

**Development and Effectiveness of Three Hydrocolloid-Lipid Emulsion Coatings on  
Preservation of Quality Characteristics in Green Bell Peppers**

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# Development and Effectiveness of Three Hydrocolloid-Lipid Emulsion Coatings on Preservation of Quality Characteristics in Green Bell Peppers

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## (ABSTRACT)

Three hydrocolloid-lipid emulsion coatings were developed using Humkote brand partially hydrogenated cottonseed and vegetable oil, and one of three combined hydrocolloid bases: xanthan gum and propylene glycol alginate (xanthan coating), locust bean gum and xanthan gum (locust bean gum coating), and maltodextrin. Sensory testing using a ranking preference test indicated that these coatings had acceptable appearance and palatability. Quality characteristics of green bell peppers (*Capsicum annum L. cv. King Arthur*) measured during the 5-week storage period included: respiration rates, chlorophyll content, surface color, puncture force, pectin (uronic acid) content, ascorbic acid (AA) and dehydroascorbic acid (DHA) content, and cumulative weight loss. No significant differences between coated and uncoated peppers were noted in tests for respiration, puncture force, hue angle, chlorophyll content, and AA content. Uncoated peppers had significantly inferior moisture retention ( $p < 0.05$ ), which caused them to be unsaleable after 8 days, while coated groups were saleable for an additional 6 to 8 days. Uncoated fruits also had greater uronic acid breakdown ( $p < 0.05$ ) and higher DHA content ( $p < 0.06$ ) than coated peppers. Significant weekly changes (all treatment groups combined) included linear increases in respiration rates ( $p < 0.01$ ) and moisture loss ( $p < 0.01$ ), increasing linear and quadratic trends in uronic acid content ( $p < 0.01$  for both trends), increasing quadratic trends for both chlorophyll and AA content ( $p < 0.05$ ,  $p < 0.01$ , respectively), and decreasing linear and quadratic ( $p < 0.05$  for both trends) in DHA content. The only significant difference between coated groups was in chroma value, with maltodextrin coated peppers appearing less vivid than locust bean coated peppers. Overall, all three coatings performed equally well during the storage study. However, coatings with higher lipid content, which included xanthan gum and locust bean gum groups, withstood humidity changes better than the maltodextrin coated peppers. Coating application provided the greatest benefits in terms of texture maintenance through water retention and prevention of pectin breakdown, despite the lack of differences observed in puncture force. Coatings may also have prevented AA oxidation as demonstrated by the higher DHA content in uncoated groups, however AA patterns do not confirm this concept. Future research should be directed toward further minimizing textural changes and maximizing coating durability.

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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 dating to 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" type 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 these 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 content, 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 lowered respiration rates, and therefore delayed senescence. In addition, coatings may have different levels of permeability to oxygen. Decreased oxygen permeability can also 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).

Composite and bi-layer coatings are the edible coatings of the future. These two types of coatings work to combine the beneficial properties of different coating ingredients to create a superior film. Composite and emulsion films of the future may consist of hydrophobic particles within a hydrophilic matrix. Such a configuration could give a water-soluble coating with good water vapor barrier properties (Baldwin, 1991). Bi-layer 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. These multi-material coatings have reduced gas exchange, and resulted 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 such coatings on whole fruits or vegetables.

## **Chapter 2: Review of Literature**

### **I. Physiological Processes Important to Post Harvest Fruit Quality**

When still attached to the plant fruits 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 (Figure 3.1). These energy sources are broken down by well known metabolic pathways (glycolysis, the Krebs 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 (Figure 3.2). 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

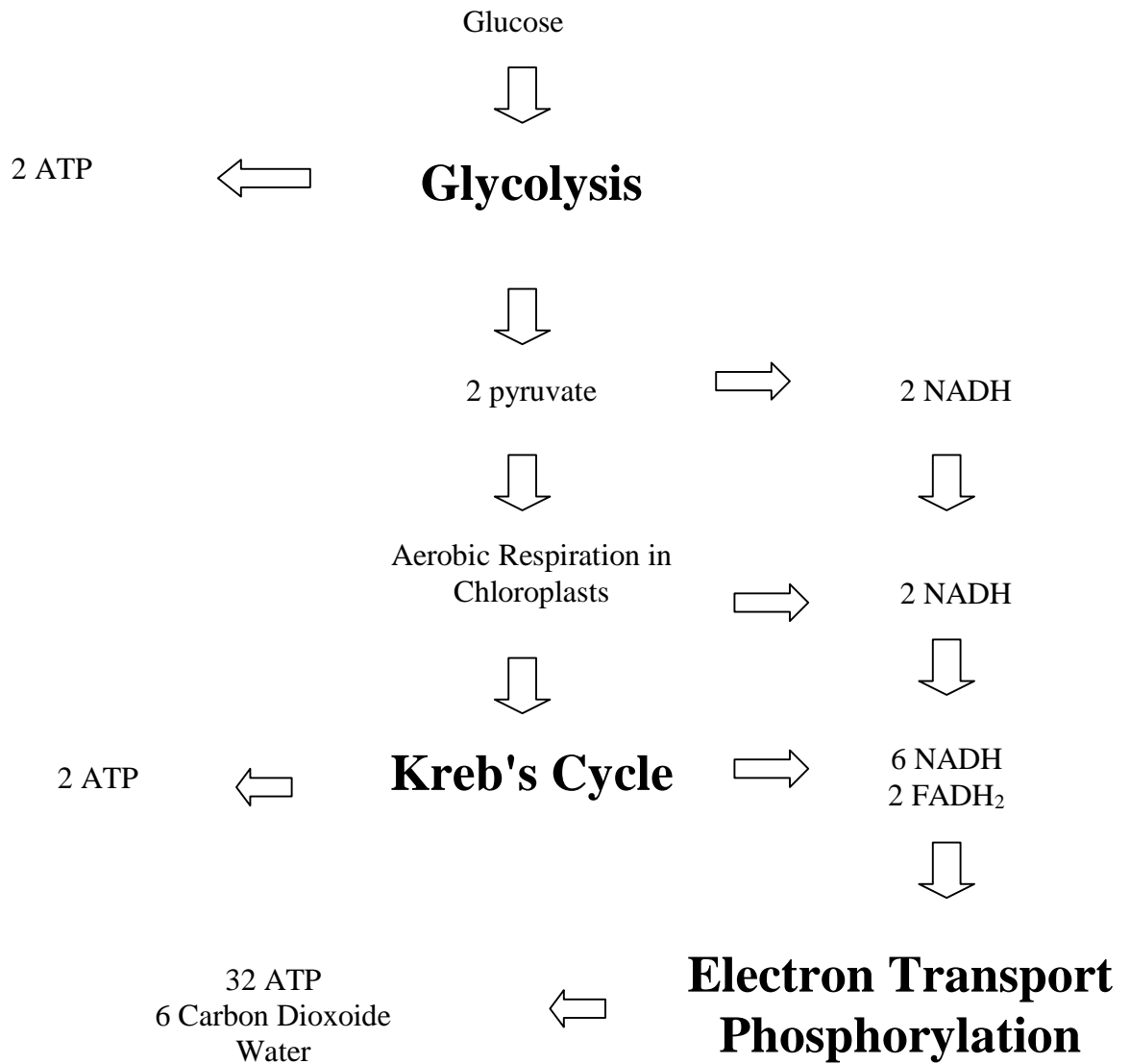


Figure 2.1: Aerobic metabolism of glucose.

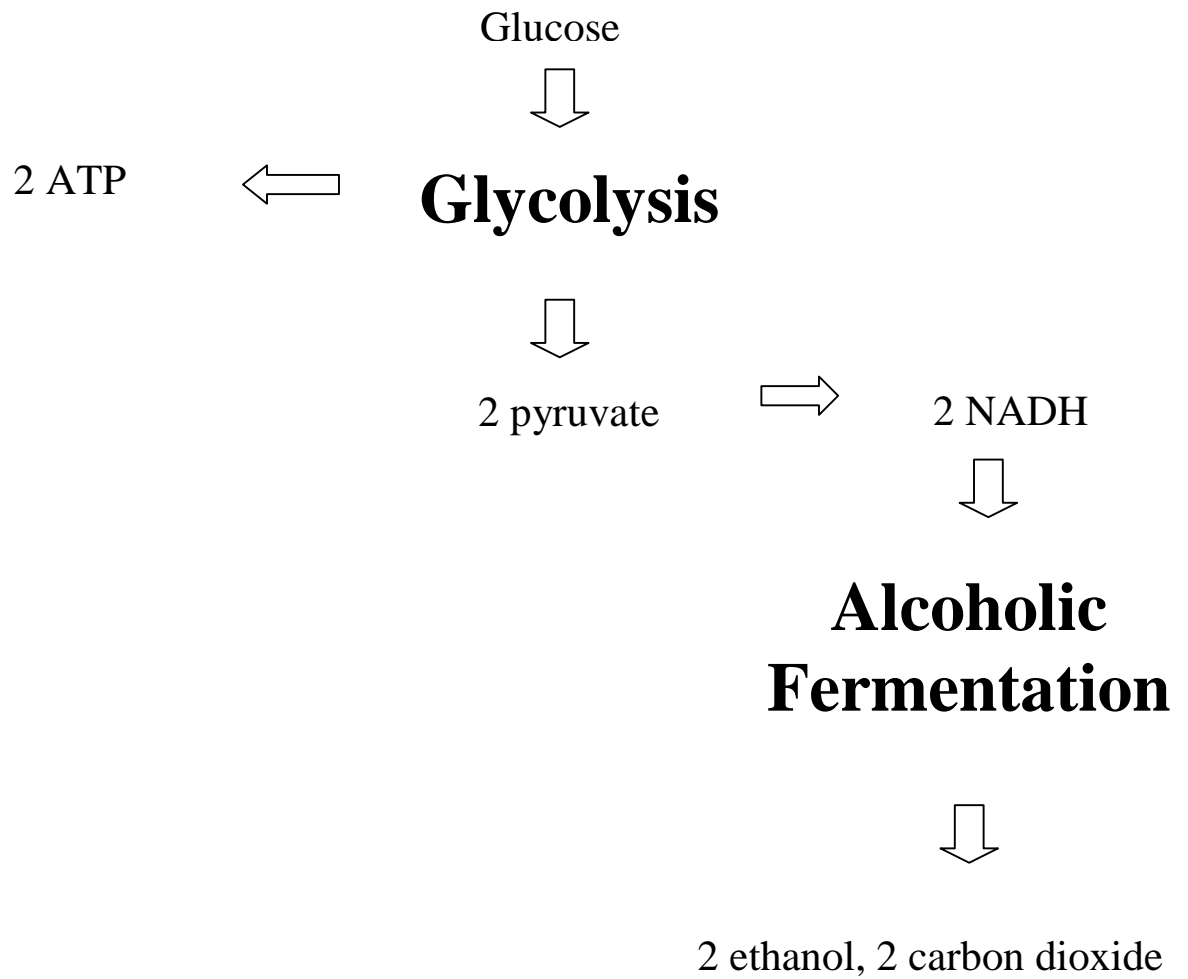


Figure 2.2: Anaerobic metabolism of glucose.



