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I am submitting herewith a thesis written by Jason Ki-Myung Noh entitled "Effect of chitosan and water soluble chitosan coatings on quality of small fruits." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

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Effect of chitosan and water soluble chitosan coatings on quality of small fruits

A Thesis presented for the

Masters of Science Degree

The University of Tennessee, Knoxville

Jason Ki-Myung Noh

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Abstract

Edible coating has been applied on the surface of fresh produce to extend shelf-life by suppressing respiration, transpiration, and microbial growth. The coatings, thus, can help decrease moisture and weight loss, and may offer a protective barrier against bacterial contamination and spoilage. Recent studies have indicated chitosan as an effective coating that extends shelf-life and improves a storability of fruits.

The objective of this study was to demonstrate the effectiveness of natural biodegradable chitosan coatings in extending shelf-life and quality of fresh small fruits, such as blueberries and grapes.

Fruits were dipped for 30 seconds in 1% chitosan in 1% aqueous acetic acid, 1% water-soluble chitosan in water, 1% acetic acid solution or tap water. Non-treated fruits served as a control treatment. Samples were stored at 4±1°C and 85±5% RH up to 24 days. Quality analysis was performed every 3 days. The analysis included measurements of texture, color, weight loss, and ethylene and CO_2 production.

Statistical analysis was conducted for all dip treatments and control fruit. For blueberries, ethylene production (0.038ppm/hr to 0.194ppm/hr; $p<0.01$) and carbon dioxide production (2.6% to 6.5%; $p<0.05$) decreased significantly for both chitosan treatments compared to control fruit. However, there was no significantly difference of fruit skin firmness among the chitosan treated samples and control treatments ($p>0.05$).

Grapes dipped in chitosan solutions had a slight but significant difference in ethylene production (0.01ppm/hr to 0.05ppm/hr; $p<0.05$) but no significant difference in CO_2 production ($p>0.50$). There was no significant changes in firmness among chitosan, soluble chitosan, and control grapes, but there were differences between grapes dipped in chitosan and water treatments and between chitosan and acetic acid treated grapes ($p<0.01$).

For all samples, Hunter b-values ($p<0.01$) were significantly different among the grapes and blueberries but no significant changes in L or a-values ($p>0.10$) among treatments. For blueberry samples, non-coated (control) had slightly bluer than chitosan treated fruits but chitosan treated grape samples were slightly more yellow color than control. There was no significant change in moisture loss from the fruit ($p>0.10$) among the treatments. The visual appearance of fruit samples did not significantly differ between water soluble chitosan treated and control but there was difference between chitosan in acetic acid treated samples and control. Chitosan in acetic acid treated samples left a thin layer film on the fruit skin that can be easily removed when washed with water.

The results suggest that chitosan coatings may be used on small fruits to maintain quality and extend shelf-life.

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1. Literature Review

1.1. Introduction

The term biopolymer is used to refer to biologically synthesized natural polymers. One example of a biopolymer is a polysaccharide, which is comprised of simple monosaccharide molecules joined to give high molecular weight polymers. Among the polysaccharides, cellulose and chitin are the two most abundant biopolymers in nature. Chitin is widely found in both animal and plant sources (Roberts 1992). Animal sources include the shells of crustaceans and mollusks, the backbone of squids and the cuticle of insects. In crustaceans, such as crabs, shrimp, and lobsters, chitin is found as a constituent of a complex network with proteins and calcium carbonate deposits to form the rigid shell. The interaction between chitin and protein is due to a polysaccharide-protein complex and presence of covalent bonding (Horst and others, 1993). Chitosan is not native to animal sources and is normally obtained by the deacetylation of shellfish derived chitin using sodium hydroxide. Most chitosan is manufactured from shellfish because a large amount of shellfish exoskeleton is available as a by-product of food processing.

Plant sources of chitin include algae, commonly known as marine diatoms, protozoa and the cell wall of several fungal species (Feofilova and others, 1996). Chitin from the diatom spines are the only form reported to be 100% poly-N-acetyl-glucosamine that is not associated with proteins and is termed chitan (McLachlan and others, 1965). A small number of fungal strains are known to produce chitosan in preference to chitin (Arcidiacono and others,

1989).

Chitin and chitosan are natural biopolymers, biodegrade easily, and have not been shown to be harmful to humans. These biodegradable polymers are usually obtained from the recovery of waste material from food processing discards (Hwang and Damodaran 1995; Sun and Payne 1996). Biopolymers, however, offer a wide range of unique applications including preservation of foods from microbial deterioration (Sams and others, 2004, Fang and others, 1994; Chen and others, 1998), excellent formation of biodegradable films and coatings (Hoagland and Parris 1996; Kittur and others, 1998), and purification of water (Muzzarelli and others, 1989). The high binding ability and antimicrobial activities of chitosan are the major functions for evaluating a new food preservation application of this natural biopolymer. Many researchers have reported that chitosan has been used as semipermeable coating material for fresh fruits and vegetables, and concluded chitosan is an excellent shelf life extender of perishable crops (Zhang and Quantick 1998; Du and others, 1997). El-Ghaouth and others (1991) and Du and others (1997) reported chitosan used to coat fresh berries has antifungal effects against *Botrytis cinerea* and *Rhizopus* sp., the common post-harvest fungal pathogens.

