However, this method of preservation is not without problems. Such consequences of irradiation include loss of color, loss of vitamin components, and increased softness or other texture changes (Salunke et al., 1991).

H. Edible Coatings

Edible coatings have been used to preserve a variety of foods, including fruits and vegetables. Today, coatings can be made from a variety of materials including lipids, proteins, and polysaccharides. The following is an overview of the types of coatings made from these materials.

H.1 Polysaccharide Coatings

Edible coatings can be made from a variety of polysaccharides. Such coatings have been used to retard moisture loss of some foods during short term storage. However, polysaccharides, being hydrophilic in nature, do not function well as physical moisture barriers. The method by which they retard moisture loss is by acting as a sacrificial moisture barrier to the atmosphere, so that the moisture content of the coated food can be maintained (Kester and Fennema, 1986). In addition to preventing moisture loss, some types of polysaccharide films are less permeable to oxygen. Decreased oxygen permeability can help preserve certain foods. Polysaccharide coatings can be made from a variety of sources ranging from seaweed extracts to connective tissue extracts of crustaceans. The following is an overview of several types of polysaccharide coatings.

Alginate, which is a polysaccharide derived from brown seaweed, provides beneficial properties when applied as an edible coating to precooked pork patties (Wanstedt et al., 1981). Such coatings increase moisture, and reduce perception of warmed over flavor induced by lipid oxidation. Lazarus et al. (1976) showed alginate coatings reduced shrinkage as well as surface microbial counts of stored lamb carcasses. Coatings made from pectin also have similar beneficial

properties (Kester and Fennema, 1986).

Carrageenan, a polysaccharide derived from red seaweed, protects against moisture loss by acting as a sacrificial moisture layer (Kester and Fennema, 1986). Coatings made of starch, particularly amylose, reduce oxygen permeability (Wolff et al., 1951). Cellulose based films reduce moisture loss and reduce the amount of oil absorbed by fried foods (Dziezak, 1991; Sanderson, 1981). Coatings have also been made from chitosan, a polysaccharide found in abundance in the shells of crustaceans. Chitosan reduces water loss, respiration, and fungal infection in bell peppers and cucumbers (Ghaouth et al., 1991). Treatment with chitosan also induces activity of chitinase enzymes which are made by the plant for the purpose of self protection against pathogens (Mauch et al., 1984).

H.2. Protein Based Films and Coatings

Edible coatings can also be made from a variety of protein sources, however, as a group, protein coatings are the least developed material for coating use. Protein coatings are in general hydrophilic and susceptible to moisture absorption, and therefore relative humidity and temperature can affect them. Sources of proteins used in these coatings include corn zein, wheat gluten, soy protein, milk proteins and animal derived proteins like collagen, keratin and gelatin (Gennadios et al., 1991).

A wide variety of food products have been coated with proteins. For example, one of the oldest known edible coatings used for packing meat products is made from the protein collagen (Gennadios et al., 1991). Casein proteins, derived from milk, have been used in emulsion based coatings to reduce water loss in zucchini (Avena-Bustillos et al., 1994). Corn zein proteins have been used to prevent oxidative rancidity, staling and sogginess in nut products (Gennadios et al., 1991). Zein proteins have also been used effectively as coatings for confectionery products (Gennadios et al., 1991). Fruit and vegetable products, however have not been coated

successfully with protein based coatings, primarily due to the fact that these types of coatings do not provide effective moisture barriers (Gennadios et al., 1991).

H.3. Lipid Based Coatings

As mentioned previously, lipid based coatings have been used for over 800 years. Historically, uses of lipid based coatings include waxing fruits as well as coating confectionery products with chocolate (Kester and Fennema, 1986; Hardenburg, 1967). Lipid coatings are mainly used for their hydrophobic properties which make them good barriers to moisture loss. In addition to preventing water loss, lipid coatings have been used to reduce respiration, thereby extending shelf life, and to improve appearance by generating a shiny product in fruits and vegetables. Lipid based coatings can be made from a wide array of lipid substances including acetylated monoglycerides, natural waxes and surfactants (Kester and Fennema, 1986).

H.3.a. Wax and Oil Based Coatings

Wax and oil based coatings include paraffin wax, candelilla wax, beeswax, carnauba wax, polyethylene wax, and mineral oil. Paraffin wax is derived from crude petroleum oil and is used for coating raw fruits and vegetables. Carnauba wax is derived from the Tree of Life's palm tree leaves. Carnauba wax has a very high melting point and is used as an additive to other waxes to increase toughness and luster. Beeswax is made by honeybees and is used in certain levels in confectioneries. Candelilla wax is derived from the candelilla plant and is used in chewing gum and candy. Polyethylene wax is produced by the oxidation of polyethylene, which is a petroleum by-product. Polyethylene wax is used primarily to make emulsion coatings. Finally, mineral oil is made of a mixture of liquid paraffinic and naphthenic hydrocarbons. Mineral oil is commonly used for coating fruits and vegetables, and as a food release agent (Hernandez, 1991).

H.3.b. Fatty Acids and Monoglycerides

Fatty acids and monoglycerides are used in coatings mainly as emulsifiers and dispersing agents. Fatty acids are generally extracted from vegetable oils, while monoglycerides are prepared by transesterification of glycerol and triglycerol (Hernandez, 1991).

H.3.c. Emulsions

Use of wax based emulsion coatings is a relatively new idea. Such emulsion coatings have excellent moisture barrier properties, however, they do not add shine to a product. Many of the emulsifiers used in such wax emulsions are derivatives of glycerol and fatty acids. Examples of such commercially available emulsifiers include polyglycerols-polystearates (Hernandez, 1991).

H.3.d. Resins and Rosins

Resins and rosins included in edible coatings are wood rosin and coumarone indene, both of which are used for coating citrus fruits. Solvent waxes are coatings made primarily of resins, small amounts of wax, and added petroleum solvent. Such coatings are water resistant and shiny due blending of the resin and wax allowed by the solvent (Hernandez, 1991).

H.3.e. Composite and Bilayer Coatings

Composite and bilayer coatings are the edible coatings of the future. These two types of coatings work to combine the beneficial properties of coating ingredients to create a superior film. Composite films of the future may consist of hydrophilic particles within a hydrophilic matrix. Such a configuration could give a water soluble coating with good water vapor barrier properties (Baldwin, 1991). Bilayer coatings, which already have been used to a limited extent, combine the water barrier properties of lipid coatings with the greaseless feel and good gas permeability characteristics of polysaccharide coatings. Such a bilayer coating has been shown to reduce gas

exchange, and result in higher internal carbon dioxide and lower oxygen concentrations in cut apple pieces (Wong et al., 1994). Unfortunately, little or no data exists showing the effects of bilayer coatings on whole fruit or vegetables.

Chapter 3:Justification and Purpose

Existing methods used to maintain high quality produce include refrigeration and controlled atmospheric storage or a combination of both. These methods both help to slow down fruit or vegetable respiration rates and help delay senescence and thereby extend shelf life (Salunkhe et al., 1991). Unfortunately, however, these methods are expensive to install, costly to maintain, as well as impractical for use with small quantities of produce. The application of edible coatings to freshly harvested produce offers a less expensive alternative with potentially equally beneficial outcomes. The use of coatings creates a modified atmosphere surrounding the commodity similar to that achieved by controlled or modified atmospheric storage conditions. The modified atmosphere created by edible coatings can protect the food from the moment it is applied, through transportation to its final retail destination, and in the home of the consumer (Smith et al., 1987).

In addition to increasing shelf life and prolonging senescence, coatings add shine and luster to commodities thus making them more attractive and appealing to consumers (Kaplan, 1986). Also, shriveling or weight loss and the textural changes that follow can be prevented by applying coatings, as has been demonstrated in green peppers (Lerdthanangkul and Krochta, 1996). Color changes that may be lost after harvest can also be preserved with coatings.

Although not a characteristic by which the quality of a harvested crop is judged, nutrient content is none the less of paramount concern. Vitamin losses, specifically vitamin C, can occur readily in the post harvest environment (Watada, 1987). Therefore edible coatings may provide a way to maintain nutritional quality.

Finally, although many benefits of applying edible coatings are known, the exact coatings to use for specific vegetables have not been well researched. The coatings which will provide maximum benefits, without jeopardizing any aspect of vegetable or fruit quality, should be further explored. For example, one problem with some coatings is limited oxygen permeability. When such a

coating is used the amount of oxygen reaching the fruit is too low to allow aerobic respiration to continue at all, which causes the plant to shift to an anaerobic pathway of respiration, fermentation. By-products of fermentation produce off flavors and aromas, and other biological disorders, all of which will shorten shelf life (Kester and Fennema, 1986). Therefore research is needed to evaluate the ability of various coatings to increase shelf life and affect the quality of fruits and vegetables post harvest.

Therefore, the purpose of this research is to determine the effect of two different lipid based coatings on the post harvest quality characteristics of green bell peppers. Specifically, the study will measure the effect of the coatings on quality changes during storage, including: texture, moisture retention, color, respiration rate, and vitamin C activity.

Chapter 4: Materials and Methods

The experiments in this study were divided into two sections, both of which served to test the ability of two different lipid-based edible coatings to preserve pepper fruits (*Capsicum annum L.*.cultivar King Arthur) during a 21 day storage period. Peppers were obtained from William's Farms, located in Lodge, South Carolina. Peppers were picked the day prior to purchase and transported in an air conditioned automobile back to Blacksburg, VA. One overnight stop was made in which the peppers were stored in air conditioning. Experiments were started two days after harvest.

The coatings selected were Pac Rite Durafresh (EcoScience Produce Systems, Orlando, FL) and Apex B (AC Humko, Memphis, TN). In general, lipid coatings, because of their hydrophobic nature act as excellent moisture barriers. The Durafresh coating is manufactured for the specific purpose of coating vegetables. It is made from aliphatic petroleum distillate (mineral oil) and other food grade ingredients. According to the manufacturer this coating functions to prolong shelf life, enhance appearance, prevent wrinkling, and allow "breathing" while substantially reducing moisture loss. The second coating, Apex B, is composed of partially hydrogenated vegetable oils (soybean and cotton seed). This coating, although not manufactured exclusively for vegetable coating, has been used for spray coating and vegetable products. The two coatings differ in their fatty acids, as well as their viscosities, drying properties, and overall appearance on the produce. The purpose for using two different lipid coatings was to determine which was more effective at preserving vegetable products during post harvest storage.

In total, six indicators of quality were tested each week. Section one consisted of measuring the ascorbic acid content, dehydroascorbic acid content, respiration rate, and weight changes in the peppers. Section two consisted of measuring color and texture changes. The values obtained from both coated pepper groups were compared to uncoated control peppers for a final comparison of storage changes. In addition, weekly changes in each group were also contrasted

with initial observations.

A. Experimental Design

Each section tested 48 green bell pepper fruits. Each variable was tested on 4 randomly selected peppers. Texture, color and respiration were measured in duplicate, only one value per pepper was recorded for the remaining variables (weight, ascorbic acid and dehydroascorbic acid content). Initial baseline values of each tested variable were established on day 0 of the test period using 12 peppers. The two coated groups and the control group were subsequently tested every seven days. For section 1, respiration rates and weight were measured on the 7th, 14th and 21st days. The same peppers used for measuring respiration rates were used for ascorbic acid and dehydroascorbic acid analysis on days 8, 15, and 22. For section two, color and compression changes were measured every seven days. Color was analyzed first and then the same piece of pepper was used to determine texture. Both tests were done on days 7, 14 and 21. A schematic outline of the experiment for sections one and two can be seen in Figures 4.1 and 4.2 respectively.

B. Preparation of Fruits

The total 48 peppers in each phase were divided into 3 groups: two coated experimental groups and one uncoated control. Coating procedures for both experimental groups were the same.