in an apple, or a large open cavity as in a pepper, is vastly different. The internal atmosphere contains 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%. This high level of carbon dioxide retards aerobic respiration due to the displacement of 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 confined gases 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 increases all oxidative processes, which in turn 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 inducing 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:

$$Q_{10} = [(R_1/R_2)] \times [10/(T_2-T_1)]$$

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 rates do not increase linearly with temperature. In fact, although the overall rate of respiration continues to increase, the rate of change between different temperature ranges actually declines until 40°C. At temperatures above 40°C the overall rate of respiration 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 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 Krebs 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 rise. In climacteric fruits the heightened respiration rate hastens the onset of the climacteric respiration peak, which is characterized by ripening (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 differ with a commodity's stage of maturity. 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 commodities 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 whereas 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. Lenticels**

Gas exchange in fruits may also occur through the lenticels, which arise in some fruit like avocados, 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 lenticels 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 lenticels 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 Altered 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 creates a negative pressure force causing 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. Tissues continue to evaporate moisture into the atmosphere, and without a source of water these cells become depleted of moisture that in turn begins to decrease internal turgor pressure. 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 transpiration rates. 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, and alter auxin transport and metabolism (Pratt and Goeschl, 1969). Despite these many roles, it is not known exactly how ethylene promotes senescence.

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**

#### **1. Color**

##### **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 a and b to form chlorophyllides as well as removes the phytol group from pheophytins (derivatives of chlorophyll) to form pheophorbides (Whitaker, 1996). This enzyme is activated after harvest by the increased heat produced in post harvest commodities. The optimum temperature for chlorophyllase activity is between 60 and 82.2°C. 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**

The red, blue and violet 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 to form oxidized pigment products (Markakis, 1982).

#### **A.1.d. Betalains**

Betalains are purplish red pigments similar in color, but not in 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 also 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, which are cleaved from triglyceride molecules by the action of lipases. These free fatty acids are open to attack by other enzymes. Lipoxygenase reacts with polyunsaturated fatty acids, and produces break down products that 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 terpenes, 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**

The texture of fruits and vegetables is provided by the cell walls of plant tissue. The cellular walls are made up of cellulose fibers which are held together by a cement like substance called pectin. Pectic substances are polymers of galacturonic acid residues. Other sugar units, including arabinose, galactose or rhamnose, may be present as side chains. 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. Second, pectic substances are enzymatically broken down to lower molecular weight substances. A decrease in the molecular weight of the pectin

polymers occurs due to the action of polygalacturonase, which cleaves the bonds between galacturonic acid residues.

During the ripening of fruit pectic compounds are solubilized. This solubilization increases both water-soluble pectin (WSP) and oxalate-soluble pectin (OSP) (Batisse, et al., 1994). A decrease in acid soluble pectin also observed to parallels the increase in WSP and OSP during ripening. Pectin in an under-ripe fruit is in the form of protopectin. The enzyme protopectinase changes the protopectin into pectin, which is the form found in ripe fruit. As the fruit begins to senescence and proceed to an overripe stage, the pectin is 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).

Texture changes result in the greatest amount of post harvest quality loss in peppers. Post harvest softening of pepper fruit can be attributed to water loss, decreased insoluble pectin and increased soluble pectin (Ben-Yehoshua, 1987). Red peppers, which are the mature state of the pepper fruit, have lower amounts of insoluble pectin and higher amounts of water-soluble pectin than the immature green peppers. Of all of the post harvest changes in peppers, the most problematic to texture is water loss. Physiological changes can be slowed or alleviated when the fruits are held in a water saturated atmosphere, indicating the importance of reducing water stress in preserving post harvest quality.

## **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



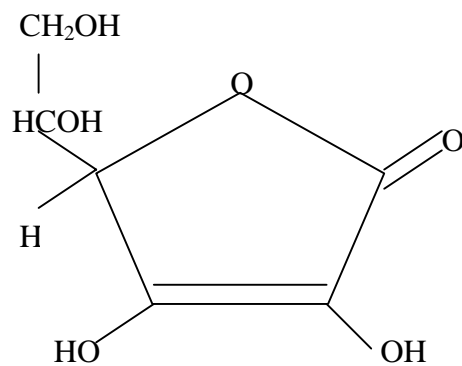
frequently overlooked as a standard of quality in harvested commodities, vitamins are frequently vulnerable to depletion in harvested vegetables.

### **B.1. Ascorbic Acid**

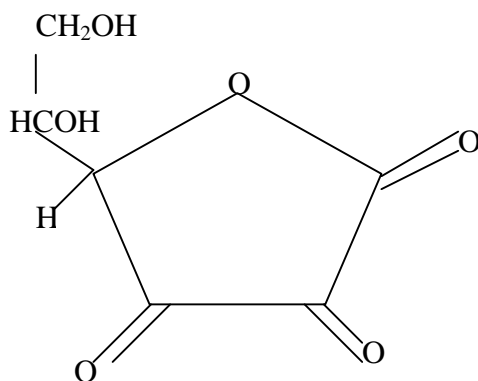
Plants synthesize ascorbic acid from D-glucose in 3 steps in which the carbon sequence of glucose is conserved (Moser and Bendich, 1991). First, carbon-2 is oxidized, by the enzyme pyranose-2-oxidase (EC 1.1.3.10) producing D-glucosone. Next, epimerization of carbon-5 occurs most likely via an oxidation-reduction reaction linked to hydrogen carriers or a keto-enol rearrangement. Finally carbon-1 is oxidized, producing L-ascorbic acid.

Ascorbic acid can be present in several forms in plant tissue. These forms include reduced ascorbic acid also known as L-ascorbic acid, monodehydro ascorbic acid (an unstable intermediate), dehydroascorbic acid, and diketogulonic acid (Figure 2.3). Ascorbic acid in the reduced form can be reversibly oxidized to the dehydroascorbic acid form during protection against oxidative damage. Dehydroascorbic acid still retains vitamin activity, however at a level lower than L-ascorbic acid. Should oxidative damage continue, dehydroascorbic acid can become irreversibly oxidized to 2,3 diketogulonic acid, a form which retains no vitamin activity (Watada, 1987).

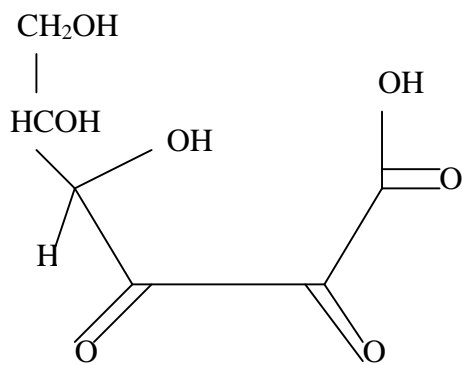
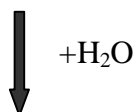
Several factors can enhance the conversion of ascorbate to 2,3 diketogulonic acid, including alkaline pH (ascorbic acid is most stable between pH 4 and pH 6) higher oxygen concentrations, the presence of metal catalysts, heat, light, and water activity. In addition to oxidative damage, enzymes may also function indirectly to lower vitamin C content. For example, ascorbic acid oxidase, an enzyme found in squash and other vegetables, 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).



**L-Ascorbic Acid**



**L-Dehydroascorbic Acid**



**2,3-Diketogulonic Acid**

Figure 2.3: Oxidation of L-Ascorbic Acid

The actual function of ascorbic acid in plants is not well known. Ascorbate does function as a precursor to oxalic acid and tartaric acid. Plants cleave ascorbate between C-2 and C-3 and form oxalate and L-threonic acid. L-threonic acid then is decarboxylated resulting in a 3-carbon acid which is recycled in hexose phosphate metabolism or is oxidized to L-tartaric acid.

Vitamin C is a required micronutrient in the human diet. The Recommended Daily Allowance (RDA) levels for adults is 60mg/day (Whitney et al., 1994a). The minimum amount needed to prevent overt symptoms of scurvy, the disease resulting from vitamin C deficiency, is 10 mg/day, however this low level does not saturate all body tissues. Vitamin C functions in many other processes in the body (Whitney et al., 1994a). Such functions include acting as an antioxidant, through which this vitamin prevents the oxidation of other materials in the body. Vitamin C is also required by enzymes that hydroxylate amino acids necessary for collagen synthesis, which makes this vitamin important in wound healing and tissue repair. In addition, vitamin C plays a role in the metabolism of several amino acids, some of which are involved in the synthesis of regulatory metabolic hormones like epinephrine and thyroxin. The metabolism of iron is also aided by ascorbic acid, which helps to keep the iron in the reduced form. Vitamin C has also been believed to help ease the severity or prevent the onset of the common cold by acting to raise the internal body temperature, acting as an antihistamine, and lowering serum iron levels (Whitney et al., 1994a).