Fresh fruit crops are widely grown throughout the temperate region of the world and are universally popular products. In climacteric fruits, ethylene and carbon dioxide play an important role as key factors of ripening. Many scientists have studied and reported the use of edible coatings to reduce the ripening and extend shelf-life of fruits.

Edible coating has been applied on the surface of fresh produce to extend shelf-life by suppressing respiration, transpiration losses, and microbial growth. It can help to decrease moisture loss. It may also offer a protective barrier against bacterial contamination and spoilage. Studies by El Ghaouth and others, 1991, Zhang and Quantick 1997, Du and others, 1997 have indicated chitosan as an effective coating that extends shelf-life and improves storability of fruits. Dipping small fruits such as strawberries and blueberries in chitosan solution can also control decay (Sams and others, 2004).

1.2. Structure of Chitin and Chitosan

The structure of chitin is a linear polysaccharide of β-(1-4)-acetamido-2-deoxy-D-glucopyranose where all residues are comprised entirely of N-acetyl-glucosamine residues or is theoretically fully acetylated. Chitosan is also a linear polysaccharide of β-(1-4)-acetamido-2-deoxy-D-glucopyranose where all residues are comprised entirely of N-glucosamine residues or is theoretically fully deacetylated. The basic structures of chitin and chitosan are shown in Figure 1.

However, traditional sources of the biopolymer do not result in 100% acetylated chitin or 100% deacetylated chitosan. The biopolymer exists as a co-polymer as represented in Figure 2. Chitin and chitosan are based on carbons in the glucopyranose ring from C-1 to C-6. In this ring, the substitution at C-2 carbon of the ring can be either with the acetamido group or amino group. Chitin or chitosan are differentiated by the acetyl content. If the number of acetamido group is more than 50%, the biopolymer is termed chitin. In chitin, the