Fruits were prepared similarly to procedures described in Lerdthanangkul and Krochta (1996). Initially, fruits were washed with a 0.5% sodium hypochlorite solution (prepared from 5% commercial bleach) for disinfection purposes, and then rinsed with water. After rinsing, the fruits were blotted dry with paper towels. Following drying, lipid coatings were applied by applying the coating to latex gloves and then smoothing it on the pepper fruits by hand. Excess coating was allowed to drain off onto paper towels. After coating, fruits were placed in cardboard boxes which are commonly used for post harvest vegetable storage. These boxes are composed of

48 peppers total

(12 used each week for 4 weeks)

Days 7, 14, 21: 12 peppers (4 from each experimental group)

↓

Respiration (duplicate) and Weight Recorded

↓

Days 8, 15, 22: 12 peppers (same fruits used above)

↓

Ascorbic Acid and Dehydroascorbic Acid Analysis

Figure 4.1: Flow diagram for phase 1: respiration, weight, ascorbic acid and dehydroascorbic acid changes.

48 peppers total

12 used each week for 4 weeks

Days 7, 14, and 21: 12 peppers (4 from each experimental group)

↓

Texture (puncture) testing (duplicate)

and

Color testing (duplicate) using peppers from texture test

Figure 4.2: Flow diagram for phase 2: color and texture changes.

cardboard coated with food grade vegetable-, petroleum-, beeswax-, and or shellac-based wax or resin. Once placed in these boxes the fruits were stored at 45°± 2° F in refrigerators located in the Human Nutrition, Foods, and Exercise Department of Virginia Polytechnic Institute and State University (Blacksburg, VA).

C. Color Analysis

Surface color analysis (Appendix A) of the bell pepper fruits was performed with a Hunter D25 L Optical Sensor Colorimeter (Reston, Va). L*, a*, and b* values were recorded (Appendix I). A 30 mm X 50 mm sample was taken from the side wall of the pepper and used in the procedure. This experiment was repeated with a second sample from the same pepper and the resulting values were analyzed as subsamples taken for each fruit. Using Statistical Analysis Sysytem (SAS), the hue angle and chroma were calculated from the L*, a* and b* values according to procedures detailed in McGuire (1992).

D. Texture Analysis

Texture (Appendix B) of green pepper fruit walls was analyzed using a puncture test performed on the Instron (Model 1011; Canton, MA). The same pepper samples that were used for color analysis were also used for texture analysis. These pieces were 30 mm X 50 mm cut from the side walls of the fruit. The sample was placed on the Instron, outside surface facing up, for the test. The pepper was punctured with a 0.78 mm diameter stainless steel cylindrical probe. The probe was programmed to have a load level of 5 kg, with a high load level of 10 kg. The high extension was 18 mm. The cross head speed was 100 mm/min and the load cell used was 50 kg. The maximum amount of force (load kg) needed to puncture the pepper sample was recorded. Two samples per pepper were tested and analyzed as subsamples.

E. Ascorbic Acid and Dehydroascorbic Acid Analysis

Using high performance liquid chromatography (HPLC), ascorbic acid content and dehydroascorbic acid content were determined (Appendix C) according to an adapted procedure) from Wimalasiri and Wills (1983).

1. Ascorbic Acid and Dehydroascorbic Acid Extraction

Ascorbic Acid (AA) and dehydroascorbic acid (DHA) were simultaneously extracted by first weighing a 25 gram sample of cut pepper pieces (approximately 1 cm X 1 cm) into a 4 ounce mini blender (Osterizer Blend 10 Pulsematic). Fifty milliliters of 3% citric acid was added to the pepper pieces and the mixture was placed on the "blend" setting for 2 minutes. After blending, the entire homogenate was transferred to centrifuge tubes. The blender parts were rinsed with 10-20 ml of 3% citric acid in order to wash remaining vegetable residue into the centrifuge tubes. The homogenate was centrifuged for 10 minutes at 7000 rpm in a Sorval RC-2 centrifuge for the purpose of reducing some of the foam created during blending. After centrifugation, the contents of the tubes were collected into a 100 ml volumetric flask. The tubes were rinsed with a small amount of 3% citric acid to wash vegetable residue into the flask. Finally, the flask was brought to volume with additional citric acid.

Solid phase extraction was then performed for the purpose of removing contaminates that may have interfered with chromatogram production. A J & W Scientific (Folsom, CA) extraction apparatus, and Octadecyl (C18, 200mg, C18, #9002; Burdick and Jackson, Muskegan, MI) solid phase extraction cartridges were used for solid phase extraction. The cartridges were activated with 4 ml of methanol and then rinsed with two consecutive washes each using 4 ml of HPLC grade water. Finally, 4 ml of homogenate was run through the filters; the first 3 ml were discarded and the remaining 1 ml was collected for analysis. This filtrate was then transferred into clear HPLC vials for use in the autosampler.

Ascorbic acid was analyzed by injecting 10 µl at 254 nm by UV detection. Run times were 8 minutes. Dehydroascorbic Acid (DHA) was analyzed using the same vegetable homogenate sampled for ascorbic acid analysis. However, 20ìl was injected and UV detection readings were carried out at 214 nm. Run times for DHA were 12 minutes.

2. Preparation of Standards

Ascorbic acid stock standard was prepared with 10 mg L-ascorbic acid (Aldrich, A9,290-2) in 10 ml of HPLC grade water. A standard curve was made using injections of 2, 5, 7, and 10 ìl. These injection volumes are equivalent to the 2, 5, 7, and 10 µg standard amounts due to the preparation of a 1:1 standard. Ascorbic acid was also used as a standard for DHA, due to its ability to also absorb at the 214 nm wavelength. A standard curve for DHA was derived from injections of 0.5, 1, 2, and 5 µl which again corresponded to a standard curve of 0.5, 1, 2, and 5 µg. (Appendix C).

For confirmation of the presence of DHA, a DHA standard was made using 10 mg of DHA (Pfaltz and Bauer, D01400) in 10 ml of 3% citric acid. The solution was heated to 60°C for the purpose of getting DHA to go into solution.

3. HPLC Specifications

The HPLC equipment was the following:

Waters Associates Brand: 600 E System Controller
484 Tunable absorbance detector
700 Satellite WISP autosampler

The column used was a Phenosphere 5 micron NH2 80 A, Phenomenex Cat. #00G-4087-E0, length X ID: 250 mm X 4.6 mm. The mobile phase was acetonitrile/water (70:30) using optima HPLC Solvents containing 0.01 M ammonium dihydrogen phosphate, and adjusting the pH to 4.3

with orthophosphoric acid. The flow rate was 2.0 ml per minute isocratic.

F. Weight Assessment

The weight of each pepper was recorded prior to respiration rate measurements using a Satorius portable scale PT 1200 (Bohemia, NY). Weight measures were used to estimate the amount of moisture lost from the fruits during the storage period.

G. Respiration Rates

Respiration rates measured the amount of carbon dioxide evolved from a single pepper at a given time (Appendix D). Pepper fruits were removed from refrigerated storage and allowed to come to room temperature for a minimum of 12 hours prior to testing. During this period the peppers were held in room 337 in the Department of Human Nutrition, Foods, and Exercise, in Wallace Hall. Returning the peppers to ambient temperature conditions was necessary for accuracy in determining respiration rates. Pepper samples were then transferred to the Department of Horticulture, Saunders Hall, for respiration analysis. The pepper was then placed in a chamber that is otherwise closed except for an opening for air to flow through the chamber via a rubber tubing at a rate of 5 liters/minute. The air passing the sample then flowed through an infrared gas analyzer (The Analytical Development Company, Model LCA2, Hoddeson, England). The difference between the carbon dioxide concentration of the incoming air and exiting air is the amount of carbon dioxide evolved by the pepper alone. The weight of the pepper is also determined in order to express the amount of carbon dioxide evolved (in milligrams) per kilogram per hour. The rate of respiration is calculated from the amount of carbon dioxide evolved according to the following equation:

Respiration Rate = $(\text{ÄCO2}.\text{F}\cdot\text{K})/\text{A}$

Where:

ÄCO2 = CO2 output - CO2 input (ambient air)

F = air flow in liters/hour

K = [44,000 mg carbon dioxide/22.4 L/mol] X (273/294)

(conversion of 1 L CO2 to 1 mg CO2)

A = kilogram weight of the fruit

H. Statistical Analysis

Data were analyzed using factorial analysis in order to determine whether the coated peppers' post harvest quality parameters differed from the uncoated control peppers. Factorial analysis was also used to determine if any of the parameters tested changed over time. Analysis of variance (ANOVA) calculations were used for the factorial analysis. Days or treatments that differed significantly at the P=0.05 level were subjected to Dunnett's difference test to compare each treatment to the nontreated control.

Chapter 5: Results and Discussion

A. Respiration

In this research edible lipid-based coatings were used to provide a physical barrier to gas exchange which generates modified atmospheric conditions within the fruit. There were significant differences ($p=0.0001$) in the respiration rates pooled over all treatments during the 21-day storage period (Table 5.1). Dunnett's one-tailed T test indicated that respiration rates on day 14 and 21 were significantly ($p<0.0001$) higher than on day 0.

One possible explanation for increased respiration may have been physical wounding. Normally, high levels of carbon dioxide inside the fruit suppress respiration due to feedback inhibition. Physical wounding and manipulation may cause an increase in respiratory rates due to disruption of the cuticle barrier which holds the carbon dioxide inside the fruit (Kader, 1987).

The presence of the hormone ethylene is also known to stimulate respiration (Kader, 1987). Ethylene synthesis can be induced by physical wounding, and thereby increase respiration rates. However, ethylene is believed to only naturally increase in post harvest commodities which fall under the category of “climacteric” fruits and vegetables. Bell peppers are not climacteric fruits; therefore, this type of increased respiration rate was not expected.

Water stress is also known to increase respiration rates (Kader, 1987). Water stress occurs when the relative humidity levels fall below the desired range. Optimum levels of relative humidity for green bell peppers are 85-95%. The humidity level in the refrigerators may possibly have been too low. However, if that had been the case increased respiration rates throughout the study would have been expected, as opposed to the sudden increase after 7 days. In addition, if water stress was occurring, textural changes should have also been observed.

Table 5.1: Respiration rates of green bell peppers during a 21 day storage period.

Day	Respiration Rate (mg CO_2 /kg/h)
0	427.67
7	467.61
14	835.75[a]
21	883.31[a]

[a]rates were significantly higher than day 1 (p=0.0001)

However, results from texture data showed no significant changes during the 21-day period (Figure 5.2). Respiratory analysis of peppers stored under controlled atmospheric conditions have shown that respiration rates are depressed in oxygen limited environments, although rates may still increase over time (Rahman et al., 1993). In contrast, Ghaouth et al. (1991) showed that respiration rates declined during storage for both uncoated and coated peppers. These same investigators also demonstrated that coated peppers exhibited an overall lower respiration rate when compared with their control counterparts. In this study, however, no significant differences ($p=0.6062$) were seen between treatments (Table 5.2). The lack of differences in respiration rates between treatments may demonstrate that the coatings were not effective at either preventing carbon dioxide from escaping the internal tissue or at lowering oxygen exposure. Lipid coatings function to preserve fruits and vegetables mainly via their ability to inhibit moisture loss. However, coatings which have higher melting points, and therefore become solid and more brittle at refrigeration temperatures may not effectively cover and seal the fruit's surface. Pinhole cracks or inconsistencies in the coating may allow for gas permeation.

One problem was noted during respiratory data collection. Each week the second (duplicate) set of data collected was consistently higher than the first set recorded. Possible explanations for this discrepancy may include recording the respiration rate prior to equilibration of the gas analyzer. Normally, during this type of analysis carbon dioxide output increases linearly while the container holding the pepper comes into equilibrium. When the level of carbon dioxide has leveled off a reading for emitted carbon dioxide should be recorded (Figure 5.1). Therefore, the first set of data may have been low due to inadequate equilibration. This was not likely the case due to the consistency of the lower results, which were always seen during the first reading. Another possibility is that temperature of the peppers may have increased during the testing period. The temperature increase may have occurred due to temperature differences between the holding room and the lab where analysis occurred. The second lab may have been slightly warmer than the holding room. As the peppers warmed, the respiration rate increased correspondingly.