## **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.

Beta carotene, when converted into vitamin A, has many functions in the body. Some of these functions include vision, maintenance of the epithelium, which in turn aids the immune system, bone and tooth growth, reproduction and cell differentiation (Whitney et al., 1994b). Beta carotene is found in yellow-orange plant foods, as well as leafy green vegetables. Recommended levels of vitamin A are expressed as retinol equivalents (RE) which is the amount of vitamin A activity, or the amount of retinol that the body can obtain from a food with preformed retinol or the precursor beta carotene. The recommended intake of vitamin A is 1000 µg retinol equivalents (RE)/day for men and 800 µg RE/day for women. One RE is equivalent to 6 µg of beta carotene.

### **B.3. Loss of Vitamins in Horticultural Crops**

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 post harvest vitamin changes. 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 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 (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**

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**

### **H.1 Edible Coating Materials**

Edible coatings have been used to preserve a variety of foods, including fruits and vegetables. Today, edible coatings can be made from several 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 number of polysaccharides. In some cases such coatings have been used to retard moisture loss during short-term storage. However, polysaccharides, being hydrophilic in nature, do not function well as physical moisture barriers, instead they function as a sacrificial moisture barrier. Here, a food is enrobed in the polysaccharide and placed into storage conditions. What moisture is lost comes from the coating layer, and therefore prevents desiccation of the actual food (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.

### **H.1.a. Cellulose**

Cellulose is a structural component in all land plants. Cellulose is made up of linear chains of glucose units joined by  $\beta$  1, 4 glycosidic linkages. Cellulose is insoluble in water for two main reasons. First, cellulose contains numerous intermolecular hydrogen bonds in the polymer, which reduces its ability to hydrogen bond with water. Second cellulose tends to readily crystallize again reducing interactions with water, and thereby reducing solubility. Nonionic cellulose ethers include methylcellulose (MC), hydroxypropyl methylcellulose (HPMC) and hydroxypropylcellulose (HPC). These cellulose ethers are water-soluble due to the addition of constituents that interfere with formation of the crystalline unit cell. These constituents are manufactured under controlled temperatures and pressures in which alkali cellulose is allowed to react with methyl chloride to form MC, or methyl chloride and propylene oxide to form HPMC or propylene oxide to form HPC. All three of these derivatives are available in powder or granular form with varied molecular weights or degrees of substitution. They are only soluble in cold water.

Lump-free solutions of these three compounds can be prepared by one of four ways. First, one can add the powder to well agitated room temperature water at a slow rate that allows individual particles to separate and become wet. Agitation is continued until all particles are dissolved and the solution is completely free of gels. Another method involves blending dry powder with any dry inert or non-polymeric soluble material used in the formulation. Blending allows for powder separation and reduces lumping. A third method consists of dispersing the cellulose ethers in a water miscible non-solvent like glycerin, ethanol or propylene glycol. The resulting slurry is then added to water. The last method requires the use of a stainless steel mixing device, developed by the company

Hercules. This device feeds the powder through a funnel into a water jet ejector and then disperses it using the turbulence of water flowing at a high-velocity (Keller, 1984).

All of the nonionic derivatives have linear polymer backbones, which allow them to form tough, flexible films that are soluble in water and resistant to lipids (Krumel and Lindsay, 1976). MC films are tough and flexible, while high levels of hydroxypropyl substitution in HPC allows for lower tensile strength and greater elongation. HPC film is also capable of injection molding and extrusion. Plasticity of these films can be improved by adding polyglycols, glycerine or propylene glycol. The films are useful in several food applications. Such films can prevent oil absorption during frying, including use on potatoes while making french fries (Sanderson, 1981). These films can also retard moisture loss and improve batter or crumb coating adhesion to the product (Dziezak, 1991). Cellulose can also be incorporated into bi-layer films. Such films have been made with HPMC and solid lipids like beeswax, paraffin, hydrogenated palm oil, or stearic acid (Kamper and Fennema, 1985).

### **H.1.b. Anionic Cellulose Ether**

Carboxymethyl cellulose (CMC) or cellulose gum is an anionic cellulose ether produced by reacting alkali cellulose with sodium monochloroacetate under controlled conditions. CMC is available in a variety of types differing in particle size, degree of substitution, viscosity, and hydration characteristics. It is soluble in hot and cold water, but is insoluble in organic solvents. Preparation of CMC is the same as mentioned for nonionic cellulose derivatives, except pH conditions must be remain between 7-9. A wide variety of ingredients may be used with CMC including proteins, sugar, starches and other hydrocolloids. CMC is rarely used to prepare unsupported films, however its ability to form strong, oil-resistant films makes it useful in many applications. For example fresh fruits and vegetables coated with a semipermeable film made of CMC and sucrose fatty acid esters (Lowings and Cutts, 1982) reduce oxygen uptake in fruits and vegetables.

### **H.1.c. Starches**

Starch is the storage form of glucose in plants. The molecular structure of starch is repeating glucose units joined by  $\alpha$  1, 4 and  $\alpha$  1, 6 glycosidic linkages. Starch in the native state is present in granules that are insoluble in cold water due to hydrogen bonding of polymer chains. When subjected to moist heat, the hydrogen bonds are broken and the starch granules absorb water. There are two primary glucose polymers in starch, amylose and amylopectin. Amylose is a linear polymer of glucose units connected by  $\alpha$ 1,4 glycosidic linkages. Normally, starches contain 18-30% amylose, however waxy starches are made primarily of amylopectin. Amylopectin is a glucose polymer consisting of straight as well as branched chains of glucose. Like amylose, the straight chains are linked by  $\alpha$ 1,4 glycosidic links, while branch points are connected by  $\alpha$ 1,6 glycosidic links. Application that require viscosity, stability and thickening utilize amylopectin, while film and gel formation require amylose. Amylose films are odorless tasteless, nontoxic and have mechanical properties similar to plastic films (Wolff et al., 1951). Amylose films have been successfully used on potatoes (Murray et al., 1971) to improve appearance, taste, and texture. Amylose films have also been used on dried raisins to prevent clumping and sticking (Moore and Robinson, 1968).

#### **H.1.d. Starch Derivatives**

Starch derivatives including modified starch, pre-gelatinized starch, and dextrans have all been used to create films. Modified starches have been used to prevent oxidation by coating candies, various dried fruits including prunes and dates, as well as nuts and beans. These modified starches were made into water-soluble transparent films from hydroxypropylated (1.1%) amylopectin starch with an amylose content  $>70\%$  (Roth and Mehlretter, 1967). Pre-gelatinized starch is an essential ingredient in powder coatings used on caramel cubes and dried fruits used in baked goods (Schieck et al., 1970). Dextrans, which are fragments of starch produced by reaction with acid, have been used as natural adhesives for coating nuts and candy (Smith, 1984).

#### **H.1.e. Pectin**

Pectin, which serves as an intracellular cement, occurs in a wide variety of higher plants. The polymer pectin is composed of repeating D-galacturonic acid residues. Pectic substances differ in their degree of methylation, which affects their solubility and ability to form gels. Schultz and colleagues (1949) have advocated the use of low methoxy pectins as film formers due to their ability to form non-sticky, visually appealing surfaces. Such coatings have high water vapor permeability and may benefit from creating a bi-layer film with lipids to lower water vapor transmission (Schultz et al. 1949).

### **H.1.f. Gums**

Many gums have been used in film formation. Gum sources include seaweed, microbial sources, and various other plant gums. Carageenan, a gum extracted from seaweed, has been used to preserve cut grapefruit for two weeks (Bryan, 1972). Alginates, which are a structural polysaccharide of brown seaweed, are good film formers, however they tend to create dry and brittle films which require additives for plasticity (Glicksman, 1983). Alginate coatings prevent moisture loss when applied as an edible coating to precooked pork patties (Wanstedt et al., 1981). Such coatings increase moisture, and reduce the perception of warmed over flavor induced by lipid oxidation. Lazarus et al. (1976) demonstrated that alginate coatings reduce shrinkage as well as surface microbial counts on stored lamb carcasses. Gum arabic has been used to eliminate the oily appearance of pecan nuts (Arnold, 1963).

Coatings have also been made from chitosan, a polysaccharide found in the shells of crustaceans. Chitosan reduces water loss, respiration rates, and fungal infection in bell peppers and cucumbers (Ghaouth et al., 1991). Treatment with chitosan also increases the activity of chitinase enzymes which help protect the plant from 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. Protein coatings are 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 and Weller, 1991). Coating of fruits and vegetables with protein films has not been done due to their limited water vapor resistance, however, composite or bi-layer coatings using proteins with hydrophobic materials may increase their usefulness.

### **H.2.a. Corn Zein**

Corn zein is derived from corn gluten by extraction with isopropyl alcohol and sodium hydroxide (Gennadios, et al., 1991). Due to its low content of polar amino acids and high content of non-polar amino acids, zein is insoluble in water and soluble in aqueous aliphatic alcohol solutions. When zein is dissolved in the correct solvents it is able to form durable, shiny films that are resistant to lipid penetration (Pomes, 1971). The film also tends to be brittle which can be remedied with the use of plasticizers like glycerin, fatty acids and acetylated monoglycerides (Reiners et al., 1973). Zein coatings combined with acetylated monoglycerides have been used to coat various nut products including sugar coated burnt peanuts (Cosler, 1957) and pecans (Andres, 1984). Other zein – coating products include freeze-dried peas, carrots, and apple slices (Cole, 1969). Zein coatings were also investigated for their ability to protect tomatoes (Park, 1991), and were found to increase their shelf life by six days. However, internal oxygen concentrations were reduced to the point of anaerobic metabolism.