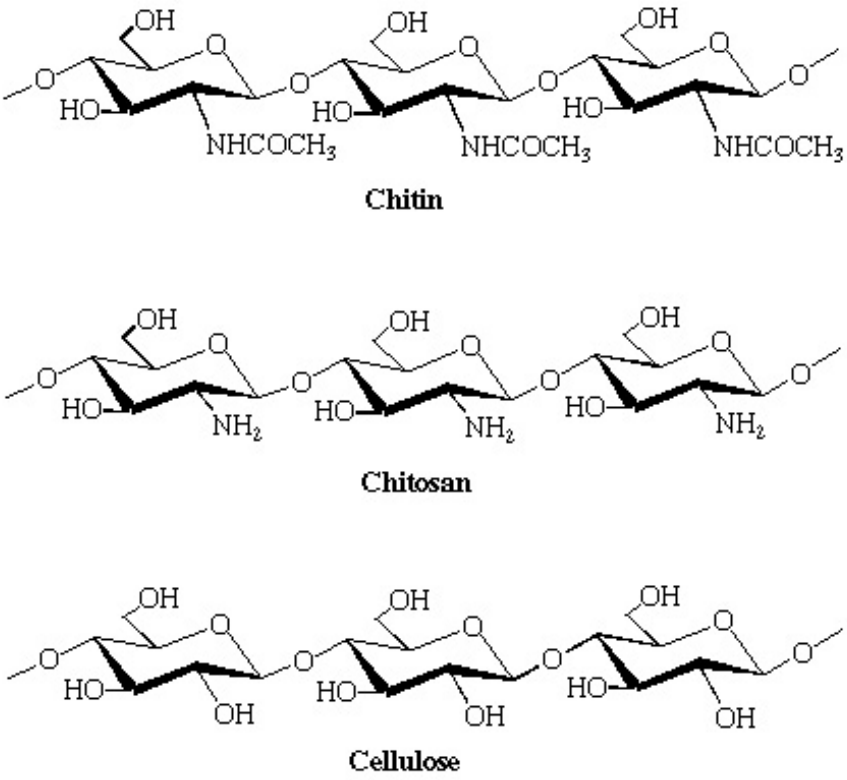

Figure 1: Structure of chitin, chitosan, and cellulose

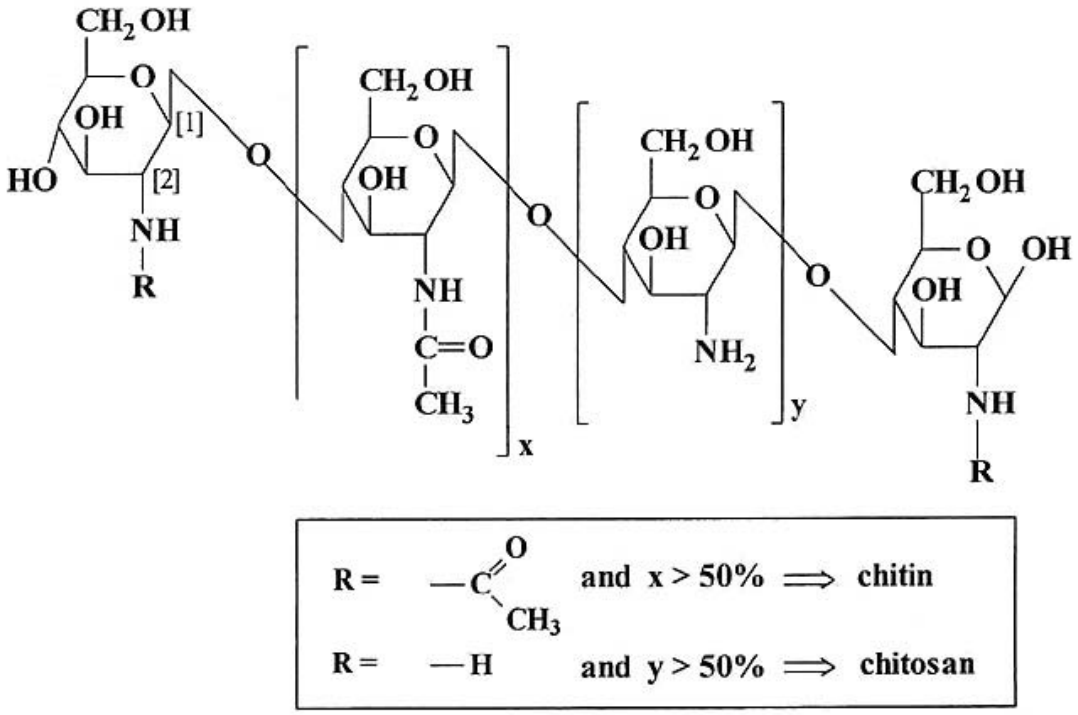

Figure 2: Chemical structure of chitin and chitosan depicting the co-polymer character of the biopolymers

number of acetamido groups is termed the degree of acetylation. When the degree of deacetylation or amino group content is more than 50%, the biopolymer is called chitosan.

Chitosan is typically insoluble in water, alkali, and organic solvents but is soluble in most aqueous solutions of organic acids such as acetic acid, formic acid, or lactic acid. The water insoluble chitosan may be over 1 million Daltons. A water-soluble chitosan has been produced which has low molecular weight (50-200 kDa). Water-soluble chitosan is desirable to use when acids are undesirable substances in products, such as some cosmetics, medicines, and foods. Muzzarelli (1988) studied chitosan with 50% deacetylation from homogeneous processing that was water-soluble, whith molecular weight of about 100 kDa.

1.2.1. Chitin Production

Chitin can be obtained from animal and plant sources. The dry shells of crabs, lobsters and shrimp contain 20-40% chitin, 30-40% of recoverable proteins and 20-30% of calcium carbonate. These are the major sources of waste from the seafood processing industry and most of today's chitosan production is produced from them. Chitin from shell fish is economically viable together with protein, pigment and mineral recovery as by-products (Khor 2001).

Chitin also can be produced from fungi. It has been estimated that fungi could provide $3.2x10^4$ metric tons of chitin annually and the supply can be potentially limitless if required (Knorr 1991). Chitin from fungal mycelia is an important alternative to shellfish sources with the benefits including a year around

supply compared to uncertainty of shellfish supply and a lower cost than shellfish chitin with less waste compared to chemical processing of shellfish (Shimahara and others, 1998).

1.2.2. Isolation of Chitin

Chitin from shellfish is the fraction of exoskeletal components which contains proteins and minerals, especially $CaCO_3$. The main components of fungal cell walls, beside, chitin are polysaccharides such as α- and β-glucan, mannan and cellulose. Chitin from shellfish is more crystalline and chemically more stable, while chitin from fungi is soft and less crystalline (Khor, 2001). Chitin from shellfish is more acetylated compared to chitin from fungi which have lower degree of acetylation.

The process of isolating chitin from shellfish requires two steps to remove major components of the shell. As shown in Figure 3, the first step is demineralization applied to remove inorganic calcium. The second step is deproteinization, necesscery to eliminate proteins from the complex with the polysaccharide. These two steps also remove small residues of trace-metals and lipids (Shimahara and others, 1988).