Table 5.2: Comparison of respiration rates of coated and uncoated peppers stored for 21 days.

Experimental Group	Respiration Rate [a] (mg CO_2 /kg/h)
Control	642.57
Apex B	634.13
Durafresh	684.06

[a] no significant differences in respiration rates between experimental groups.

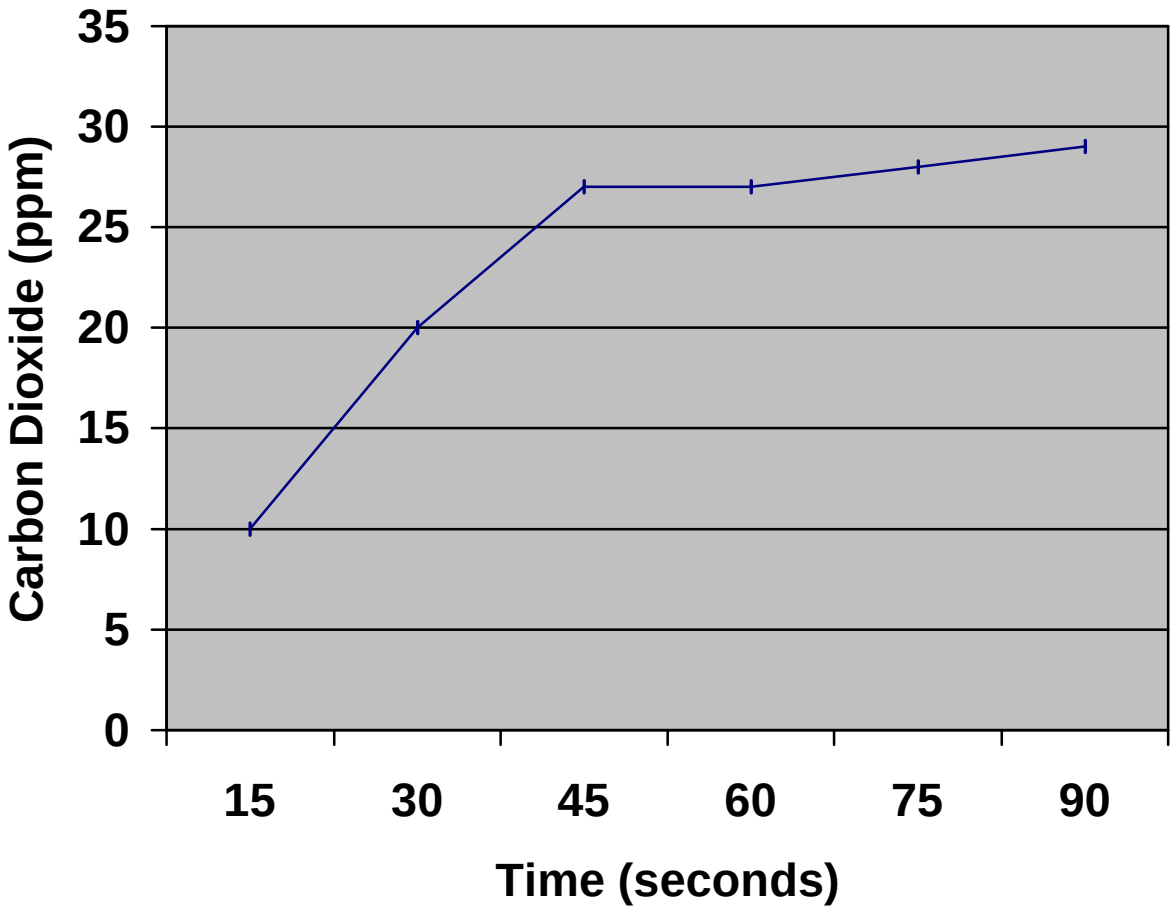


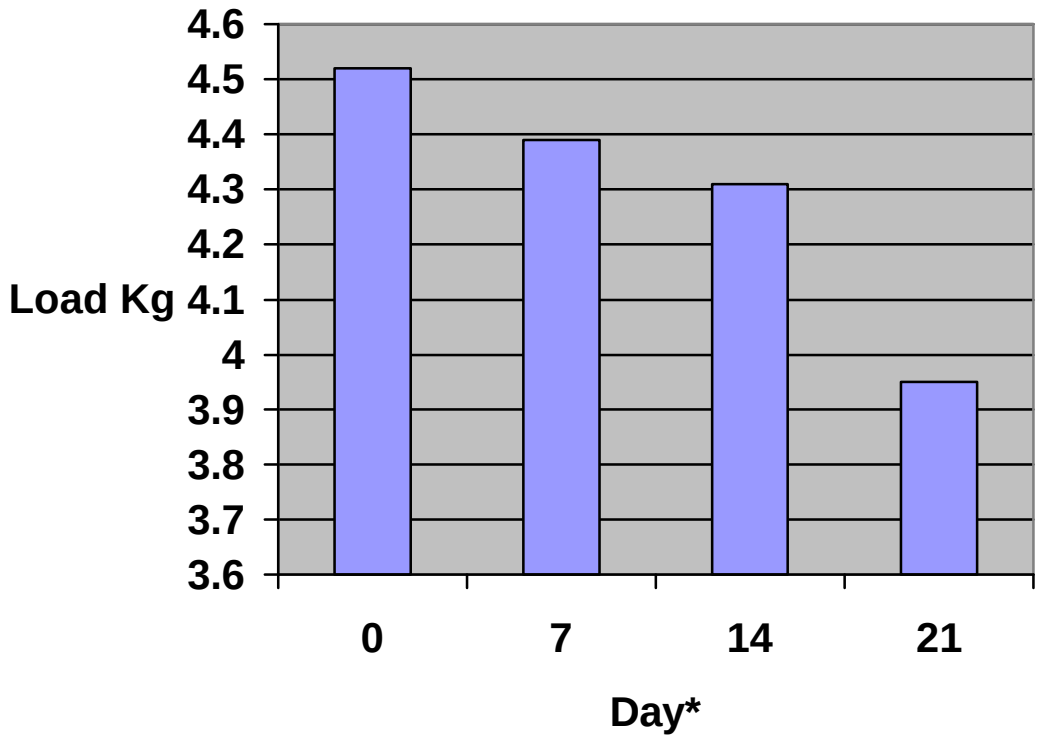
Figure 5.1: Schematic diagram indicating fluctuating changes in carbon dioxide levels during respiration analysis.

B. Texture

A post harvest change in texture can occur due to loss of moisture through transpiration, as well as enzymatic changes. For peppers in particular, one of the primary causes of declined marketability is loss of moisture and wilting (Kader, 1983). Fruits and vegetables that are coated with lipid substances are less likely to lose moisture due to the coating's hydrophobic nature which seals in water.

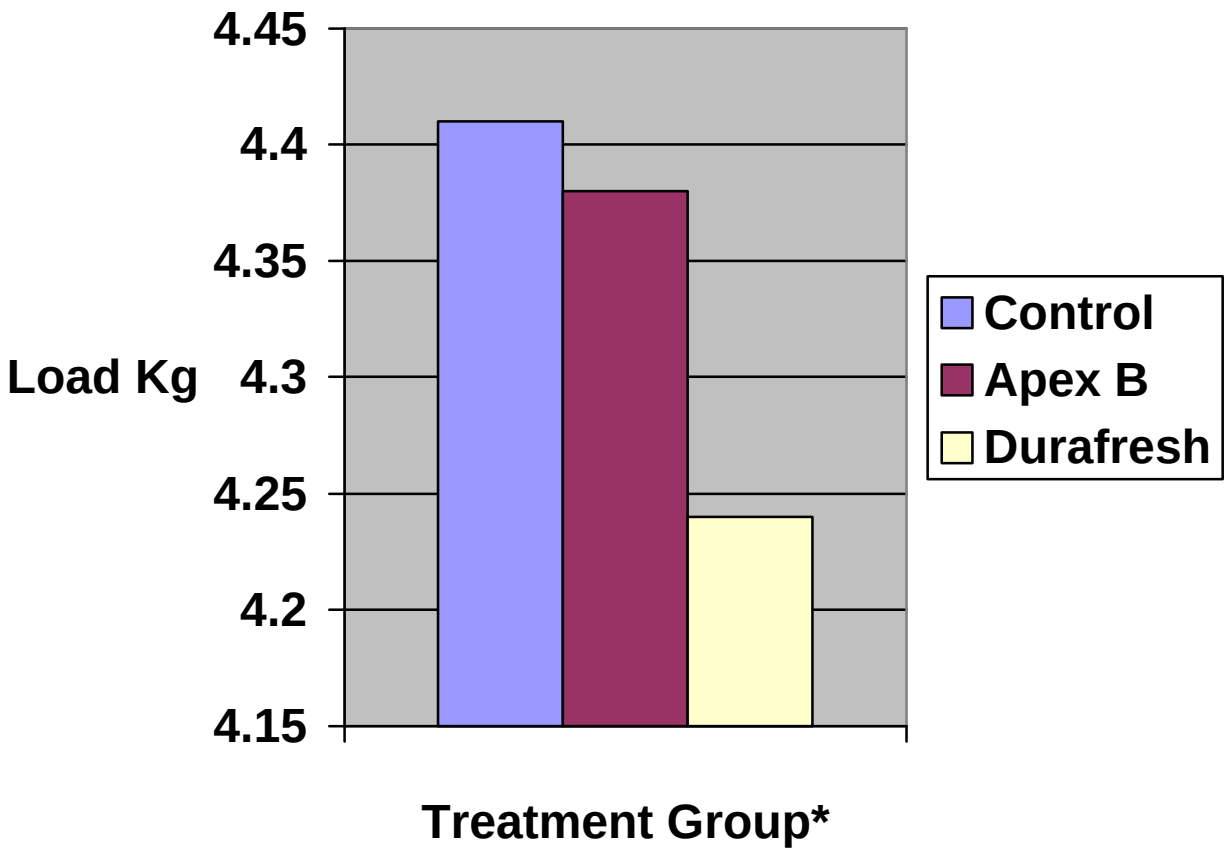
Texture testing was conducted to predict the force required to puncture samples with a cylindrical probe. Presumably, the more moisture the fruit lost, and the more advanced the enzymatic breakdown of pectin, the lower the required force necessary to puncture the fruit. However, upon statistical analysis no texture differences were observed throughout the study or in any of the experimental groups (Figures 5.2 and 5.3, respectively). Despite the lack of significant results, changes in texture were evident over the storage period. At the beginning of the third week, fruits in all 3 experimental groups were beginning to look dimpled and wilted. A few were even degraded to the point of being soft, watery, and unable to be fully penetrated by the probe. Therefore, although the probe was measuring maximum force to puncture, these soft fruits exhibited a rubbery quality, and upon impact with the probe, they were merely bent with the pressure instead of crisply being penetrated. Evidence of rubbery texture can be seen in the greater displacement distance required for maximum force to be reached (Figure 5.4). The figure shows that a fresh pepper may reach maximum force when compressed 6 millimeters, while a mushy old pepper may reach the same maximum force in 12 millimeters. The delay could have been due to the rubbery bending of the tissue.

The fact that the coated groups did not differ in puncture pressure was unexpected. Many vegetables and fruits, including citrus fruits, apples, potatoes, tomatoes, as well as peppers have been covered with lipid coatings to retain moisture. Lerdthanangkul and Krochta (1996) examined the preservative effects of various types of coatings, including the



*No significant differences between days (p=0.1881)

Figure 5.2: Comparison of textural changes in green peppers during 21-day storage.



*No significant differences between groups (p=0.7905).

Figure 5.3: Comparison of textural differences between treatment groups of green bell peppers stored for 21 days.