### **H.2.b. Wheat Gluten**

Gluten proteins, which are primarily found in the wheat kernel endosperm, can be extracted with alcohol. Gluten proteins are composed of two primary fractions, glutenin and gliadin. Gliadin proteins are globular in structure and of low molecular weight.

They are known for their ability to promote extensibility in doughs. Gliadins contain a high number of glutamine residues, which allows for hydrogen bonding between gliadin chains. Hydrophobic interactions between non-polar residues and the presence of a small number of basic and acidic groups results in the decreased water solubility of gliadin (Krull and Wall, 1969).

Glutenins are high molecular weight proteins which promote dough elasticity by the formation of intramolecular disulfide bonds. Amino acids found in glutenins are similar to those found in gliadins: high amounts of glutamine, as well as high levels of proline and other nonpolar amino acids.

Wheat gluten films are made by dissolving the proteins in aqueous ethanol, and then drying the dispersion. Homogenous film-forming solutions require either acidic or alkaline conditions along with agitation and heating of the mixture (Gontard et al., 1992; Gennadios et al., 1993). Film structure is generated by the reformation of disulfide bonds that were originally destroyed by the addition of reducing agents during film mixing. Reformation occurs by sulfhydryl-disulfide interchange reactions and re-oxidation in the air. Film structure is also aided by hydrogen and hydrophobic bonds (Gennadios, et al., 1991). Plasticizers may be added to increase film flexibility.

### **H.2.c. Soy Proteins**

Soy protein isolates are produced by alkali extraction of protein meal, an end product of soybean processing. The primary amino acid residues in soy protein are glutamic acid and aspartic acid (Boldwell and Hopkins, 1985). Soy-based edible films can be produced on the surface of heated soymilk. Films prepared from aqueous protein dispersions are thought to form a film held together by hydrophobic and hydrogen bonds created through protein polymerization and solvent evaporation.

### **H.2.d. Collagen**

Collagen is a fibrous protein derived from the connective tissues of animals. Three amino acid residues predominate in collagen: glycine, proline, and hydroxyproline. Collagen can be extracted using mild acid or alkali conditions or neutral salts. These films are primarily used in the meat industry as casings for sausages. Gelatin is a collagen product produced through partial hydrolysis of collagen, which involves thermal denaturation to cleave hydrogen and electrostatic bonds, and then hydrolytic breakdown of covalent bonds (Eastoe and Leach, 1977). Gelatin films have been used to coat fruit components of yogurt for the purpose of ingredient separation (Shifrin, 1968).

### **H.2.e. Milk Proteins**

Milk proteins are composed of two main fractions: whey proteins and casein proteins. Caseins are complex proteins which can be divided into 4 subfractions:  $\alpha_{s1}$  -  $\alpha_{s2}$  -,  $\beta$ - and  $\kappa$ - caseins. These proteins form hydrated micelles that are dispersed throughout milk. The exact organization of the micelles is not known. Casein proteins can easily form films in aqueous solutions. Film formation is attributed to the random coiled structure of the protein, as well as its ability to hydrogen bond. Resulting films are transparent, flavorless, and flexible. Casein proteins have been used in emulsion based coatings to reduce water loss in zucchini (Avena-Bustillos et al., 1994). Casein coatings have also been tested for their ability to reduce moisture loss from raisins (Watters and Brekke, 1996) however, results showed the coatings were unable to maintain moisture content. Casein and acetylated monoglycerides have also been tested on cut, peeled apple pieces, for their ability to prevent moisture loss and enzymatic browning (Krochta et al., 1990). Results indicated the coating prevented water loss, and when mixed with ascorbic acid, also prevented browning.

Whey proteins are simple proteins made of  $\alpha$ -lactoglobulin,  $\beta$ -lactoglobulin, immunoglobulins, and proteose-peptones. The film-forming capabilities of each separate whey protein has not been examined, however, as a whole whey proteins can be processed to produce transparent, flavorless, flexible films similar to those made from caseins. Plasticizers, which serve to reduce internal hydrogen bonding, have been added

to whey films to increase film flexibility and water vapor permeability (McHugh, et al., 1994). Lipids, including acetylated monoglycerides, waxes and fatty acids, have also been incorporated into whey films (McHugh and Krochta 1994). The addition of lipids resulted in decreased water vapor permeability, with the greatest lowering effect observed with the addition of beeswax emulsion, and high levels of homogenization.

### **H.3. Lipid Based Coatings**

As mentioned previously, lipid based coatings have been used for over 800 years. Historically, uses of lipid coatings included 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 skin on fruits and vegetables. Lipid-based coatings can be made from a wide variety of lipids 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. Synthetic paraffin wax is also available and can be made from catalytic polymerization of ethylene. Both synthetic and natural paraffins are FDA approved as food additives as long as certain specifications including ultraviolet absorbency and molecular weight levels are met. 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. Carnauba wax is considered GRAS as a food additive. 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 as emulsifiers and dispersing agents. Fatty acids are extracted from vegetable oils, while monoglycerides are prepared by transesterification of glycerol and triglycerides (Hernandez, 1991). Long chain alcohols are added to coatings due to their hydrophobic nature and high melting points. For the same reasons, long chain fatty acids are also used as coating additives (Hagenmaier and Shaw, 1990)

### **H.3.c. Emulsions**

Use of wax based emulsion coatings is a relatively new idea, and therefore little research has been done on preparation and composition of such films. Emulsion coatings have excellent moisture barrier properties; however, they do not add shine to a product. Many of the emulsifiers used in wax emulsions are derivatives of glycerol and fatty acids. Examples of commercially available emulsifiers include polyglycerols-polystearates (Hernandez, 1991).

Emulsion coatings may be subdivided into macro- and micro-emulsions. Macro emulsions have a particle size range of  $2 \times 10^3 - 10^5 \text{ \AA}$ , and microemulsions have particle sizes of 1000-2000  $\text{\AA}$ . Formation of small wax droplets in microemulsions depends on the interaction of the dispersed phase and the emulsifier, while globule size in macroemulsions relies on the mechanical method of dispersion, including high pressure homogenization or high-speed stirring. Wax emulsions can be applied by various methods including spraying, brushing, dipping, and foam application. Carnauba and beeswax can be dispersed easily into microemulsions due to their high hydroxyl and ester

group content. However, emulsion formation requires the selection of appropriate emulsifiers. Microemulsions usually utilize two emulsifiers: one that is soluble in both the continuous and dispersed phases, and a co-surfactant, usually alcohol. Small droplet size in a microemulsion results in a uniform film that dries to a glossy finish. The inversion process (Prince, 1977) is the most common method of making wax-in-water microemulsions.

Selection of an appropriate emulsifier for microemulsions is based on two criteria. The first is the hydrophobic-lipophylic balance (HLB). Emulsifiers are classified by their HLB value, which may range from 1-40 depending on the relative portions of hydrophobic and hydrophilic portions of the emulsifier. Hydrophilic emulsifiers have high HLB values, while hydrophobic emulsifiers have low values. Emulsifiers with high HLB values are usually used with oil in water emulsions, while emulsifiers with low HLB values are used with water in oil emulsions.

The second criteria is the phase inversion temperature (PIT). PIT refers to the temperature at which an oil in water emulsion turns into a water in oil emulsion. Interfacial tension is minimal at this temperature, allowing for production of very small dispersed droplets. Examples of emulsifiers include sodium alkyl sulfate and stearyl alcohol. In addition, many natural waxes including carnauba and beeswax, are also used as emulsifiers.

#### **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. Rosins are derived from oleoresins of pine trees as an exudate or a by-product of the wood pulp industry. Resins can be modified by hydrogenation, polymerization, isomerization and decarboxylation all of which improve thermoplasticity and make the film resistant to color and oxidative changes. Coumarone indene is a coal or petroleum by product. It is resistant to alkali conditions, dilute acid and moisture due to its aliphatic structure.

Solvent waxes are coatings made primarily of resins, small amounts of wax, and added petroleum solvent. Solvents aid in blending of resins and waxes to produce glossy, water-resistant coatings that are easy to apply and dry quickly (Hernandez, 1991). Despite these desirable properties, which make them good choices for coating fruits and vegetables, these coatings are not used very much due to their expense and propensity for migration into food products (Kaplan, 1986).

#### **H.4. Composite and Bi-layer Coatings**

In any edible film used for moisture barrier properties it is necessary to achieve a continuous lipid layer. Formation of a continuous lipid layer on an uneven, porous food surface is difficult, namely because the lipid may penetrate the food surface, which results in decreased uniformity (Greener and Fennema, 1989a). To combat this problem researchers advocate using a two-step approach in which a base layer is applied first to seal the surface of the food, and then a lipid layer is added on top. The base layer acts as an anchoring substance or matrix on which the lipid sits. The type of base layer used has been varied in many studies, and usually is made up of either a protein or a hydrocolloid. Regardless of the content of the base layer, it is important that the material be viscous enough to remain on the applied surface and seal off any surface cracks or irregularities. Furthermore, the film must not be brittle or prone to cracking. To combat this problem plasticizers are commonly added. Lipids used in bi-layer coatings need to be tough yet pliable, so that they may be easily applied to the surface of the food. Ethyl cellulose has been added to waxes because it reduces crystal formation (Bennet, 1975), as well as increases the viscosity of the lipid.