In order to obtain an acceptable isolation of chitin, the selections of shells of crabs and lobsters are important in determining the quality of the final isolated material (Khor, 2001). Shells of the same size and species are chosen. Cleaning and drying of the shells is followed by thorough crushing. The small shell pieces are treated with hydrochloric acid to remove calcium carbonate, and proteins are

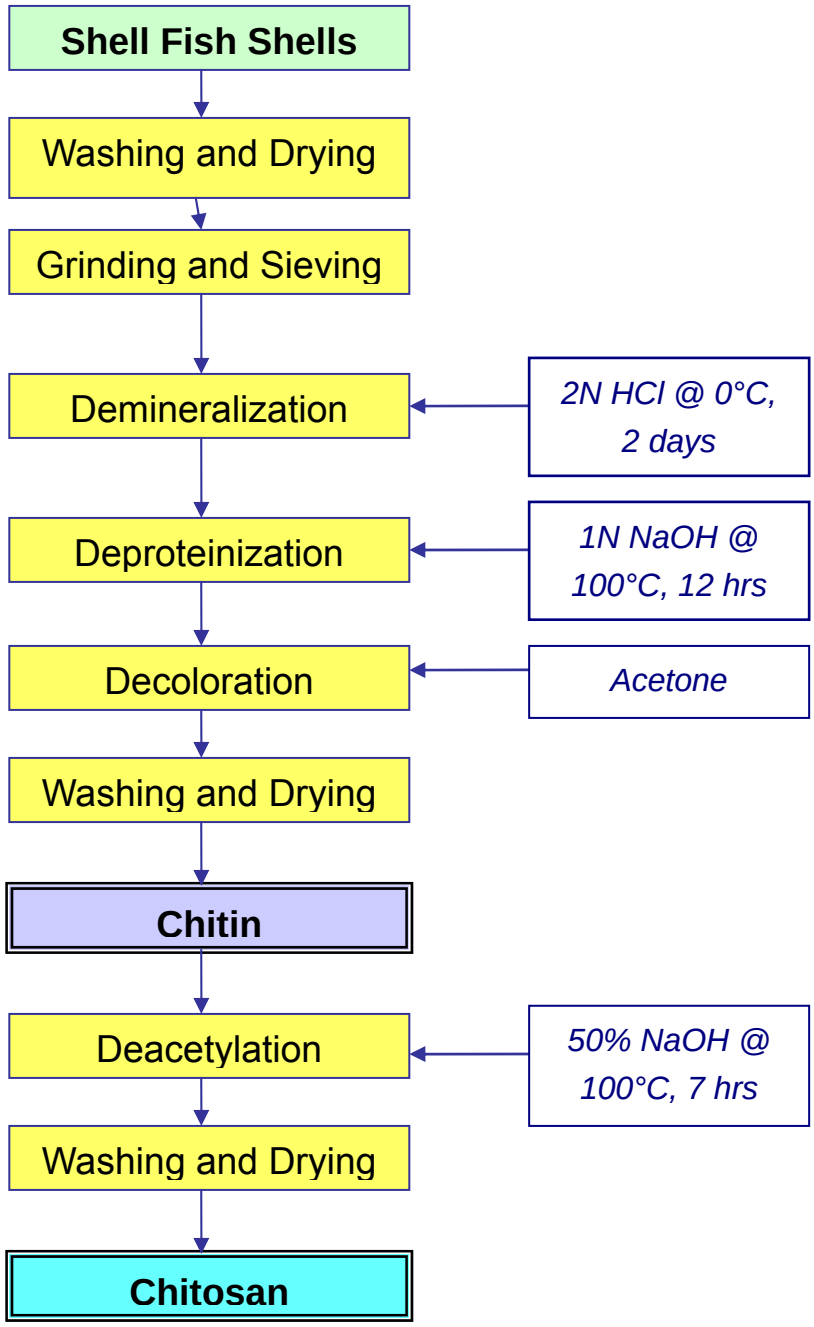

Figure 3: Chitin and chitosan manufacturing process

removed with sodium hydroxide. Other organic materials lipids and pigments are removed by extraction with ethanol or acetone after the demineralization and deproteinization.

1.2.3. Demineralization

Demineralization is the removal of minerals, primarily calcium carbonate. This process involves the decomposition of calcium carbonate into water-soluble calcium salts with release of carbon dioxide. The common reagent is hydrochloric acid (2N HCl at 0°C for two days) that produces water-soluble calcium chloride ($CaCl_2$) (No and Mayers, 1997).

1.2.4. Deproteinization

Deproteinization breaks the covalent bonds between chitin and protein linkages by using NaOH. A 1M aqueous solution of NaOH is the common solution for the deproteinization of chitin. NaOH, however, results in partial deacetylation of chitin and hydrolysis of the biopolymer that lowers the molecular weight of chitin (Brine and others, 1981).

1.2.5. Deacetylation of Chitin into Chitosan

Deacetylation of chitin into chitosan is usually done by treating chitin with 50% NaOH at 100°C for several hours, cooling and washing with water until neutral pH. This process is usually repeated twice. Chitosan is extracted with 2% acetic acid solution, filtered and precipitated in distilled water to give purified

chitosan that is dried and stored (No and Meyers, 2001).

1.3. Application of Chitosan Coating

Chitin and chitosan are white and odorless powders. Chitosan coating can be safely used to extend shelf life and improve the quality of fresh, frozen and fabricated foods due to a non-toxic and biodegradable nature (Kester and Fennema, 1986; Labuza and Breene, 1989). These coatings can provide supplementary and sometimes controlling physiological, morphological and physicochemical changes in food products (Kittur and others, 1998).