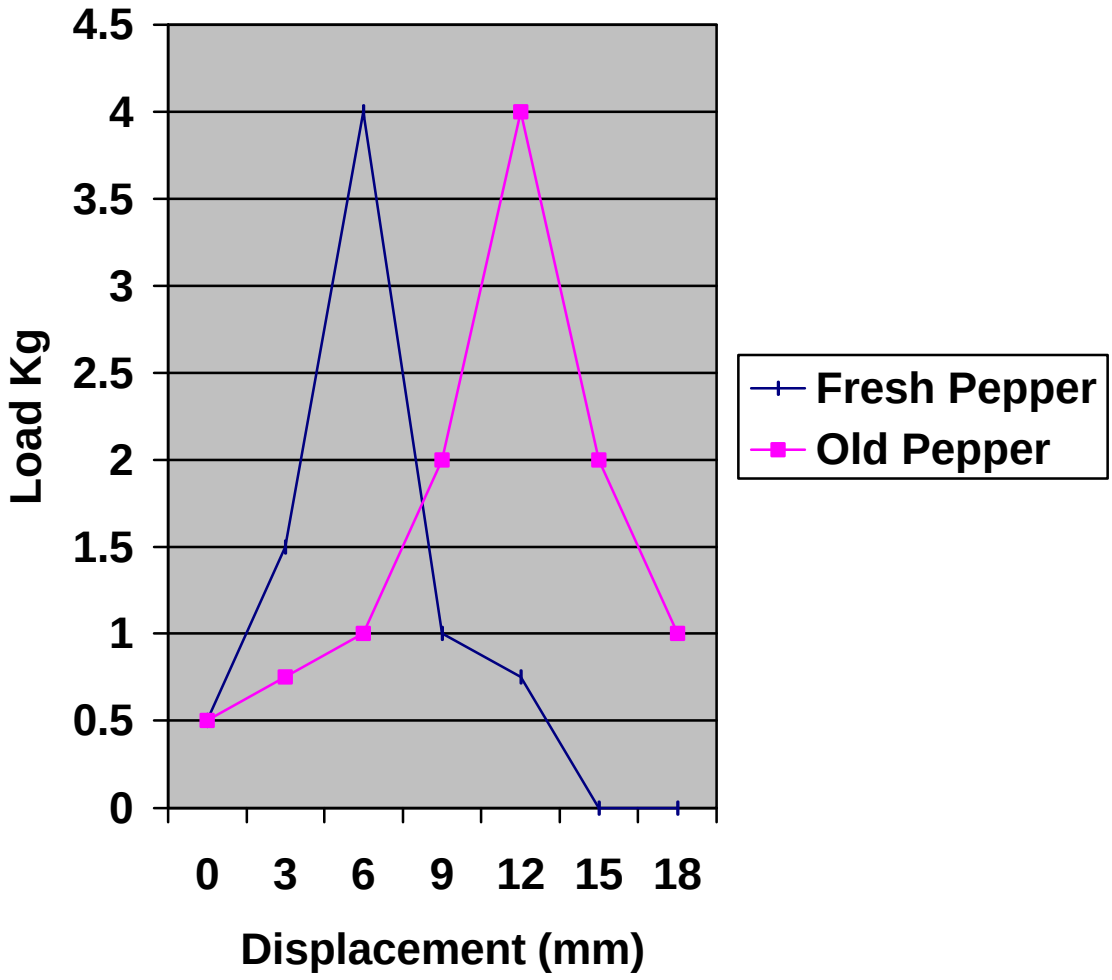


Figure 5.4: Comparison of old and fresh pepper texture patterns.

Durafresh coating used in this study, as well as protein and polysaccharide based coatings. Their results suggested that the Durafresh coating was the most effective due to its ability to prevent desiccation. The investigators measured texture with an Instron using a non-destructive deformation test. A sensory panel also subjectively asssessed texture. Both types of texture analysis firmly concluded that the Durafresh coated peppers demonstrated significantly less softening and higher firmness scores. Results from their study also showed that only the Durafresh coated fruits were still marketable after the storage period.

Therefore, although no significant texture changes were recorded during this storage study, the lack of difference observed may be due to the type of test used. Another type of objective test, like the nondestructive deformation test, or perhaps sensory testing may have been a more accurate measure for textural changes.

C. Ascorbic Acid

Ascorbic acid is a water-soluble vitamin that is easily oxidized. The vitamin may exist in several forms that vary in level of activity. L-ascorbic acid has the highest vitamin activity; dehydroascorbic acid has 25% less vitamin activity than L-ascorbic acid, and diketogulonic acid has no vitamin activity. L-ascorbic acid can be reversibly oxidized into dehydroascorbic acid, which can become irreversibly oxidized into diketogulonic acid. Oxidation of ascorbic acid may be caused by several factors including exposure to oxygen, metals, light, heat, and alkaline pH. Edible coatings applied during the study were expected to prevent transfer of gases between the fruit and the atmosphere. Therefore, the coatings should help prevent oxidation of vitamin C by hindering the fruit's exposure to oxygen, altering enzymatic activity and slowing the respiration process.

Initial ascorbate content of fresh peppers averaged 78.72 mg/100g (Table 5.3). Normally, green bell peppers contain anywhere from 125-200 mg ascorbic acid per 100grams of pepper.

Table 5.3: Ascorbic acid content of green bell peppers during storage.

Storage Day	Ascorbic Acid Content ± standard error (mg/100g)[a]
0	78.72±18.86
7	76.42±26.69
14	89.50±25.05
21	98.31±27.88

[a]no significant differences in ascorbic acid over the 21 day storage period (p=0.1061).

Therefore values were lower than expected (Moser and Bendich, 1991). Several factors may have contributed to lower vitamin C content. First, the method of ascorbate extraction may have lead to reduced recovery. Several samples were analyzed twice during one day to determine if ascorbate was being lost during the time from extraction to analysis. Results indicated that the ascorbate level was lower during the second sampling procedure, despite efforts to prevent light and air exposure (Table 5.4).

Vitamin C was extracted from 12 pepper fruits on the particular testing day. Because of the length of time to prepare the samples, a lapse of 1-3 hours occurred between the extractions of the first and last sample. This large amount of time may have allowed the first samples to have been exposed to greater amounts of light and oxygen. Despite these possibilities, Wimalasiri and Wills (1983) stated that the vitamin remains stable for over three hours in the extracting fluid which was citric acid.

To rectify these problems, during days 7 and 14, tin foil was used to shield the homogenate from light. However, the vials used for HPLC analysis were clear. Samples remained in these clear vials during autosampling for approximately one hour after extraction. Although only a small amount of light can enter the autosampler, perhaps the presence of the light may have enhanced vitamin C breakdown. In addition, in order to minimize the amount of time that the homogenates waited prior to HPLC analysis, subgroups of each sample were prepared and analyzed prior to preparation of later samples.

Another potential methodology related cause of lowered ascorbate levels might have been the loss of ascorbate during the solid phase extraction step. Although ascorbic acid is not supposed to be filtered out, differences were observed with one sample that was analyzed with and without filtration. Of the two samples tested, one sample remained unchanged; however, another sample had a lower level of vitamin C (Table 5.5).

Table 5.4: Comparison of ascorbic acid differences between identical samples.

Sample	**Ascorbate (mg/100g) Sample 1**	**Ascorbate (mg/100g) Sample 2**
Control #1 Day 7	115.1	99.44
Control #2 Day 7	124.3	110.92
Control #1 Day 21	141.20	120.9

Table 5.5: Vitamin C changes observed between filtered and unfiltered samples.

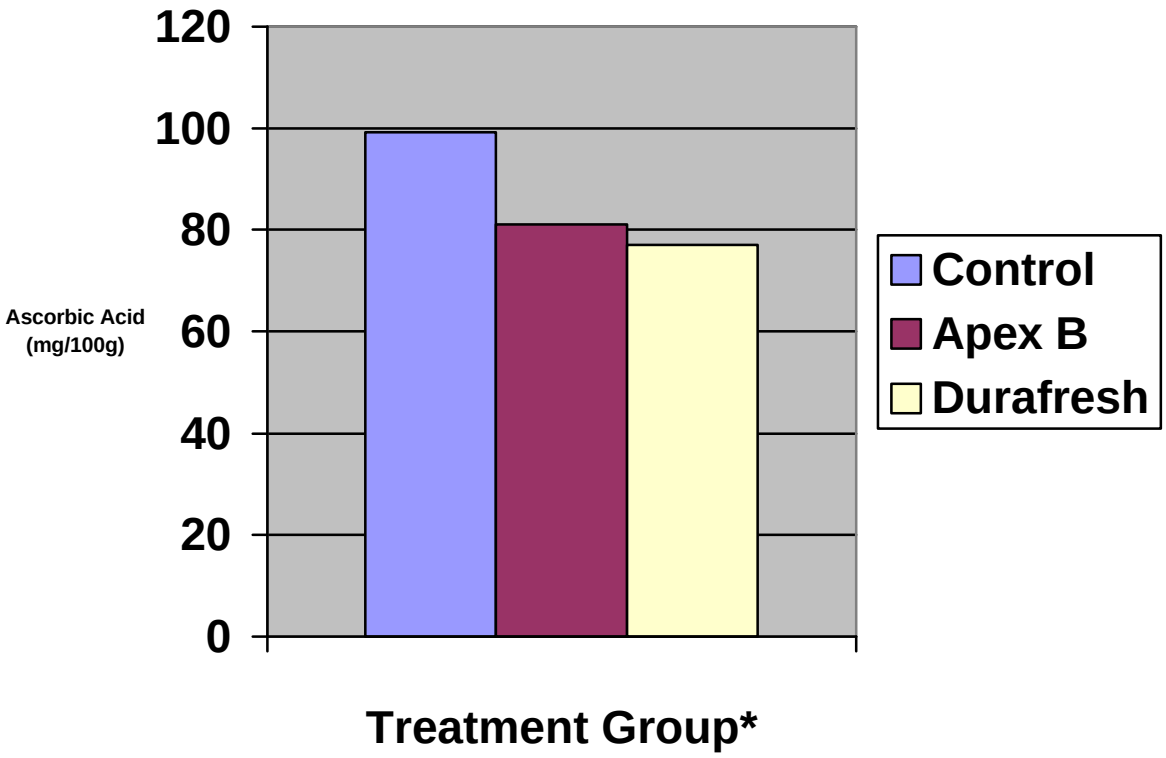
Sample	**Ascorbic Acid (mg/100g) Filtered Sample**	**Ascorbic Acid (mg/100g) Unfiltered Sample**
Apex B #3, Day 14	67.44	76.24
Control #3, Day 14	102.04	100.96

Lowered vitamin levels may have also been caused due to the exposure of the pepper to metal, including the metal blade of the blender, and the knife blade used to prepare the samples. Metals are known to catalyze oxidation of ascorbate.

Other possible explanations for lower vitamin C levels may involve the growing conditions of these particular peppers. Poor quality soil, inadequate rain fall and sun exposure may have contributed to lowered vitamin synthesis in the fruits, and thus lowered overall levels at the time of harvest. Also, the variety of pepper used in this study (*King Arthur)* may have had a naturally low level of ascorbate, or a high level of ascorbic acid oxidase activity. Both of these factors could have caused a lowering in vitamin C levels. However, when the same variety, and a smaller number, were examined from a different producer, vitamin levels were consistently above 125mg/100g.

Results (Table 5.3) indicated that ascorbate levels did not differ significantly during storage ($p=0.1061$). However, ascorbic acid content appeared to be increasing during the study. It was originally hypothesized that ascorbate levels would decrease with time due to vitamin oxidation. The lack of significant differences over time suggests that oxidation is not occurring at an appreciable rate, however as will be discussed later, the oxidation product of vitamin C, dehydroascorbic acid, did increase with time. Ascorbic acid has been demonstrated to accumulate in certain fruits after harvest, therefore, the slight increase in the amount of vitamin content during the study may be attributed to post harvest synthesis.