##### **H.4.a. Creation of Bi-layer Films**

Lipid films either in the molten form or with added ethanol (a solvent) are applied to the surface of a previously dried base matrix. Base films are created by different procedures depending upon the substance being used. Typically, the base film is made by creating a

solution of either carbohydrate or protein compounds with a solvent added. The solvent forms a film upon drying. This solution may contain added emulsifiers, or plasticizing agents. Once mixed, the base film is warmed and cast onto the desired surface and dried to evaporate the solvent. Lipid materials are added to the base film using either the solvent method or the molten lipid method. In the solvent method the lipid is added to ethanol while being agitated. The previously dried base films are pre-heated and the lipid in ethanol suspension is cast over the base film. Once applied, the solvent is allowed to evaporate. In the molten lipid method the lipid is melted and spread onto a dried base film. A pre-heated Teflon sheet may be used to cover the surface of the film in order to produce a uniform surface. The film is then cooled before the Teflon sheet is removed.

However, with fruits and vegetables these types of “films” may not be entirely appropriate. First, such films require two casting steps, one for the base matrix and one for the lipid film. Both of these steps involve a heated drying step that may take as long as 20-30minutes. Such a procedure may be too involved for common place horticultural produce practices. In order to justify the use of edible coatings on harvested commodities they must be both practical and easy to apply as well as non labor intensive. Therefore, instead of typical “film” products, which are likened to shrink wrap and saran, coatings offer a more practical approach. Coatings may be applied by dripping or foaming the product onto the fruit and then dispersing the product into a uniform layer by use of brushes and rollers. Coatings are already being applied to horticulture products in this manner, as is the case with apples. Therefore, it may be possible to use existing machinery to apply such coating mixtures, and thus added expense will be reduced.

The use of more than one coating ingredient, whether in a bi-layer or emulsion system, is beneficial. Bi-layer coatings and emulsion-based coatings have many advantages over traditional monolayer coatings. First, these coatings can provide added durability through use of a support matrix. Second, these coatings may be multifunctional, and allow gasses to pass, while maintaining moisture. Lastly, these coatings may adhere more efficiently to produce. The use of lipids on produce has been known to reduce moisture loss. However, lipid coatings may be problematic for several reasons. They

may become brittle and develop pin-holes which allow for moisture and gas loss. Lipids may also oxidize and produce off flavors. Sensory properties of lipids, including their dull appearance and waxy taste, may also not be undesirable. Therefore, additives to these coatings may help alleviate these negative aspects. A variety of ingredients have been investigated as possible components of bi-layer films or emulsions. Such ingredients include milk proteins, acetylated monoglycerides, waxes, cellulose derivatives, and other lipids, hydrocolloids and proteins.

The milk protein, casein, has been tested in a variety of bi-layer systems. Avena-Bustillos and Krochta (1993) investigated the ability of acetylated monoglyceride and caseinate salts to prevent water transfer. In these coatings, the casein proteins act as an emulsifier and aids in stabilizing the protein lipid emulsion. These coatings are also easier to apply to products than single-component coatings. Casein materials alone are hydrophilic, and are therefore a poor moisture barrier, however when combined with lipids, which are hydrophobic the moisture barrier properties were enhanced. In this emulsion the coating provides structural cohesion.

Calcium crosslinking of casein can help to adjust the gas barrier properties of caseinate and lipid coatings. Crosslinking can be accomplished by changing the pH to the isoelectric point that maximizes casein protein interactions. Increased crosslinking proved to be an effective measure of reducing water vapor permeability (Avena-Bustillos and Krochta, 1993).

The use of caseinate and acetylated monoglyceride films has also been tested on apples and celery (Avena-Bustillos et al., 1997). Results from this study indicated that 1.5% calcium caseinate and 1.5% acetylated monoglyceride solutions reduced moisture loss in celery, but not in apples. The lack of significant reduction in water vapor permeability in apples may be due to their initial high water vapor resistance. Therefore, products with already high water vapor resistance may not benefit from these coatings.

The use of wheat gluten and lipid bi-layer films was investigated by Gontrad et al. (1995). In this film, wheat gluten provided a structural layer, while the lipid layer functioned to prevent moisture loss. Wheat gluten films are transparent and are mechanically strong, however, as with other proteins, they are hydrophilic and therefore are not acceptable as the sole prevention of moisture loss (Gontrad et al., 1994). Results showed that solid lipids, including molten beeswax and paraffin placed on the protein, provided the best at moisture retention. This composite coating was made using wheat gluten, glycerol and diacetyl tartaric ester of monoglyceride (DATEM) as the base layer and beeswax as the upper layer.

Greener and Fennema (1989a) examined barrier properties of films based on methylcellulose and beeswax. Methylcellulose lends strength to the film, while the lipid prevents moisture loss. Results indicated the films with the least water vapor permeability (WVP) (at 97% relative humidity) were composed of a double beeswax coating applied to a molten fatty acid-hydrocolloid base. Another film with good WVP was made from fatty acid and hydrocolloid in water-ethanol solution. Films made from beeswax applied to molten to a methylcellulose base, or in ethanol applied to methylcellulose base had much higher WVP rates, indicating that these films may not be as well suited to prevent moisture loss.

#### **H.4.b. Coating Application**

Many coating methods are available. The type of method used depends on the nature of the coating constituents and the characteristics of the commodity to be coated. The following section will review several common coatings methods including dipping, foam application, spray, drip, and controlled drop application..

##### **Dip Application**

The dip application method involves submerging small quantities of produce into a vat of coating solution. Citrus fruits were the first types of fruit to be coated by this method

(Platenius, 1939) however, other types of fruits, including tomatoes, rutabagas, and peppers have also been coated by dipping. Coating continuity is of paramount importance in the dip method, and can be only be achieved by complete wetting of the entire fruit. Following dipping, commodities are dried either at room temperature or with aid of a drier. Several problems may occur using the dipping method, including build up of trash, dirt, and microorganisms in the dipping tank. To remedy such problems sieves are used to remove debris. Other problems with dipping may include coating dilution due to addition of water from the fruit or vegetable's surface. Lastly, coating applications from the dip method are usually thick, which may pose problems with respiration and storage characteristics (Grant and Burns, 1994).

### Foam Application

Foam coatings are made by adding a foaming agent to the coating or by blowing compressed air in to the coating tank (Hartman and Isenberg, 1956; Long and Leggo, 1959). The foam is continually agitated and then dropped onto the commodity as it rolls by on rollers. Brushes and cloth flaps smooth the coating over the fruit while excess is removed and recycled. Unfortunately, the act of evenly distributing the coating may be difficult due to the constant movement of the fruit. The fruit is dried as in dip application, however the process is not as extensive as dip coating due to the lower water content of foam coatings.

### Spray Application

Most coatings are applied by spray application. Such coatings are sprayed through nozzles onto produce that is traveling by on rotating brushes. The brush set up is important to coating application and distribution. Straight-cut brushes are more effective at coating distribution than round brushes, while spiral cut brushes may be used on irregularly shaped produce to facilitate tumbling and ensure even coverage. The number of brushes is also important. Too many brushes will remove too much coating, while too few will not generate even coverage. Typically brush beds are made of 12-14 brushes,

composed of equal portions of polyethylene and horsehair bristles. The distance between bristles is important, with recommended distances of no more than 0.95 cm between bristles. Brush wear should be checked often due to the tendency for natural bristles to fall away from the brush (Grant and Burns, 1994)

Coating flow from spray nozzles can be emitted as a full cone, tapered, even-edged flat or air atomizing in nature. Spray delivery allows for uniform coating of commodities, however, air currents between nozzles and fruits and vegetables can affect the coating application, and therefore should be minimized. Adjustments to flow and bed fill should also be made when spray coating commodities of different surface areas.

### Drip Application

Drip application is the most economical coating application method (Grant and Burns, 1994). The drip method involves dropping various sized coating droplets onto a commodity or brush. Coatings are then dispersed by the rolling action of the brushes.

### Controlled Drop Application

This method of application has been used with only certain types of produce. Controlled drop application involves delivering the coating to a rotating disk that disperses the coating into smaller sized droplets, which are then delivered through a spray nozzle to the food. The speed of the rotating disk will determine the coating droplet size; larger droplets are delivered by a slower rotation speed. Citrus commodities as well as apples have been coated using this method (Grant and Burns, 1994).

Regardless of which coating method is used, several factors are important for successful coating. Clean pumps, nozzles and brushes are essential to prevent clogs and product buildup. Buildup of coatings on rollers is particularly detrimental due to hardening of the coating, which may injure commodities passing over them. Prior to coating, all commodities should be free of surface water that will dilute the coating and may cause



foaming of the coating materials should fungicides be present. Brush wear and tear should also be monitored continually. Worn brushes will not give complete coating coverage. Making sure that commodities are thoroughly cleaned can also ensure complete coating. Sufficient drying time is also necessary. Insufficiently dried coatings may be sticky or tacky, which when handled can create incomplete coating coverage points. Therefore, drying time coupled with the correct temperature, humidity and airflow are necessary for proper coating application.

#### **H.4.c. Coating Permeability Testing**

The permeability properties of coatings and films are essential to their functionality. Therefore, measurement of these properties is necessary for evaluating a coatings' potential applications. The following is a review of the methods developed for analyzing film permeability to water vapor, gas and lipid substances.

##### **H.4.c.1. Permeability of Films to Water Vapor**

Water vapor permeability (WVP) is defined by ASTM E96-80 as the rate of water vapor transmission through a unit area of flat material of unit thickness induced by a unit vapor pressure difference between two specific surfaces, under specified temperature and humidity conditions (ASTM, 1980). This definition is expressed by the following equation:

$$\frac{(\text{amount of water vapor}) (\text{thickness of film})}{(\text{film area}) (\text{time})(\text{differential partial pressure of water vapor})}$$

Methods for measuring water vapor permeability include gravimetric techniques, infrared sensor techniques, coulometric cells, spectrophotometric techniques, and gas chromatographic systems.