The mechanisms of coating to extend shelf-life and functionality of foods or food additives includes controlling moisture transfer between food and surrounding environment, controlling rate of respiration, controlling release of chemical agents such as antimicrobial substances or antioxidants, high impermeability to certain substances like fats and oils, structural reinforcement of foods and increased stability of flavor compounds agents by forming microcapsules (Kester and Fennema, 1986; Labuza and Breene, 1989). Also, El Ghaouth and others (1992) reported that chitosan coating has ability to form semipermeable film. Thus it can help to modify the internal atmosphere as well as decrease the transpiration loss and delay the ripening of fruits.

Several researchers have reported the effect of chitosan coating on storability and quality of fresh fruits. Du and others (1997) reported extending the storage life and better controlling decay of peaches, pears and kiwifruits by chitosan application. Also, extension of the storage life of cucumbers, bell

peppers, strawberries, and tomatoes can occur after application of chitosan coating (El Ghaouth and others, 1991, 1992). The results of their studies indicate that reduction of ethylene and carbon dioxide production occurs with decreased respiration rates, along with inhibition of fungal growth and delayed ripening.

Chitosan coating on fresh fruits can provide modified atmosphere storage and reduce quality changes through control of the internal gas composition of the fruits. The coating offers a protective barrier against bacterial contamination and moisture transfer to extend the shelf life (Bhale and others, 2003). Other researchers reported that chitosan coatings help to reduce transpiration and control weight loss (Drake and others, 1988; Sumnu and others, 1995), to slow down ripening and extend shelf life by controlling respiration rate and ethylene production (Yaman and Bayindirli, 2001, Jiang and Li, 2001), to reduce the symptoms of fruit injury, browning and rotting (Zhang and Quantick, 1997), and to provide the fruit a glossy or matte finish (Bai and others, 2003).

1.4. Environmental Factors Influencing Fruit Ripening

1.4.1. Effect of Atmosphere

Atmospheric composition of O_2, CO_2, and C_2H_4 can affect respiration rate and storage life of fresh fruits. An atmosphere that contains less than 8% O_2 decreases ethylene production, and above 5% CO_2 delays many responses to ethylene by fruit tissues that would increase ripening (Knee 2002). However, a minimum of 1 to 3% O_2 atmosphere is required for many fruits to avoid the change from aerobic to anaerobic respiration. An anaerobic condition causes

the replacement of glycolytic pathway to Krebs cycle, and pyruvic acid is decarboxylated to form acetaldehyde, CO_2, and ethanol, which leads to off-flavors (Kader 1986).

1.4.2. Effect of Humidity

The respiration and transpiration (water loss) are partially depending on the temperature and humidity of the environment surroundings. Relative humidity (RH) controls the fruit transpiration. It influences water loss, decay development, some physiological disorders, and uniform fruit ripening. Transpiration is the process of water movement from fruit cells to surrounding atmosphere, which follows high to low water concentration. Fruits should be stored in high relative humidity environments (85-95% RH) to minimize water loss, weight loss, and shriveling (Woods 1990).

1.4.3. Effect of Temperature

Temperature is the most important factor in maintaining the quality of fresh fruits and minimizing post-harvest losses. Low temperature reduces the ethylene production and the metabolism which slows ripening processes of fruits (Larrigaudiere and others, 1997). Temperature also affects the growth and the spread of pathogens and decay. Certain fungi that can cause the disorder of fruits do not grow at low temperatures (Sommer 1985). Thus providing good temperature environment is important for reducing spoilage, decay and ripening on post-harvest fruits.

1.5. Atmosphere Determination by Gas Chromatography using Headspace Method

Gas Chromatography (GC) has been used for identification of fatty acids, triglycerides, cholesterol, gases, water, alcohols, simple sugars, oligosaccharides, amino acids, vitamins, pesticides, herbicides, antioxidants, flavor compounds, food additives, and more. Gas Chromatography is ideally used to the analysis of thermally stable volatile compounds. The advantage of using GC is the wide variety of detectors that can provide either sensitivity or selectivity of specific compounds during analysis.

Gas Chromatography is the most widely used technique for the separation and analysis of volatile compounds. Currently many scientists use a technique referred to as headspace method, is being used for the separation and identification of chemical compounds in food since it is one of the simplest methods of isolating volatile compounds from foods, but other methods also have been used for food analysis. The headspace method requires the collection of head space vapor above the food samples (sample can be solid or liquid) that is then directly injected into a GC. Many researchers reported using GC head space analysis to measure the respiration rate and ethylene production for fruits (Jiang and Li 2000; Wszwlaki and Mitcham 2000; Tian and others, 1997; Rogiers and Knowles 1998; Bruno and others, 2004).

1.5.1. Gas Chromatography Basics

In gas chromatography, the oven controls the temperature of the column. The injection is made at a lower oven temperature and is then temperature is programmed to increase to an elevated temperature. The compound elution time and resolution are dependent upon temperature, so temperature programmed runs are common. The higher temperature will cause the sample to elute faster and provides lower resolution. Oven temperature program rates can range from as little as 0.1 ºC/min to the maximum temperature heating rate that the GC can provide. The most common rate is 2 to 10 ºC/min.