Despite the lack of change in vitamin content observed during storage, significant differences (Figure 5.5) were seen between treatment groups ($p=0.0279$). However, the treatments differed in a manner contrary to what was expected. Theoretically, coated groups should retain more vitamin C than uncoated groups. Data indicated that the uncoated control actually had higher levels of vitamin C than either of the coated groups.



*Apex B and Durafresh treatments were significantly different from the control (p=0.0279).

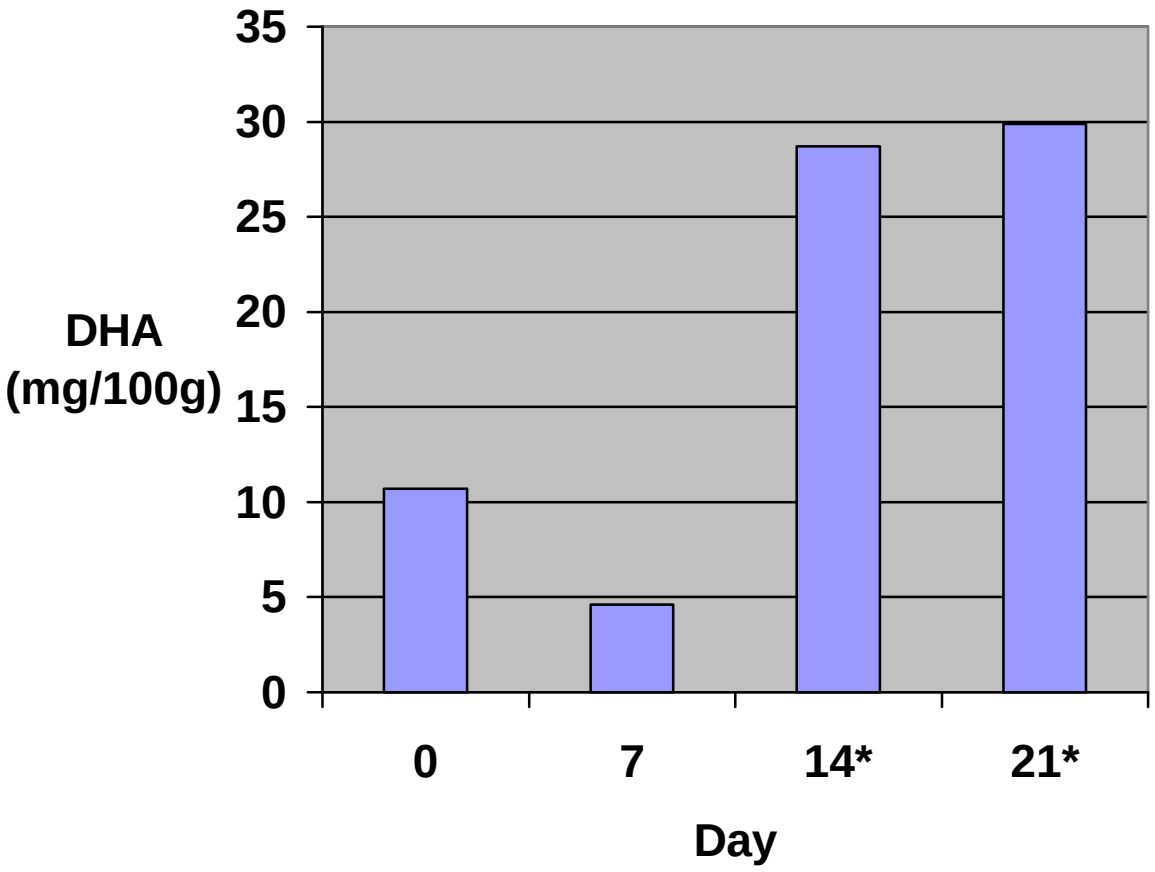
Figure 5.5: Comparison of ascorbic acid (mg/100g) content of three different treatment groups of green bell peppers stored for 21 days.

Most sources indicate processes which help to increase carbon dioxide levels, and at the same time displace oxygen surrounding the fruit, should help prevent vitamin oxidation (Watada, 1987; Moser and Bendich, 1991). The purpose of the two selected coatings was to help preserve vitamin content and prevent oxidation. However, Wang (1977) suggests that elevated carbon dioxide levels may contribute to decreased vitamin synthesis. Specifically, Wang (1977) determined that high levels of carbon dioxide inhibited ascorbic acid accumulation in peppers stored at 13° C. Such an effect may have occurred in this study.

D. Dehydroascorbic Acid

Fresh green peppers normally contain undetectable levels of dehydroascorbic acid (DHA) (Wilamlasiri and Wills, 1983; Matthews and Wall, 1978). DHA is one of the products formed from oxidation of L-ascorbic acid. Although DHA does have vitamin activity due to its reduction to L-ascorbic acid in the body, high levels indicate the beginning of oxidative breakdown (Gregory, 1996). Further oxidation of DHA creates diketogulonic acid (DKG) which has no vitamin activity.

Dehydroascorbic acid levels differed significantly over the 21 day storage period ($p=0.0001$) (Figure 5.6). According to theory, dehydroascorbic acid (DHA) should accumulate during storage due to the oxidation of L-ascorbic acid. As previously mentioned, ascorbic acid did not change over the storage time. However, as expected, DHA content was higher on days 14 and 21. This large increase may be due to either a sudden accumulation of DHA due to oxidation of ascorbic acid, or changes in methodology that may have made quantification more accurate. Modifications included holding homogenate samples in the dark, and preparing the samples in smaller groups to limit light and oxygen exposure. DHA is known to be very unstable, and upon standing will breakdown into diketogulonic acid. Therefore, it is possible that the precautionary steps used may have helped to preserve DHA for analysis.



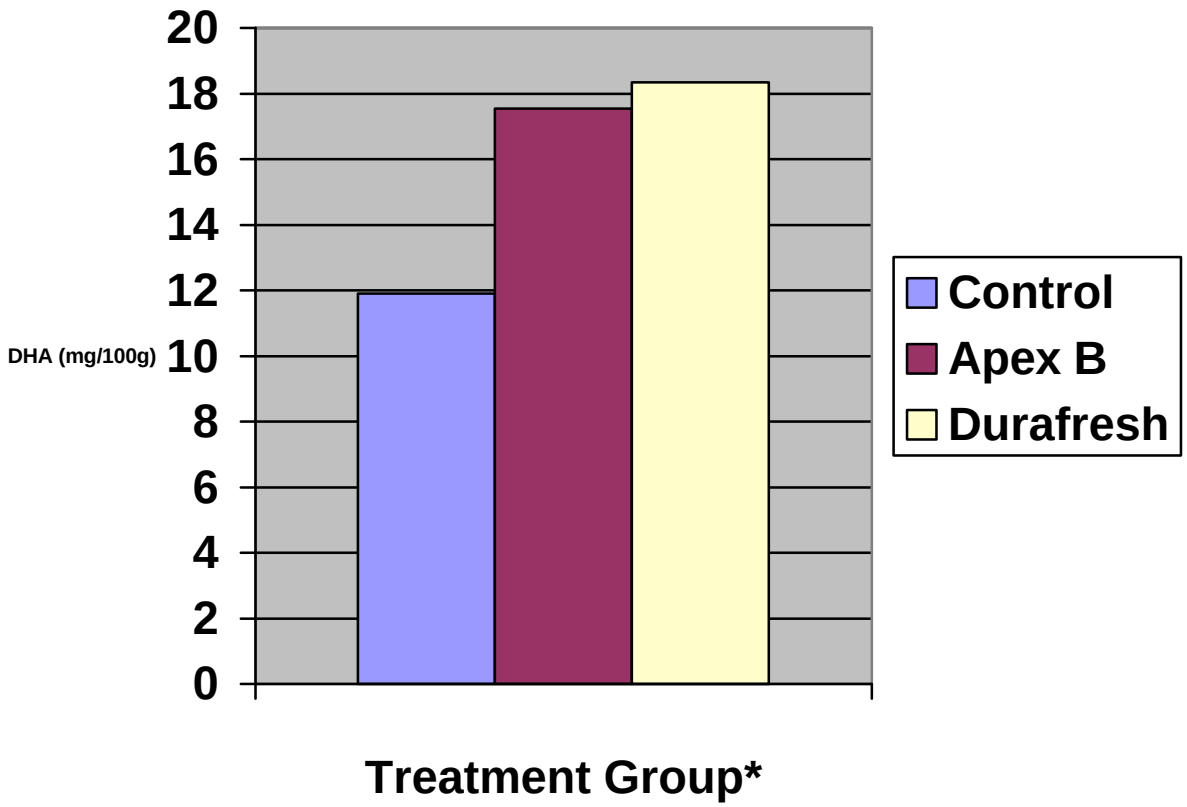
*significantly different from control (p=0.0001)

Figure 5.6: Changes in dehydroascorbic acid (DHA) content of green bell peppers measured during a 21 day storage period.

Treatments also influenced DHA content (Figure 5.7). The two coated treatments differed significantly from the control ($p=0.0126$). However, like the ascorbic acid, the treatment groups did not perform as expected. It was hypothesized that the coatings would act as barriers to the atmosphere and oxidation of AA into DHA would be less likely to occur. However, the DHA levels were actually higher in the coated groups. The lower levels of ascorbic acid in these treatment groups did coincide with increased DHA levels. The reason for the coated groups exhibiting more vitamin oxidation is still puzzling. As mentioned previously, ascorbic acid levels may have dropped in the coated groups due to synthesis suppression brought about by high carbon dioxide, however, this theory should be rejected in lieu of the DHA data. High DHA levels indicate oxidative breakdown, and contradict the notion of decreased synthesis. If synthesis was actually being suppressed by elevated carbon dioxide then the level of DHA should have remained low throughout the storage period.

Additionally, L-ascorbic acid levels in tomatoes have been shown to increase during ripening, with a corresponding decrease in DHA levels (Watada, 1987). Although the tomato is a climacteric fruit and the pepper is not, green peppers also have shown varied contents of vitamin C associated with maturity. Green peppers change from their immature form (green color) to the mature form (red color) on the plant. The red fruits have approximately 50% more vitamin C per fruit. Therefore, it is possible to consider that vitamin C production in peppers may still be ongoing as the fruit matures. If the peppers were still accumulating vitamin C, even in its post harvest state, then a slight increase in its content would be expected. DHA would still have been predicted to decrease with time.

Increased respiration levels recorded on the 14^{th} and 21^{st} days (Table 5.1) may also have been responsible for the increased DHA. Increased respiration may have altered other biological functions, including enzymatic activity, which was not examined, and this combination may have caused AA to be oxidized. Although the data does not support the breakdown of AA during this period, further analyses of biological changes should be conducted.



*Apex B and Durafresh differed significantly from the control (p=0.0126).

Figure 5.7: Comparison of dehydroascorbic acid content of three treatment groups of green bell peppers.

Statistical interaction between treatments and time (p=0.0545) indicated that during on day 14 treatments had a significantly higher DHA content than the control (Figure 5.8). However in on day 21, only the Durafresh had significantly higher DHA levels. Possible reasons for the increased levels are difficult to postulate. The coating may be interacting with the fruit and producing conditions which stimulate DHA production. The exact type of interaction is hard to surmise due to the lack of differences observed in the other parameters measured.