#### **Gravimetric Techniques**

Gravimetric techniques are commonly used to assess water vapor permeability of edible films. Advantages of these techniques include control of experimental parameters, and low cost. Two versions of this technique are used, the Desiccant method and the Water method. These tests have been standardized by ASTM E96 (1980). In the Desiccant method the edible film seals off a test dish containing desiccant. The test dish is then placed into a temperature and humidity-controlled chamber. In the Water method the dish contains distilled water or a salt solution instead of desiccant. Water vapor transmission in both methods is determined by periodically weighing the dishes after equilibrium has been reached and a steady rate of moisture transmission is obtained. Several precautions are necessary to obtain accurate results. First, water vapor permeability (WVP) assessment of some films requires a correction method to account for edible films with low resistance to mass transfer. In addition, sealing of the film to the test dish should be done so that no breaks are allowed. For this purpose sealing is commonly done using molten wax, or silicon vacuum grease. Factors that must be controlled during this test procedure include temperature, humidity and air circulation. ASTM recommends velocities of at least 500 ft/minute. Relative humidity should be monitored using a hygrometer inside the test chamber, and maintaining  $RH \pm 2\%$  throughout testing.

### Infrared Sensor Technique

Infrared sensor techniques utilize instruments capable of rapidly determining WVP. Such instruments are produced by Modern Controls Inc. (MODCON) (Minneapolis, MN). There are two main systems, the Infrared Diffusometer (IRD) series and the Permatran-W series. The IRD series is made of two chambers separated by the film. The first chamber is a dry chamber with a RH of 0%. The second chamber has a known relative humidity and temperature. Two bands in the infrared spectral region are monitored during testing. One band indicates where water molecules absorb the other is where they do not. The absorption spectra is monitored to determine the time required to cause a given increase

in water vapor concentration in the dry chamber. From this information the water vapor movement per unit area is calculated.

The Permatran-W method also utilizes the two-chamber system. Here, dry air is moved throughout the dry chamber. Water vapor that diffuses through the film enters the dry air stream and is carried to a pressure-modulated infrared sensor, which measures the fraction of infrared energy absorbed by the water vapor. An electrical signal with an amplitude proportional to the water vapor concentration is produced.

### Coulometric Cells

Another instrument, called the Minneapolis Honeywell tester (The St. Regis Company), can also be used to determine WVP. This instrument operates by monitoring the changing humidity in a controlled air space above the film being tested. As with the Infrared Sensor techniques a two-chamber system is used. A bottom chamber has a layer of water which maintains 100% RH, while the top chamber contains a RH sensor made of two platinum electrodes wound around a glass tube coated with a thin layer of phosphorus pentoxide. The tube absorbs water that permeates the film and passes into this chamber. The absorbed moisture is then electrolyzed and decomposed into hydrogen and oxygen. Changes in electrical resistance are measured to determine RH changes. The rate of water vapor movement between chambers is monitored by the rate of increase in RH in the upper chamber over time.

### Spectrophotometric Techniques

A spectrophotometric technique for determining WVP was developed by Holland and Santangelo (1984). Here a detector film is clamped between two pieces of test film in a simple cell. The detector film is made of regenerated cellulose soaked in a 2.6-2.8 M aqueous solution of cobalt chloride, which is then air-dried and stored in a desiccator. Blue color of the detector film fades proportionately to the amount of water vapor present. Color changes can be monitored spectrophotometrically at 690 nm. Although

this method is sensitive and works well for nonpolar polymeric materials, limitations exist when measuring films that are more permeable to WVP than the detector film.

#### **H.4.c.2. Gas Permeability of Edible Coatings and Films**

Most research on gas permeability of edible films deals with oxygen and carbon dioxide gases due to their effects on respiration and oxidation rates of foods. Permeability of films to gases is defined the same as permeability of films to water vapor: the rate of gas transmission through a unit of flat material of unit thickness, induced by a unit vapor pressure difference between two specific surfaces, under specified temperature and pressure conditions. Methods of assessing gas permeability include manometric methods, volumetric methods, gas chromatography, coulometric methods, infrared detection and bioluminescence.

Manometric methods are applicable only when testing flat materials at 0% RH. This method is commonly called the Dow Cell technique (ASTM, 1988), and consists of various Dow gas transmission cell consoles, which may be purchased from Custom Scientific Instruments, Inc. (Cedar Knolls, NJ). In this technique the sample is placed in a gas transmission cell between two chambers. One gas chamber is pressurized with the gas being tested, and the other chamber is made a vacuum. The rate of gas transmission is measured by recording changes in capillary pressure. These changes are related to calibration data, which is obtained by measuring the volume of gas that passes through both a standard specimen and the test specimen (at a specified temperature and pressure) into the initially evacuated calibrated manometer. Problems with this method include difficulty in calibration, and temperature and pressure changes, which result in breakage when measuring fragile films.

#### **Volumetric Method**

The volumetric method is commonly referred to as the Linde Cell. This test is similar to the Dow cell, however here the low-pressure chamber is maintained near atmospheric

pressure and the transmission of gas through the test specimen is indicated by a change in volume. These instruments are also made by Custom Scientific Instruments, Inc. (Cedar Knolls, NJ). This method is very operator dependent, and therefore not commonly used.

### Isostatic Concentration Methods

Isostatic methods maintain constant atmospheric pressures in both chambers of the permeation cell. This method is useful when testing fragile films. In addition, because there is no absolute pressure gradient forced on the film, testing conditions are more true to application conditions. Gas permeability is determined by creating a partial pressure or concentration gradient between the two chambers, and then simultaneously determining oxygen and carbon dioxide transmission rates (Landrock and Proctor, 1952). This method is based on Dalton's law of partial pressure, which states that gases in mixtures behave independently of one another and their total pressure is based on the sum of their partial pressures. Again a two-chamber test cell is used with the film separating the two chambers. The film is held in place with screw clamps and rubber gaskets to ensure a leak-proof system, and wire screens are placed above and below the test film to prevent distortion of the film during testing. Nitrogen gas is passed through the lower chamber, while the test gas is passed through the upper chamber. All gases flow through glass humidity towers to control RH of the chambers. Test gases that diffused through the film accumulate in the lower chamber. Gas content of the lower chamber is analyzed using Orsat gas analysis buret system. Oxygen is measured using "Oxysorbent" and carbon dioxide is measured using potassium hydroxide.

### Gas Chromatography

Chromatographic analysis of gas samples can be determined by passing samples through a specific detector in a gas chromatograph, or by directly injecting samples. Again the test cell is made of two chambers separated by the film. Both gas chambers have an inlet and an outlet for gas flushing. The lower test chamber has a sampling port. Nitrogen

gas dried over calcium chloride is passed through both sides of the chamber until no detectable gas is apparent. Next a stream of test gas is passed through the upper compartment of the cell while the lower compartment is sealed. At regular intervals, gas samples are withdrawn from the lower compartment and are analyzed by gas chromatography. Advantages of this system include increased accuracy, repeated sampling of cell test gases, increased sensitivity, and rapid collection of data (Karel et al., 1963). Many improvements have been made to this system including automated sampling, and test cell improvements. Other advantages of this system include low cost, and increased sensitivity.

### Coulometric

Coulometric methods are the most commonly used to determine gas permeability properties. MOCON Corporation (Minneapolis, MN) makes Oxtran analysis units. These units operate under the principle of Faraday's law. Four electrons are released electrochemically for every oxygen molecule passing through the sensor. These electrons produce a current that is passed through a resistor, creating a voltage that can be quantified. These systems are extremely sensitive, and can precisely control temperature and RH. Disadvantages include potential leaks in the system, which may produce unreliable baseline levels, and high cost.

### Infrared Detection

Carbon dioxide permeability is easily measured by infrared detection due to its absorption of infrared radiation at a specific wavelength. MOCON produces equipment to measure the carbon dioxide transmission rates of films.

### Bioluminescence

Use of bioluminescence to determine gas transmission rates through films was developed by Miltz and Ultizur (1980). This method is based on luminous bacteria emitting light in

vivo. The bacteria are confined in a pouch of the film being tested. The intensity of the emitted light is measured and quantified, and is proportional to the oxygen transmission through the film.

#### **H.4.c. 3. Lipid Barrier Properties**

Hydrophilic films are expected to have good lipid barrier properties. No commercial method of measuring lipid barrier capabilities has been developed, however several custom methods are used, including the rate method and the permeability method.

##### Rate Method

A standard method of rate of grease transmission through flexible materials has been developed by ASTM (1988). In this method the barrier material is exposed to a cotton patch covered with grease on one side. Weights are placed on top of the cotton patch and the time required to show a visible change on a ground glass back up plate is measured. This test is rapid and sensitive to small amounts of grease, however data reproducibility is low, and results are operator dependent.

##### Permeability Method

Nelson and Fennema (1991) developed a method to test lipid barrier properties of methylcellulose edible films using a two-chamber cell. Here, the inner and outer cells each contain two different oils. A water jacket is used to enclose the chambers allowing for temperature control. Films are placed between two stainless steel screens separating the inner and outer chambers. Oil samples are analyzed at the beginning and end of the test using an AOAC method (1984). The rate of lipid migration was determined by measuring UV absorbance of the oil at 234 nm.

### **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, 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.

Although many benefits of applying edible coatings are known, the exact coatings to use for specific vegetables have not been well researched. The coatings that 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 optimize the formulation of a coating that will allow sufficient oxygen permeability as well as inhibit moisture loss.

Multi-component coatings can combine the oxygen permeability characteristics of protein or carbohydrate films with the water retention characteristics of lipid films. Numerous studies have been conducted to determine the optimum conditions and components of composite films. However, such studies typically only show data related to the ability of the materials to form a film. The practical use and testing of such films on foods has been limited to prepared foods like pizza (Kester and Fennema, 1989b) and baked products like brownies (Greener and Fennema, 1989b). Trials of composite coatings on whole fruits or vegetables have been scarce.