The GC column usually used is the capillary column. It is hollow fused silica glass (less than 100 ppm impurities) tube ranging in length from 5 to 100 m. The wall is very thin, 25 μm, so that they can be flexible. The column outer walls are coated with a polyamide material to enhance strength and reduce breakage. Column inner diameters are typically 0.1 mm (microbore), 0.2-0.32 mm (normal capillary), or 0.53 mm (megabore). Liquid coating is chemically bonded to the glass walls and internally cross-linked at phase thickness ranging from 0.1 to 5 μm. Many scientists are using capillary column to analyze ethylene and carbon dioxide production for fruits and vegetables (DeEll and others, 2005; Wild and others, 2005; Liu and others, 2004).

There are several detectors available for GC. Each offers certain advantages in either sensitivity or selectivity. The most common detectors are flame ionization (FID), thermal conductivity (TCD), and electron capture (ECD) detectors. TCD is universal detector that is used in food applications. ECD is

used for halogenated compound with nitro or conjugated double bonds. ECD is used commonly in determining the pesticide residues. FID responds to organics on weight basis. Food analysts are most often working with organic compounds. Thus, FID is most common detector for food analysis. It is good sensitivity, wide linear range in response for quantitation.

There are three types of carrier gas that are used in GC. Nitrogen is the most efficient but has an optimum at a low flow velocity, which causes long analysis time. Helium is the next best choice and is the most commonly used carrier. Scientists are used helium as a career gas for ethylene and CO_2 analysis for fruits (DeEll and others, 2005; Wild and others, 2005; Liu and others, 2004). Hydrogen, however, is generally the best choice since it offers high efficiency and small dependency on flow velocity. Hydrogen is not commonly used as a carrier gas due to being flammable. GC should operate at the maximum carrier gas velocity that provides resolution

1.6. Quality Attributes of Fruits

Appearance quality factors of fruits are size, shape, color, and freedom from defects and decay. Defects can begin before harvest as a result of damage by insects, diseases, birds, hail, and/or chemical injuries. Post-harvest defects can be physical, physiological or pathological (Snowdon 1990). Textural quality factors can be firmness, crispness, juiciness and mealiness. Flavor or eating quality depends upon sweetness, sourness or acidity, astringency (phenolic compounds) and aroma. Off-flavors can be result from accumulation of

fermentative metabolites such as acetaldehyde, ethanol, and ethyl acetate. Nutritional quality is related to contents of vitamins, minerals, dietary fiber and phytochemicals (Eskin 1991; Kader 1999; Seymour and others, 1993).

Consumers are looking for good quality fruits that look good shape, firm, texture, and pleasant flavor and high nutritive value. The producers and handlers are concerned first with appearance, textural quality, and long post-harvest life. Maturity at harvest is the most important factor that determines storage-life and final fruit quality. Immature fruits are often occurs shriveling and mechanical damage and loss quality when ripe. Overripe fruits often become soft and mealy with unwanted flavor added after harvest. Any fruit picked either too early or too late in its season is more susceptible to physiological disorder and has shorter storage-life than fruit picked at the proper maturity (Kader 1999).

1.6.1. Factors Influencing Quality

Many pre-harvest and post-harvest factors influence the composition and quality of fruits such as genetic factors (selection of cultivars), pre-harvest climatic conditions and cultural practices, maturity at harvest and harvesting method, post-harvest handling procedures, storages, and processing methods (Goldman and others, 1999; Lee and Kader 2000).

The effects of pre-harvest climatic conditions and cultural practices on post-harvest quality of fruits have been reviewed by a couple of researchers (Crisosto and others, 1997; Goldman and others, 1999; Lee and Kadar 2000). According to these researchers, in general, lower light intensity during growth

leads to lower contents of ascorbic acid and sugars in fruits. Temperature influences the uptake and metabolism of mineral nutrients by plants. Rainfall affects the water supply to the plant and may cause fruit cracking. Soil type, mulching, irrigation and fertilization influence the water and nutrient supply to the plant (Ferguson and others, 1999).

Fruits should be stored in optimal ranges of temperature and relative humidity is the important factor in maintaining the quality and minimizing post-harvest losses. Temperature management is the most effective tool for extending the storage life of fresh fruits. At low temperature, the metabolism of fruit is slowed down and extends the storage life.

Controlling the respiration is important to reduce the ripening speed. Every 10 °C decrease in temperature will reduce respiration activity by factor of two- to three-fold (Mitchell 1992). Another benefit of lowering temperature is reducing ethylene production. The ethylene synthesizing enzymes, 1-aminocyclopropane carboxylic acid (ACC) oxidase and ACC systhase, are sensitive to low temperature and as temperature is lowered, less ethylene will be produced (Larrigauduere and others, 1997). Fruits are also less sensitive to ethylene at low than at ambient temperatures (Zhou and others, 2001). During ripening, sugars increase and volatile constituents, such as flavors and odors develop. However, delays between harvesting and cooling or processing can result in direct losses due to water loss and decay, and indirect losses in flavor and nutritional quality.