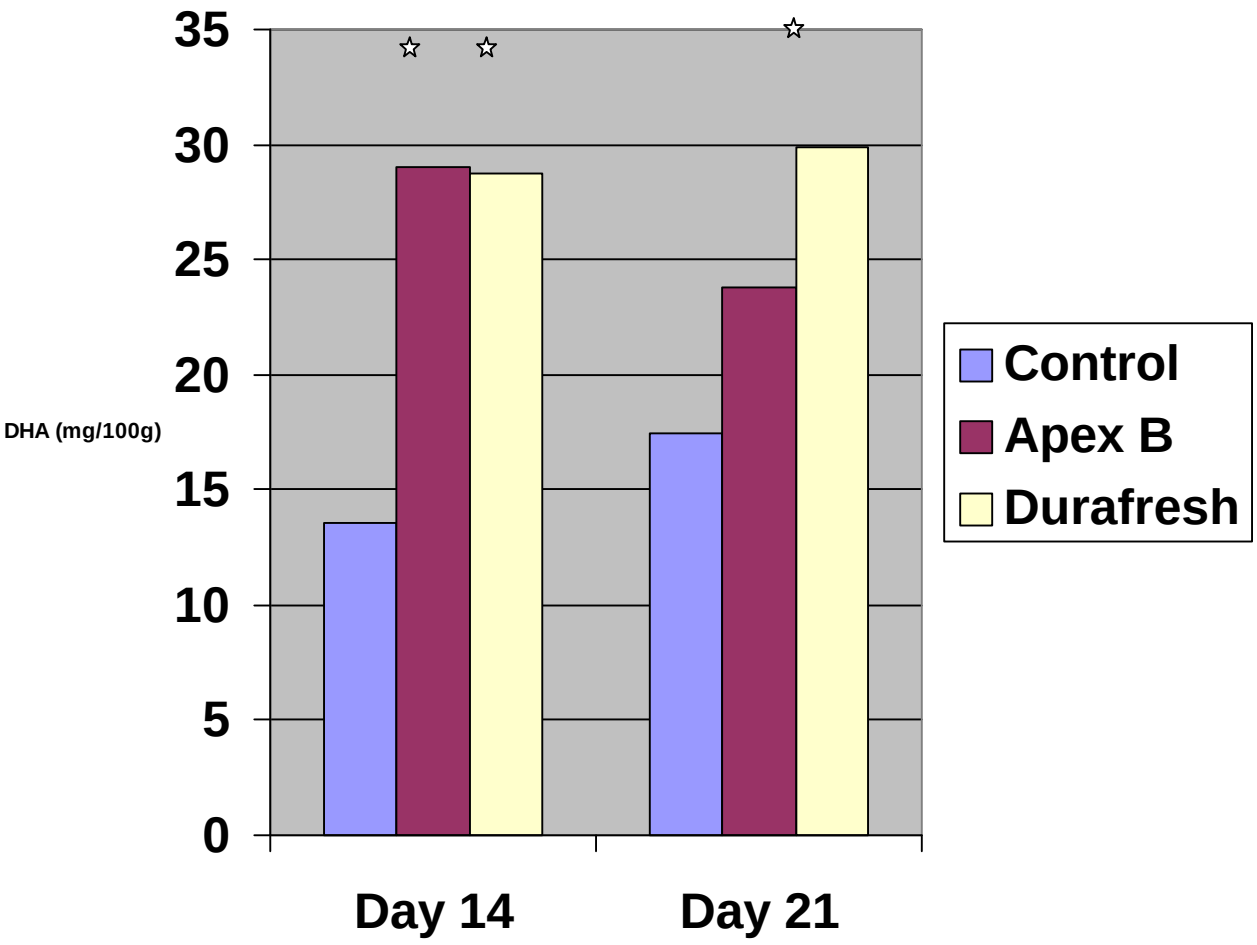
E. Weight

Post harvest weight changes in fruits and vegetables are usually due to loss of water through transpiration. This loss of water can lead to wilting and shriveling which both reduce a commodity's marketability. Lipid coatings act as a barrier to moisture loss, and have been shown to significantly reduce post harvest appearance and texture changes (Lerdthanangkul and Krochta, 1996).

Weight measurements (Table 5.6) in peppers during the 21-day storage showed no significant differences (p=0.6064). In fact, the weight of the fruits varied greatly. Changes in average weights could not be ascertained due to the large standard deviation. Data regarding weights values between treatments (Figure 5.9) were not significant (p=0.5459). Again the sample size was too small to determine whether variations existed between groups.

F. Color Analysis

Color changes in green peppers may be caused by photodegradation that occurs when chlorophyll pigments are bleached by light and oxygen. Enzymatic degradation of chlorophyll may also occur. Coatings are believed to help limit color changes by protecting the peppers from excessive



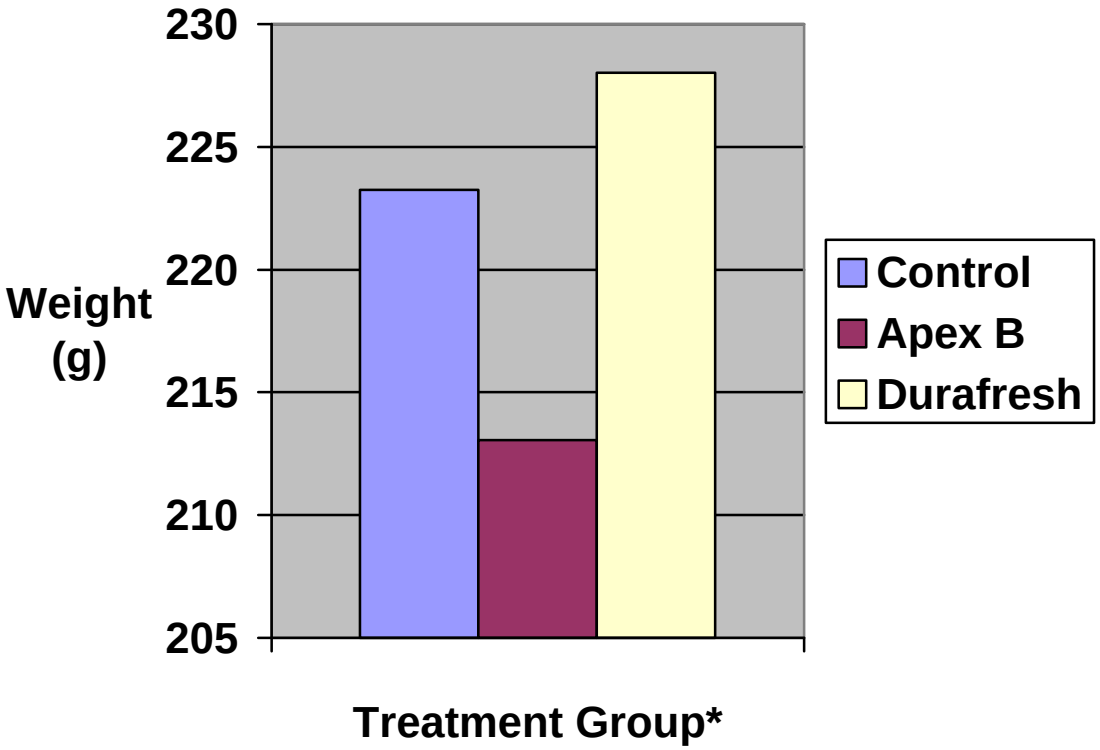
☆ Day differed significantly from control (p=0.0545)

Figure 5.8: Comparison of dehydroascobic acid (DHA) changes between treatment groups and control peppers observed on days 14 and 21.

Table 5.6: Average weekly weight (grams) of green peppers stored for 21 davs.[a]

Treatment Group	Day 0	Day 7	Day14	Day 21
Control	226.68±50.73	238.15±55.72	228.10±26.92	200.05±15.33
Apex B	184.50±23.15	229.30±59.77	201.13±36.50	237.33±33.99
Durafresh	214.98±24.46	214.13±34.33	254.08±49.19	228.93±27.97

[a]no significant differences between days or treatments (p=0.6064,p=0.5459, respectively)



*No significant differences between treatment groups (p=0.5459).

Figure 5.9: Average weight of pepper samples from each treatment group.

oxygen exposure, and therefore, are expected to prevent bleaching. Color changes were evaluated in terms of hue angle and chromaticity values, and were both calculated from traditional L*, a* and b* color measures. Hue angles denote the color of the sample such that a hue angle of 0° = purple, 90° = yellow, 180° = blue-green, and 270° = blue. Chroma values indicate color saturation or intensity. Higher numbers indicate a more vivid color, whereas lower numbers correspond to dull colors.

1. Hue Angle

Hue angle (Table 5.7) did not differ significantly during the storage period ($p=0.3438$). Data values were located between numbers designating yellow and blue-green, and therefore a green color was indicated. Lack of difference in the color during the 21 days indicated that no bleaching occurred. Although photodegradation usually occurs in the presence of light and oxygen, refrigerator storage, which was in the dark, most likely eliminated the likelihood for this occurrence.

Results (Figure 5.10) also showed that treatments did not differ significantly in hue angle ($p=0.9515$). Coatings were expected to help maintain color by preventing oxygen exposure, however, as indicated by the results coatings had no effect on color changes. In fact, peppers in all treatment groups maintained their green color throughout the storage period.

2. Chromaticity

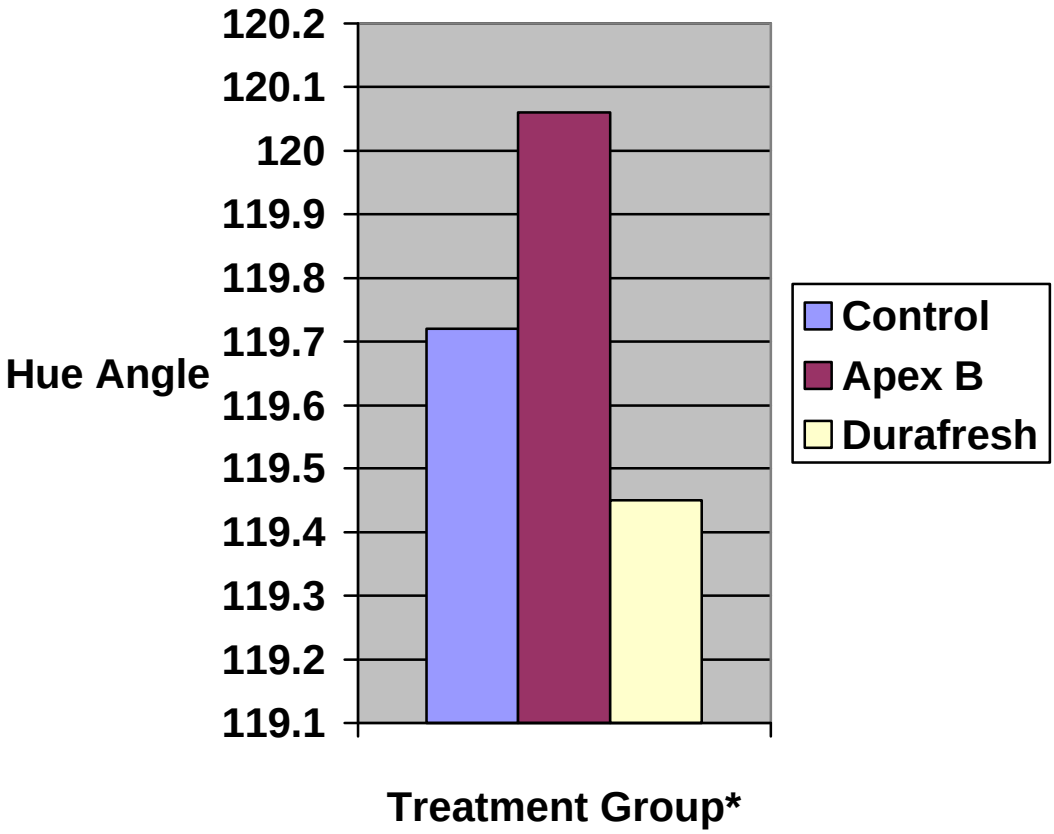
Chromaticity values (Table 5.8) differed significantly during the storage period ($p=0.0097$). Dunnett's test. Data indicated that day 14 had significantly lower chromaticity values, indicating a slightly duller appearance, than day 0. A dull appearance brought about by chlorophyll degradation may have been expected in the later weeks of the study. However, the fact that day 14

Table 5.7: Changes in hue angle of green bell peppers during 21-day storage period.