Composite coatings that are easy to apply and do not require extensive drying procedures are needed. Ease of film or coating application is paramount for use in horticultural practices. Ideally, coatings that may be applied by dripping or foaming the product onto the fruit and then dispersing the product into a uniform layer by use of brushes and rollers. This type of coating application would be most practical. Coatings are already being applied to horticulture products in this manner, as is the case with apples. Therefore, it may be possible to use existing machinery to apply such coating mixtures, and thus added expense will be reduced.

Coating of produce may be limited by coating texture. Consumers are unlikely to accept coatings that peel and are thick enough to be detected on the produce. This is particularly problematic for commodities in which the peel is eaten. Therefore, development of a relatively undetectable coating is needed.

Therefore, the purpose of this research was to formulate composite coatings that combine the optimum characteristics of both hydrocolloids and lipids. Coating prototypes will then be tested to evaluate consumer acceptability, as well as the coating's ability to preserve post harvest quality characteristics of green bell peppers. Specifically, the study will measure the effect of the coatings on quality changes of green bell peppers during storage, including: puncture force, pectin content, surface color, chlorophyll content, moisture retention, respiration rate, and vitamin C activity.

## **Chapter 4: Materials and Methods**

### **I. Experimental Design**

This experiment was divided into three steps. Section one involved development of lipid and polysaccharide emulsion coatings. From the formulations created, four coatings were selected for sensory testing based on aesthetic properties, adhesion to the fruit's surface, and maintenance of integrity during refrigerated storage. The coatings considered acceptable by sensory panelists were used in the storage study portion of the experiment. In the third step three treatments were tested: an uncoated control group of peppers, and three coated groups. Randomly selected peppers were tested each week, for five weeks, to assess quality changes. Statistical analysis was used to determine if coated peppers maintained higher quality during storage than control peppers. An outline of these methods can be seen in Figure 4.1.

### **II. Coating Materials**

#### **A. Polysaccharides**

Polysaccharides were selected as coating ingredients for several reasons.

Polysaccharides improve initial coating application, aid in coating adhesion to fruit surfaces, as well as maintain coating integrity during storage. They also function to alter gas and moisture barrier properties of the coating. Some gums offer the ability to increase viscosity, stabilize emulsions, and promote cling to fruit surfaces. Starch derivatives are known film-forming agents, and were therefore selected for this ability.

The polysaccharides selected as potential components included:

- carboxy methyl cellulose (TIC Gums, Belcamp, MD)
- locust bean gum (TIC Gums, Belcamp, MD)
- acid-modified starch (Pure Cote B790, Grain Processing Corporation, Muscatine, IA)
- propylene glycol alginate (TIC Gums, Belcamp, MD)
- guar gum (TIC Gums, Belcamp, MD)

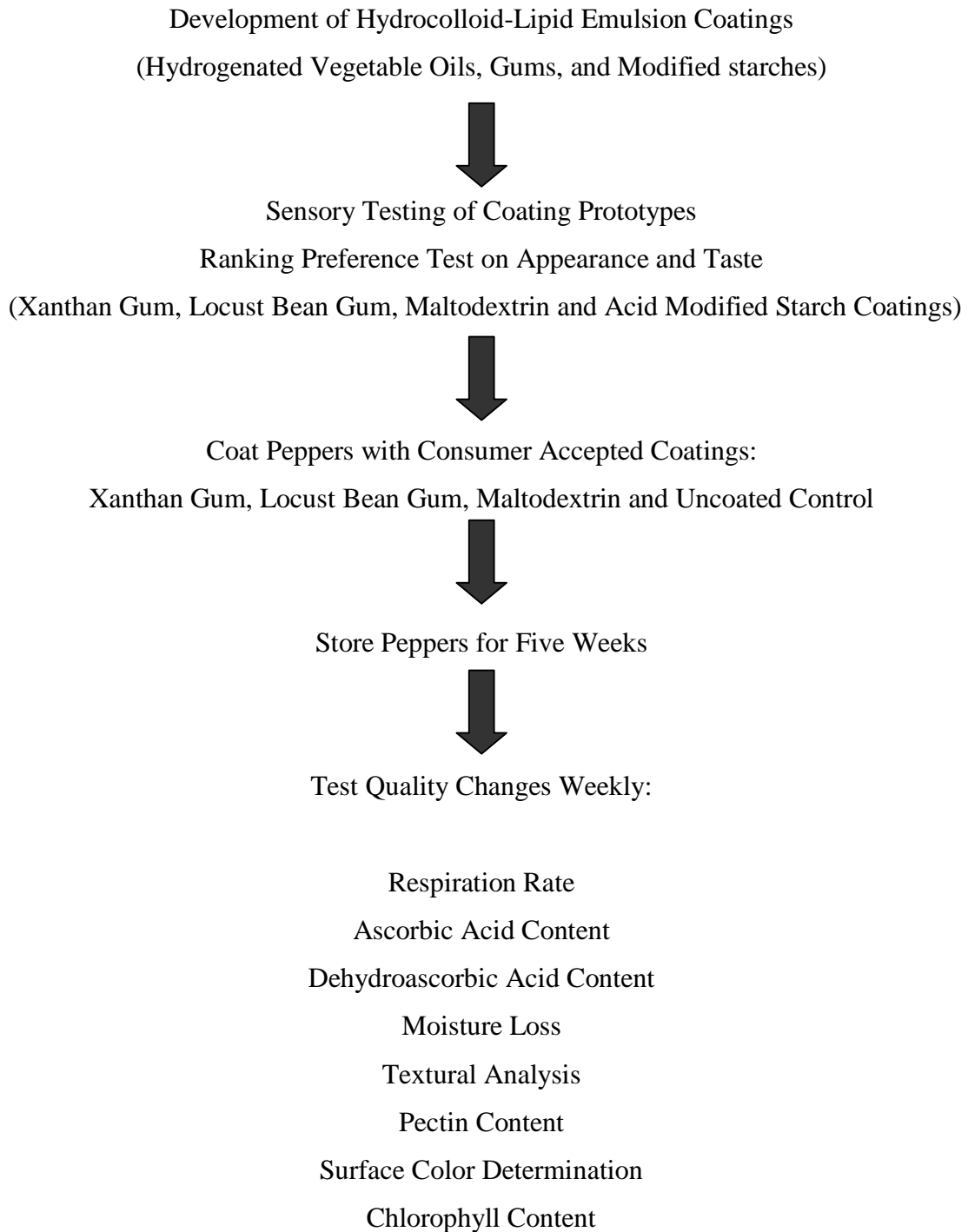


Figure 4.1: Flow Chart of Experimental Steps

- xanthan gum (TIC Gums, Belcamp, MD)
- maltodextrin (Star Dri 18, Staley, Decatur, IL)

## **B. Lipids**

Lipids were selected based on their ability to cling to the fruit and their potential as a moisture barrier. Lipid coating materials included one liquid fat (Food Lion vegetable oil) and four solid fats that varied in their solid fat content at room temperature. Astral (AC Humko, Memphis, TN), Humkote (AC Humko, Memphis, TN) and Food Lion brand shortening (Salisbury, NC) were all partially hydrogenated soybean and cottonseed blends selected for their common ability to adhere to pepper surfaces, potential ability to prevent moisture loss, and their differences in solid fat content. Parafin wax (Gulf Was, Gulf Lite and Wizard Inc., Memphis, TN) was also tested based on its ability to coat other foods like candies and confections.

## **C. Emulsifiers and Additives**

Three emulsifiers were selected based on their ability to stabilize the gum and lipid dispersions. Super G 10 and Super G (AC Humko, Memphis, TN) were composed of mono and diglycerides, which function to maintain oil-in-water emulsions. The polysaccharides xanthan gum (TIC Gums, Belcamp, MD) and propylene glycol alginate (TIC Gums, Belcamp, MD) were also tested for their emulsifying capacity. Glycerol (Vitusa, Berkley Heights, NJ) was used to plasticize coatings that became brittle during drying or cold storage.

## **III. Coating Application**

Coatings were prepared according to the formulas provided in Table 4.1. A detailed description of the formulation of these coatings can be found in the Results and Discussion section, Chapter five. These four coatings were each applied by spreading a

Table 4.1: Coating prototype formulations.

	<b>Coating Type</b>			
	<b>Xanthan Gum</b>	<b>Locust Bean Gum</b>	<b>Maltodextrin</b>	<b>Acid Modified Starch</b>
<b>Ingredient</b>	<b>Percentage of Ingredient Present</b>			
Humkote	28.0	20.0	5.8	3.5
Super G 10	10.0	18.0	5.8	3.5
Maltodextrin	0	0	11.6	0
Propylene Glycol Alginate	1.2	0	0.69	0
Locust Bean Gum	0	1.0	0	0
Xanthan Gum	0.6	1.0	0.34	0
Acid Modified Starch	0	0	0	15.0
Distilled Water	60.2	60.0	40.7	73.0
Glycerol	0	0	0	5.0

thin layer of the material onto the fruit's surface. Coatings were allowed to dry at room temperature with the aid of a fan. If necessary, coatings were buffed to produce an even layer on the fruit.

#### **IV. Sensory Testing**

Sensory testing was conducted to determine consumer preference for coating prototypes. One pepper piece (approximately 2 cm X 2 cm) from each prototype was presented to untrained panelists on a paper plate. Pepper pieces were identified by 3-digit random numbers. After reading and signing consent forms (Appendix A), panelists were asked to taste the samples and rank them on a scorecard (Appendix B) according to their preference for each piece. Sensory testing was done on freshly coated peppers, as well as peppers that had been stored for ten days. Data were analyzed using analysis of variance to determine if preference differed for any of the coatings. If coatings differed, Tukey's honest significant difference test was used in multiple comparison testing to determine which coatings differed. All prototypes that were consistently highly preferred were used in the storage study portion of the testing.