After harvest, fruit constantly lose water to the environment, which causes

the weight loss. Many products show visible signs of wilting or shriveling after losing 3 to 5% of their initial weight (Mitchell 1992). The rate of water lose is controlled by the vapor pressure difference between the fruit and the surrounding air, which is affected by temperature and relative humidity. Low temperature will help to reduce the weight loss more than high relative humidity.

Temperature also affects the rate of growth of pathogens and decay. The lower the temperature, the slower the metabolism can be occurred. Certain plant pathogen, fungi, can cause losses do not grow at low temperatures. *Rhizopus stolonifera* ceases growth below 5 °C and germinating spores can be killed at 0 °C (Dennis and Cohen 1976). *Botrytis cinerea* can survive at 0 °C but it develops very slowly (Sommer 1985). Low temperature storage is important for reducing decay on harvested fruits. However, temperature below the optimal range for fruit can cause freezing or chilling injury, and temperatures above it shorten storage life. Also, wide temperature changes can result in water condensing on the stored product and more rapid water loss.

Responses to atmospheric modification vary greatly among plant species, organ type and developmental stage, and duration and temperature of exposure (Beaudry 1999; Kader and others, 1989). Maintaining optimal range of oxygen, carbon dioxide and ethylene concentration around the commodity extends post-harvest life by 50% to 100% relative to air control. Exposure to ethylene induces faster ripening (Saltveit 1999).

1.6.2. Ethylene Effects on Fruit Quality

Ethylene (C_2H_4) is a gaseous unsaturated hydrocarbon compound. It is colorless, has a faint color, and has a slightly sweet taste. Yang (1985) reported that ethylene is the simplest compound and is considering as a phytohormone. It is involved in plant growth such as germination, seedling growth, leaf growth, senescence, fruit ripening, flowering, and stress response (Straeten and Montagu 1991). Ethylene production rates are depend on the fruits but generally it increases with maturity at harvest, physical injuries, disease incidence, increased temperature up to 30 °C, and water stress (Kader 1992).

Ethylene is the most important regulator of fruit ripening for the post-harvest fruits. Ethylene is simple gaseous hydrocarbon that can diffuse into and out of plant tissues, and can affect quality factors of fruit products such as color, texture and flavor. Effects of ethylene can be beneficial or not depending on the fruits and its uses. However, beneficial effects of ethylene on quality of fresh fruits promote red color development, degreening and stimulation of ripening, but the attributes are detrimental if expressed via acceleration of senescence, stimulation of chlorophyll loss and excessive softening (Saltveit 1999).

Ethylene biosynthesis starts with the amino acid called methionine. It uses ATP to produce S-adenosyl methionine (SAM) which converts SAM to cyclic amiono acid, 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase. ACC then converts into ethylene by the reaction of ACC oxidase. The production rates of ethylene are depends on the fruit types. It generally increases with maturity of ripening, physical injuries, disease occurrence, water stress, and

higher temperatures. However, ethylene production rate can be reduced by storing at low temperature (32-40 °F), by O_2 reduction (less than 8%) and above 1% CO_2 levels (Kader 1992).

1.7. Fruit Color

Fruit color changes can involve combinations of chlorophyll breakdown and the synthesis and degradation of carotenoids and phenolic pigments such as anthocyanins (Lancaster and others, 1997). Red color development in apple has been associated with chlorophyll decline unmasking anthocyanins (Marsh and others, 1996). Color changes also can be affected by nitrogen and potassium nutrition. Nitrogen has been directly associated with maintaining green color in fruit (Crisosto and others, 1997). Increased nitrogen fertilization of peaches can results in improved Hunter ‘a-’, ‘b-’ and chroma values for fruit puree (Olienyk and others, 1997).

1.8. Firmness

Firmness is an important indicator of fruit quality. Many researchers have studied methods to maintain fruit firmness during post-harvest storage and shelf-life. Greater firmness (slower rate of softening) in fruits has been associated with high calcium concentrations (Hopkirk and others, 1990; Richardson 1997). Berries are responsive to post-harvest calcium dips, which help maintain firmness in strawberries (Garcia and others, 1996) and blueberries (Hanson and others 1993). The firmness is probably caused by binding of calcium to pectic

polymers in cell wall (Ferguson 1984; Fallahi and others, 1997). Also, nitrogen can be associated with fruit firmness. High nitrogen contents of fruit have been negatively associated with firmness for berries (Prange and DeEll 1997).