Day	Hue Angle[a,b]
0	118.40±4.64
7	120.63±4.64
14	121.73±5.35
21	118.25±13.15

[a] Hue angle measures: 0° = purple, 90° = yellow, 180° = blue-green, 270° = blue

[b] no significant differences between days were observed (p=0.3438)



*Treatments did not differ significantly (p=0.9515)

Figure 5.10: Comparison of hue angle values for three treatment groups of green bell peppers.

Table 5.8: Comparison of chromaticity changes in green bell peppers stored for 21 days.

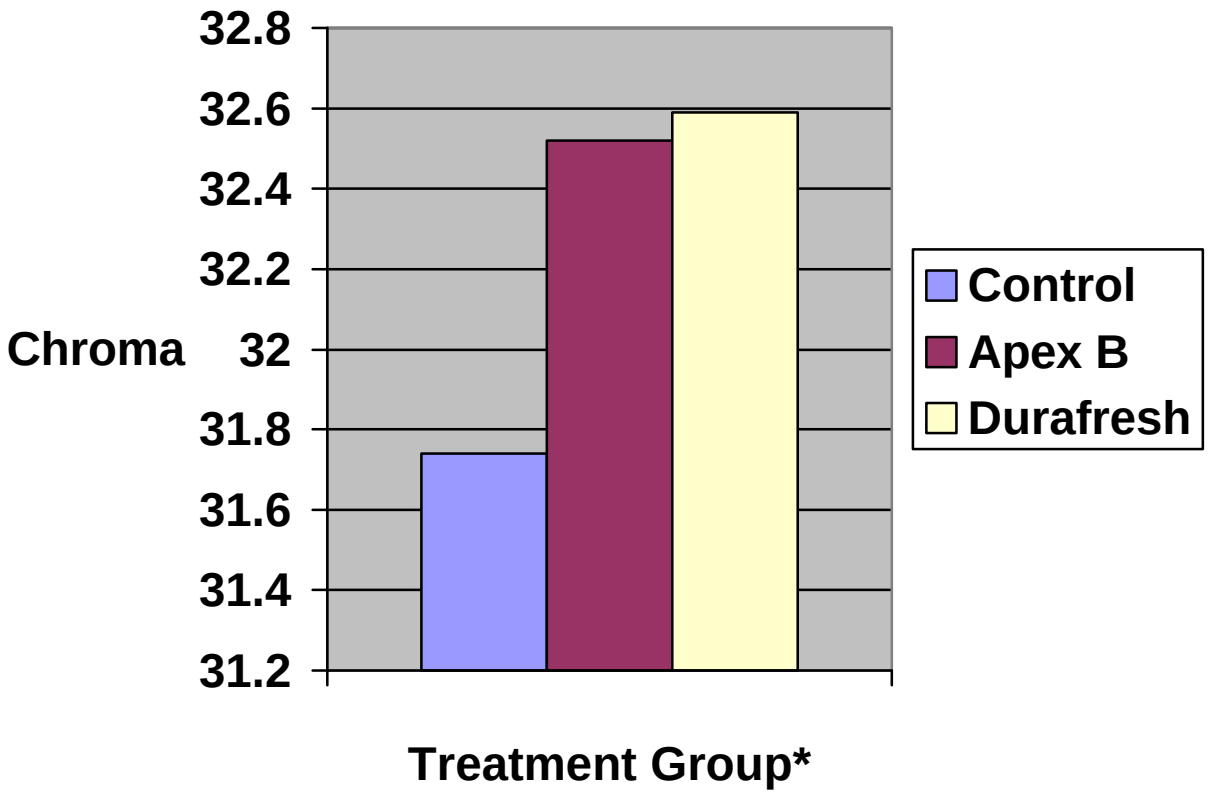
Day	Chromaticity [a]
0	33.88±7.50
7	35.25±6.07
14	29.16±6.75[b]
21	30.83±6.43

[a] = low chromaticity values indicate dullness, high values indicate bright, vivid colors.

[b=] significantly different from control (day 0) p=0.0097

alone, and not day 24, differed from the control, was unusual. Possible explanations for this day's difference may be due to methodology errors made during that sampling period. Such errors may have included incorrect calibration of the colorimeter.

Chromaticity values were not significantly affected by treatment ($p=0.8464$) (Figure 5.11). This figure shows that the treatments had similar values indicating a dull fruit color. The coatings were expected to prevent color degradation. However, because no differences were observed, the coatings did not offer any color retention benefits.



*No significant differences between treatment groups (p=0.8464).

Figure 5.11: Comparison of chroma values for treatment groups of green peppers stored for 21 days.

Chapter 5: Conclusions and Suggestions for Future Research

Generally, actual results differed greatly from anticipated results. For the parameters measured, uncoated peppers displayed a similar keeping quality compared to coated samples. In most cases, including weight, texture, and hue angle no differences between treatments or changes during the duration of the study were observed, whereas, the other parameters displayed results that completely contradicted the expected hypothesis.

Weight loss over time was expected due to loss of moisture. In addition, coated groups were expected to weigh more due to the moisture retention properties of the lipid coatings. Lack of variation in weight may have been due to the small sampling size which resulted in large standard deviation values. Also, a lack of coating integrity and uniformity may have allowed moisture to escape from the pepper.

Normally, peppers are expected to soften over time due to enzymatic changes and loss of water. Lack of observed textural differences between weeks may have been due to the type of test used to measure texture. In the future, texture may be better evaluated by other types of compression tests, possibly including whole fruit compression, or by sensory analysis. Edible coatings were hypothesized to help maintain texture by sealing in moisture. As with weight changes, lack of texture differences between groups may have been due to lack of coating uniformity.

Color changes, as measured by hue angle, were also expected due to bleaching of chlorophyll by oxygen and photodegradation. However, due to dark storage conditions, photodegradation may have been minimal. Bleaching of chlorophyll by oxygen was believed to be alleviated in coated groups by prevention of oxygen exposure. However, if coatings were not uniform, oxygen may have been able to permeate. Intensity of color, measured by chromaticity showed no variation between treatments. Weekly differences in chromaticity were seen. Day 14 was found to be significantly less vivid when compared to the other days. This difference may have been due to

procedural errors that may have occurred on that testing day, including incorrect calibration of the equipment.

Normally, in fruits and vegetables ascorbic acid can be easily oxidized into dehydroascorbic acid. Storage results were expected to show a progressive decline in ascorbic acid levels with a corresponding increase in DHA levels. However, no changes in ascorbic acid content were observed during the study, although DHA was shown to increase with time. Lack of ascorbic acid changes, as well as overall low vitamin C levels, may have been due to methodology problems. Changes in ascorbate and dehydroascorbic acid procedures may help to increase recovery. Such changes include using amber vials in the autsampler, keeping homogenates in the dark, limiting sample exposure to oxygen, and utilizing a chelating agent to eliminate catalytic metals from interfering with vitamin levels.

Lipid coatings were also expected to maintain higher levels of ascorbic acid due to exclusion of oxygen and prevention of DHA accumulation. Contrary to this premise, coated samples contained significantly lower levels of ascorbic acid, and higher levels of DHA than the uncoated control group. Possible explanations for this discrepancy are not clear, but may have been due to coating composition.

Respiration rates were found to increase significantly on the 14^{th} and 21^{st} days. These changes may have been indicative of natural metabolic changes occurring during senescence. However, problems with respiration analysis, including inconsistent data, may be remedied with methodology changes. Respiration measures should be conducted after all samples have come to equilibrium in the room in which they will be analyzed. A longer equilibration period prior to reading of carbon dioxide content may yield more reproducible results.

Other suggestions for future research include additional methodology changes as well as further exploration of post harvest physiology changes. Methodology recommendations include

increasing the sample size. Standard deviation values were very large in this investigation. Use of a larger sampling pool would anticipate a more narrow standard deviation. Additionally, for some tests, pooling of data may be helpful. Analysis of ascorbic acid and dehydroascorbic acid in particular may benefit from pooling of results due to the large variance between samples.

Further analysis of biological parameters may also enhance the understanding of post harvest physiological conditions. Such tests may include analysis of ascorbic acid oxidase activity to monitor degradation of vitamin C. Textural changes could be monitored by analysis of pectinase and cellulase activity. Color degradation could also be analyzed by monitoring cholorphyllase activity.

Additional sensory work to determine consumer acceptance for the coated fruits and vegetables should also be done. Sensory work could help determine if the coatings were objectionable to consumers in terms of sticky or waxy feel they may impart to the vegetable or fruit.

Finally, use of higher quality coatings may also be considered in the future. Coating composition can have a direct effect on gas exchange and moisture barrier properties. Coatings composed of polysaccharides or proteins offer better gas permeability characteristics, while lipids are still considered superior for moisture retention. Lipids, when used alone, are prone to cracking and pinholes which interrupt their surface barrier characteristics. Therefore, a combination of various substances into a composite or bilayer coating could utilize the best characteristics of each material, and produce a functional coating that will maintain produce characteristics.

Appendix A - Color Measurements

1. Sample Preparation:

Obtain four random peppers from each experimental group (12 peppers total). On a cutting board cut 2 side wall pieces (30 x 50 mm) from each pepper for duplicate measures. Avoid overly curved or internally fibrous sections. In total 24 pieces will be sampled, 8 from each experimental group.

2. Hunter Colorimeter Method:

1. Obtain laptop computer from Room 329, Wallace Hall.
2. Connect laptop cables (power supply, printer cable and DC power supply cable).
3. Connect the power cord to the sensory interface unit (SIU). Leave the SIU in the standby position until ready to run samples. The system will need to warm up for 30 minutes.
4. Turn the power on to the computer and printer.
5. Place the SIU in operate mode.
6. Follow computer instructions for zeroing the machine.
7. Place the black tile under the specimen port and press F1 (read).
8. Place the white tile under the specimen port and press F1 (read). The instrument is now zeroed and should be in the measurement mode.
9. Read cut samples (30 X 50 mm) by placing them in the specimen port, external side up.
10. Record L*, a*, and b* values.
11. Repeat with remaining samples.

Appendix B - Texture Measurements

1. Sample Preparation:

1. Samples prepared for color measurements will be used for Texture measurements (see Appendix I).

2. Instron Procedure

1. Turn on the Instron power button, computer, monitor, and printer, after the computer runs through a series of checks the main menu should appear on the computer.
2. Turn the GPIB button until the red light above it is lit. This enables the GPIB to interface with the computer.
3. Using the mouse, choose the section labeled "TEST" Test a Sample.
4. Fill in the information that appears on the screen: Operator (enter your last name), Sample ID (enter sample code), Method (enter "compressive"), and Method number 20. Method 20 is a preprogrammed method stored on this computer that runs the puncture test described in the materials and methods section of this thesis.
5. Check parameters describing sample, change if necessary. If correct press F10 (ok).
6. Place the sample under the puncture probe assembly (stainless steel probe (0.78mm diameter), and puncture platform.
7. Adjust the plate distance by turning off the GPIB button and using the up and down buttons. Turn the GPIB back on before starting the test.
8. Press enter to begin acquisition. The probe will move down until it punctures the sample. After the probe has reached its final distance the computer screen will read "remove the sample and press enter to return the cross head."
9. Place the next sample on the platform, press F10 to continue, and F10 again for "ok", and then hit enter to begin acquisition again.

10. Repeat for remaining samples. Press ESC to print data.

Appendix C - Ascorbic Acid and Dehydroascorbic acid Analysis Procedure

1. Extraction of Ascorbic Acid and Dehydroascorbic Acid

1. Obtain 12 peppers from refrigerated storage (for this experiment the 12 peppers used for respiration measures the previous day were used).
2. Cut a side wall section from the pepper into pieces approximately 1cm x 1cm.
3. Weigh 25g of the cut pepper pieces into the blender,
4. Cover pieces with 50 ml of 3% citric acid.
5. Blend for 2 minutes on the "blend" setting.
6. Pour homogenate into Teflon centrifuge tubes. Rinse any residue into the tubes with 5-10 ml 3% citric acid.
7. Centrifuge samples for 10 minutes at 7000 rpm.
8. Pour samples into 100ml volumetric flask. Rinse residue into flask with 3% citric acid.
9. Bring homogenate up to volume using more citric acid.
10. Repeat steps 1-9 with remaining peppers.