#### **V. Analysis of Quality Changes**

##### **A. Texture**

##### **A.1. Puncture Force Analysis**

Texture of pepper fruit walls was analyzed using a puncture test performed on the Instron (Model 1011; Canton, MA) (Appendix C). The same pepper samples that were used for puncture testing were also used for pectin analysis. Two pepper pieces (approximately 30 mm X 50 mm) were cut from opposite sides of the fruit. The samples were placed on the test platform, outside surface facing up. The pepper was punctured with a stainless steel cylindrical probe 0.50 cm in diameter. The probe was programmed to have a load level of 5 kg, a high load level of 10 kg, and a high extension of 18 mm. The crosshead 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.

## **A.2. Pectin Analysis**

Pectin changes occurring during the storage period were analyzed by the method of Huber (1983) (Appendix D). Theoretically, enzymes reduce high molecular weight pectin polymers into numerous lower molecular weight fragments. The degradation of high molecular weight polymers results in softening of the plant tissue. Analysis of uronic acid content of stored pepper samples was used to determine the extent of enzymatic pectin degradation, and its affect on pepper texture. Uronic acid quantification was conducted as follows:

Pectin polysaccharides were isolated by grinding 8 grams of edible pepper tissue in 20 ml of cold (-20°C) acetone for 2 minutes in a homogenizer (Polytron, Kinmen) set at speed 6. The homogenate was then pulled through a glass fiber filter (Whatman, 0.45 µm PTFE filter) with the assistance of a vacuum pump. The tube the pepper was ground in was then rinsed with an additional 20 ml of cold acetone. Next the residue was washed with 100 ml of 80% acetone, followed by a second washing with 100 ml of 100% acetone. The acetone insoluble residue (AIR) was then removed from the filter paper and suspended in cold (4°C) 2 phenol: 1 acetic acid: 1 water (w/v/v) for 5 minutes to inactivate enzymes. Next, acetone was added to a final concentration of 80%. The AIR was again filtered through a glass-fiber paper with vacuum assist, dried at room temperature, and then stored in a dessicator over phosphorus pentoxide.

### **Uronic Acid Extraction**

Pectin polysaccharides were extracted by suspending approximately 40 mg of the dried acetone powder from above in 3 ml of sodium acetate EDTA buffer (50mM acetate, 40 mM EDTA, pH 4.5) for 4 hours at room temperature with stirring. The suspension was then centrifuged to settle particulates.

### **Quantitative Analysis of Uronic Acids**



Uronic acids were quantified according to the procedures of Blumenkrantz and Asboe-Hansen (1973). A 0.2 ml portion of each uronic acid sample was mixed with 1.2 ml of sulfuric acid/tetraborate (0.0125M tetraborate in sulfuric acid). The mixture was then mixed on a vortex and then heated at 100°C for 5 minutes. After heating, the mixture was cooled in an ice bath. Next, 20 µl of m-hydroxydiphenyl reagent (0.15% meta-hydroxy-diphenyl in 0.5% NaOH) was added, the sample was vortexed and the absorbance was read at exactly 5 minutes at 520 nm. A blank composed of 20µl of 0.5% NaOH (which replaced the reagent) was used to zero the spectrophotometer. A standard curve was established using 1, 4, 5, 10, 20 and 40 µg standards. The absorbance of the samples was used to determine their uronic acid concentration from the standard curve.

## **B. Color Analysis**

### **B.1. Surface Color Analysis**

Fruit surface color was analyzed using a Hunter D25 L Optical Sensor Colorimeter (Reston, Va) (Appendix E). Hunter L\*, a\*, and b\* values were recorded from two sample slices (approximately 30 mm X 50 mm) cut from opposite walls of the pepper. Using Statistical Analysis System (SAS), the hue angle and chroma were calculated from the L\*, a\* and b\* values according to procedures detailed in McGuire (1992).

### **B.2. Chlorophyll**

Total chlorophyll analysis was based on the procedure of Lancaster et al. (1997) (Appendix F). A one gram sample of pepper skin was homogenized in 15 ml of cold acetone for 2 minutes at speed 6. The extracted fluid was collected in a graduated cylinder. The residue was then re-extracted with 5 ml of 80% acetone. The extracted fluids were combined and the volume was then brought to 30 ml using 80% acetone. Spectrophotometric analysis on filtrate samples was done at 645nm and 663 nm. Total chlorophyll content was calculated according to the following equation (Holden, 1976).

Total chlorophyll (mg/l) = 20.2D<sub>645</sub> + 8.02 D<sub>663</sub>

### **C. Ascorbic Acid and Dehydroascorbic Acid Analysis**

Ascorbic acid and dehydroascorbic acid content were determined using a high performance liquid chromatography (HPLC) procedure adapted from Wimalasiri and Wills (1983) (Appendix G).

#### HPLC Specifications

The HPLC equipment included the following:

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

The column used was a Bondclone 10 micron CHO (Phenomenex, Cat. #00H-3240-C0, length X ID: 300 mm X 3.9 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.

#### Ascorbic Acid and Dehydroascorbic Acid Extraction

Ascorbic Acid (AA) and dehydroascorbic acid (DHA) were simultaneously extracted by first weighing 25 grams 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 and 2 drops of antifoaming agent (Antifoam A-5758, Sigma Chemical Company, St. Louis, MO) were 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 a 100 ml volumetric flask, and brought to volume with additional citric acid.

For ascorbic acid analysis the pepper homogenate was passed through a syringe filter (Acrodisk, CR, PTFE) and then added to clear HPLC sample vials. Ascorbic acid was then analyzed by injecting 10 µl of sample, and reading at 254 nm by UV detection. Run times were 8 minutes.

Prior to analysis of dehydroascorbic acid, solid phase extraction was performed to remove contaminants that interfere with chromatogram production. An extraction apparatus (A J & W Scientific Folsom, CA) and Octadecyl (C18, 200mg, C18, #9002; Burdick and Jackson, Muskegan, MI) solid phase extraction cartridges were used. 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 were 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 where 20µl were injected and UV detection readings were carried out at 214 nm. Run times for DHA were 12 minutes.

#### Preparation of Standards

An ascorbic acid standard was prepared with 10 mg L-ascorbic acid (Aldrich, A9,290-2) in 10 ml of 50% acetonitrile water. A standard curve was made using injections of 2, 5, 7, and 10 µl. These injection volumes were equivalent to the 2, 5, 7, and 10 µg standard amounts due to the preparation of a 1:1 standard. A 1:10 dilution of dehydroascorbic acid standard (Aldrich) was prepared and injected using 2, 5, 7 and 10 ul amounts.

#### **D. Respiration Rate**

Respiration rates were calculated by measuring fruit carbon dioxide production (Appendix H). Respiration rates vary with changes in temperature therefore, pepper fruits were removed from refrigeration and allowed to equilibrate to room temperature (22°C) prior to analysis. After equilibration, two peppers were first weighed and then placed in a chamber with two rubber tubes attached. One tube delivered outside air to the

chamber at a rate of 5 liters/minute, while a second tube transferred air and gases produced by the peppers to an infrared gas analyzer (The Analytical Development Company, Model LCA2, Hoddeson, England). The amount of carbon dioxide produced by the peppers was equal to the difference in the carbon dioxide levels between ambient air and air that had flowed through the chamber. The rate of respiration was calculated from the amount of carbon dioxide evolved according to the following equation:

$$\text{Respiration Rate} = (\Delta\text{CO}_2 \cdot \text{F} \cdot \text{K}) / \text{A}$$

Where:

$$\Delta\text{CO}_2 = \text{CO}_2 \text{ output} - \text{CO}_2 \text{ input (ambient air)}$$

F = air flow in liters/hour

$$\text{K} = [44,000 \text{ mg carbon dioxide} / 22.4 \text{ L/mol}] \times (273/294)$$

(conversion of 1 L CO<sub>2</sub> to 1 mg CO<sub>2</sub>)

A = kilogram weight of the fruit

## **E. Weight Changes**

Overall moisture loss was determined by repeatedly weighing the same set of forty peppers (ten peppers from each treatment group) every three days.

## **VI. Statistical Analysis**

### Sampling

Color and texture analysis both involved randomly selecting 9 peppers from each treatment (36 peppers total). Two sub-samples from each fruit were used for analysis. The same peppers that were used for color testing were also used for chlorophyll measurements, and the peppers used for texture analysis were also used in pectin analysis.

Peppers used for testing of pectin, ascorbic acid, dehydroascorbic acid, and chlorophyll were all sampled the same way. Ascorbic acid and dehydroascorbic acid test

were performed on the same samples. Here, 9 peppers from each treatment were randomly selected. The nine peppers were divided into three groups of 3 peppers. Each group of three peppers was cut into pieces from which a 25 gram sample was measured out. A total of 3 samples (which were each composite samples of three individual fruits) from each treatment was analyzed.

Respiration analysis involved using 8 peppers from each treatment. The eight peppers were divided into 4 groups of 2 peppers, which were analyzed together.

### Analysis

Analysis of variance (ANOVA) was used to determine if any changes between treatment groups or changes during the storage period occurred. Dunnet's multiple comparison tests was used to determine if control groups differ significantly from treated groups on any given week of the study. Contrasts were run to determine if there were any trends in experimental values during the five-week study. Interaction effects for treatment and week of study were calculated for each set of data. When interaction was significant, the interaction term was used to determine significance of the week and treatment effects. Correlation testing was done between ascorbic acid and dehydroascorbic acid data.