2. Solid Phase Extraction

1. Hook vacuum hose to filtration apparatus.
2. Attach filtration cartridges and collection tubes with holes in the bottom.
3. Turn on vacuum pump.
4. Pipette 4 ml of methanol into each filtration cartridge; allow to run through cartridge.
5. Pipette 4 ml of HPLC grade water through each cartridge; allow to run through. Repeat with 4 more ml of HPLC water.
6. Shake homogenate in volumetric to thoroughly mix. Pour 3 ml of homogenate into cartridges allow to run through. Some stirring with a glass rod or spatula may aid filtration.
7. Break vacuum seal; add collection tube inserts for collecting the remaining material collected.

8. Pour approximately 1 ml of homogenate into the cartridge; allow to collect in tubes.
9. Remove collected fluid, save for HPLC analysis.

3. Preparing and Loading HPLC Chromatograph and Autosampler

1. Turn on the Helium and Nitrogen Gases
2. Turn on computer, printer, controller, detectors and autosampler. If detector says "fail" press "auto zero." Wait for the (cal) light to go off on the UV detector. This indicates that the detector is calibrated.
3. On the controller press (set-up). From this screen press (system config). Scan the system by pressing (rescan). The following configuration should appear UV #1 484, 21; Autoinjector 712,5.
4. Press (set-up) to return to the main menu page of the controller. Press (Direct).
5. Place solvent lines in the appropriate solvents, one is the delivery line the other the sparge line. Let solvents purge for at least 30 minutes at 100 ml/min. Set this under the sparge section of the menu pages. Do not set any flow at this time, flow should =0 ml/min.

Acetonitrile/water (70:30) –use Optima HPLC solvents containing 0.01M Ammonium Dihydrogen Phosphate. Adjust to pH 4.3 with orthophosphoric acid.

6. At the computer, type in (Base) at the C>. The computer will ask if it is okay to delete files, answer: YES. Next, the computer will ask if it should initialize the plotter? Answer: NO.
7. Baseline should appear on the screen, press enter.
8. From the Main Menu Page on the computer, select "load a method". If the ascorbic acid method does not automatically appear you will have to load the method. For ascorbic acid load method C:Max\Data3, for dehydroascorbic acid load method C:Max\Data5.
9. Once the method is loaded check the sections of the file to see that it is correct for the method you chose.
10. Set the column temperature and pressure limits under the (External Control) section. For ascorbic acid and dehydroascorbic acid the temperature should be set at 22° C, and the

pressure limit should be 3000 psi. Select (Gradient). Move to (set up) and pull down the (pump controller) menu. Make changes if necessary and click (okay).

11. On the computer, under the sample queue set the section under FROM to (Acqu) for running samples. Fill the Sample Queue with Sample Name, File Name, etc. To print or retrieve a file from disk put in (Disk). Vial should be set in sequence 1, 2, 3, Etc., as they are loaded into the autosampler tray.
12. It may be necessary to prime the pumps with solvent before starting the flow rate. To do this, loosen the prime valve and use the prime syringe to draw 5 ml of solvent through the pumps. Close the prime valve. It may be also helpful to pump some solvent through the pumps but not through the column. Open the pressure release valve and pump solvent at 5 ml/min. Never put this high of a flow through the column. Set flow back to 1 ml/min and then close pressure release valve. Set the %A and %B solvent to the percentages for the start of the isocratic run (for this study 100% A, 0% B).
13. Allow column to equilibrate for approximately 15 minutes or until the pressure stabilizes. Once the column has reach the correct temperature (22°C) and the pressure has stabilized load the sample tray with samples.
14. Prepare sample tray by pipetting samples into glass inserts inserted into vials with a spring and Teflon cap.
15. On the computer select (RUN), execute methods, press start, and click (Okay) to begin background acquisition.
16. Let samples run through sampler. Screen should say “waiting for acquisition to start,” the controller should beep, and then read (inject wait), when the sample is injected the controller will read (an injection occurred). The computer will read (acquiring), and the controller will then read (running).
17. When the analysis is complete for all samples quit the monitor program by selecting (quit). Go to “execute methods”, edit queue wide parameters and select “printer” for output. Also select “re-integrate peak, save peak, and format peak integration.” Go back into execute methods and click (run). Printer should start.

18. For analyzing dehydroascorbic acid click (load) and retype the file name of the method: c:max\data5. Change the sample queue and execute methods.

4. Calculating Ascorbic Acid and Dehydroascorbic Acid Content

1. Determine each sample's AA and DHA content by plugging the peak area for either DHA or AA into their respective standard curves. Sample standard curves for AA and DHA can be seen in Figures C1 and C2 respectively.
2. Values derived from the curve will be in ug/g, convert samples into units of mg/100g of pepper.

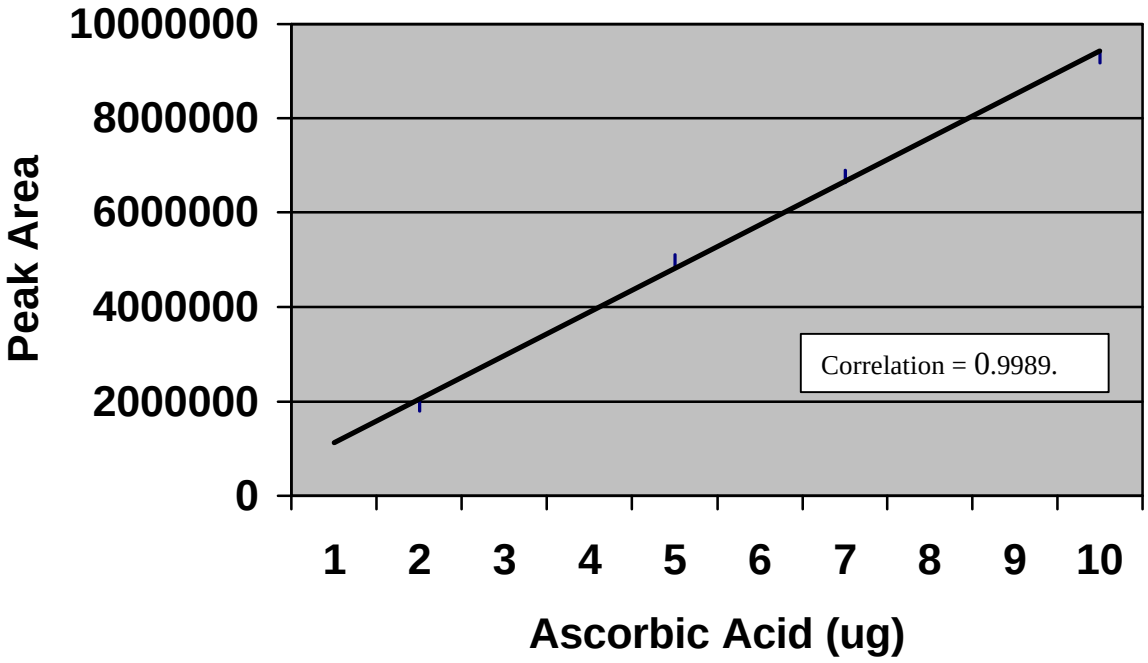


Figure C.1 Ascorbic acid standard curve.

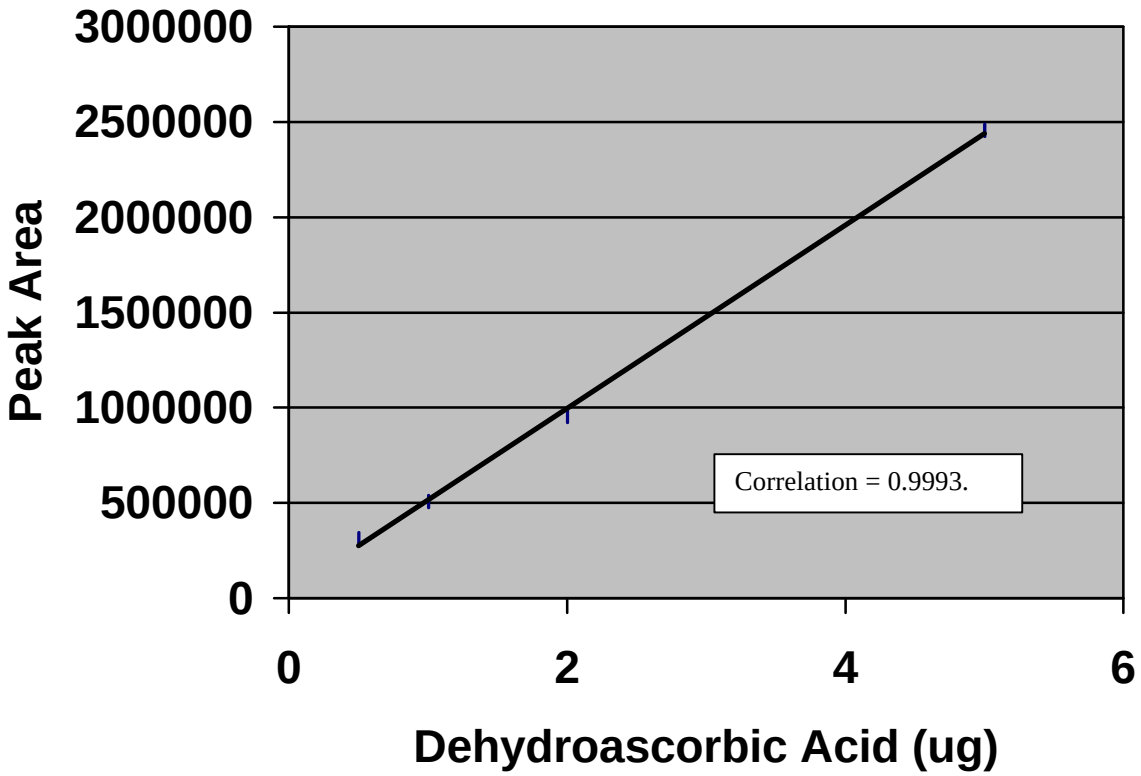


Figure C.2: Dehydroascorbic acid standard curve.

Appendix D - Respiration Analysis

1. Remove 12 peppers (4 from each experimental group) from refrigerated storage 12 hours prior to testing. Allow to come to room temperature in Room , Department of Human Nutrition, Foods, and Exercise, Wallace Hall, Virginia Tech, Blacksburg, VA.
2. Transfer samples to the Horticulture Department laboratory, 2nd floor, Saunders Hall, Virginia Tech, Blacksburg, VA.
3. Turn on Infrared gas analyzer equipment: Plug in power supply, turn on external air source.
4. Adjust airflow for 5 liter/minute using the attached gauges.
5. Record weight for all samples (to be used in respiration calculation).
6. Place samples, one pepper fruit at a time, in the metal container. Seal container with plastic lid.
7. Adjust the tubing so the analyzer is reading the external air source. Record the external air carbon dioxide value (ppm) after the level has stopped fluctuating.
8. Switch the tubing from the external air source to the source running from the metal container holding the pepper.
9. Wait for the reading to stabilize and then record the carbon dioxide output of the fruit.
10. Repeat for all samples, read samples an additional time for duplicate readings.

Calculate the respiration rate:

Respiration Rate = (ÄCO2.F.K)/A

Where:

ÄCO2 = CO2 output - CO2 input (ambient air)

F = air flow in liters/hour

K = [44,000 mg carbon dioxide/22.4 L/mol] X (273/294)

(Conversion of 1 L CO2 to 1 mg CO2)

A = kilogram weight of the fruit

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