1.9. Small Fruits

1.9.1. Blueberries

Berries are considered soft fruits. There are three popular types of blueberries in the market. The high-bush blueberry (*Vaccinium corymbosum*) is the most popular, and then low-bush (*V. angustifolium*) and rabbit-eye (*V. ashei*) species. The species of *V. angustifolium* are grown in the northern parts of United States, and *V. ashei* species are grown widely in southeastern United States (Ballington and others, 1982). Cappellini and others (1972) reported that blueberries picked early in the harvest season are commonly infected by *Alternaria tenuis*, and harvested late are commonly infected by *Botrytis cinerea* and *Glomerella cingulata*. Blueberry should be harvested plump, firm, and uniformly colored (light blue to dark blue), free from injury and decay. However, green or red color indicates unripeness; overripe berries are dull and usually soft skin.

Kader (1985) studied recommended storage condition for various types of fruits. He reported the range of optimal storage condition for blueberries were 10-20% CO_2, 0.1 to 1.0 µl/kg hr ethylene in 32-40 °F.

1.9.2. Grapes

Grapes were one of the earliest fruits grown by humans. It is the most widely cultivated fruit crop in the world. Among the total production, 68% of grapes are used for wine, 20% for table grapes (fresh fruits), 11% for raisins, and 1% for fresh juice (Olmo 1993). The grape vine belongs to the genus *Vitis* of the family Vitaceae. The genus *Vitis* includes two subgenera: *Euvitis* or true grapes and *Muscadinia* (Winkler and others, 1974). The grape consists of an epicarp (skin), a juicy and mesocarp, and an endocarp, the tissue surrounding the seeds. During the ripening periods, grapes begin sugar accumulation, loss of acids, softening, skin color changing, and cell expansion (Salunkhe 1984). Grapes are nonclimacteric fruit which will not develop color or taste after harvest. During the early stage of growth, the respiration of grapes is very active, but the respiration slows down as they grow. Kader (1985) recommended storage condition for grapes of 1-5% CO_2, 0.1 µl/kg hr ethylene in 32-40 °F.

Grapes are harvested based on the texture of the pulp, peel, easy separation of the berries from bunches, and characteristic aroma. High quality of grapes should have well-developed clusters, be well filled, fresh appearance, firm, plump, and have the typical shape and uniform color for the cultivar. Most common fungus to infect the grape is *Botrytis cinerea,* which causes gray rot (Cappellini and others 1986).

2. Objectives

The objective of this study was to demonstrate the effectiveness of natural biodegradable chitosan coating in extending shelf-life and quality of fresh small fruits, such as blueberries and grapes. This study determined (1) ethylene and CO_2 production, (2) weight loss, (3) color changes, and (4) firmness of small fruits to evaluate quality changes in blueberries and grapes.

There were four dipping treatments performed in this study:
1% chitosan in 1% aqueous acetic acid, 1% water-soluble chitosan in water, 1% acetic acid, and tap water, and non-treated (no dipping) berries served as control.

3. Materials and Methods

3.1. Materials

Blueberries were harvested at the optimum stage of maturity at the University of Tennessee, Agriculture Experimental Station (Crossville, TN). After harvesting, the blueberries were transported to the Food Science and Technology pilot plant and placed in a cooler (4 °C) for over night to remove the field heat. Grapes were obtained within 48 hrs of commercial harvest and stored at 4 °C until use within 24 hrs.

Acetic acid was obtained from Fisher Scientific (Pittsburgh, PA). All other materials were of analytical grade and obtained from Fisher Scientific (Pittsburgh, PA).

3.1.1. Chitosan

Two different chitosan coatings were purchased: medium molecular weight chitosan (average molecular weight 400 kDa and about 200 mPa.s in 1% acetic acid at 20 °C) was obtained from Fluka Chemical Co. (Luausanne, Switzerland). Medium-low molecular weight water-soluble chitosan (average molecular weight 120 kDa (Kim, 2004)) was obtained from EZ Life Science Co., Ltd. (Seoul, South Korea).

3.2. Sample Preparations

3.2.1. Preparation of Chitosan Solution

The following procedure of making chitosan solutions containing 1% chitosan (wt/v) in 1% (v/v) acetic acid was: addition of 1 g of chitosan powder into 90 ml of tap water while agitating on a stir plate and heating until temperature reached 100 °C. The dispersion was cooled to room temperature (25 °C) while stirring and addition of 10 ml of 10% acetic acid occurred to produce a 1% acetic acid in the final solution. The solution was stirred overnight at room temperature (25 °C) to ensure complete solubilization of the chitosan molecules.

3.2.2. Preparation of Water Soluble Chitosan Solution

The procedure for producing chitosan solutions containing 1% water-soluble chitosan (wt/v) required addition of 1 g of water-soluble chitosan powder into 100 ml of tap water while stirring. The solution was stirred overnight at room temperature (25 °C) to ensure complete solubilization of the chitosan molecules.

3.2.3. Preparation of Other Solutions

The procedure for producing 1% acetic acid (v/v) required addition of 10ml of 10% acetic acid in 90 ml of tap water while stirring for 10 min.

3.2.4. Preparation of Fruits

Each type of fruit (blueberry and grape) was divided into five groups for five different treatments (1% chitosan in 1% aqueous acetic acid, 1% water-

soluble chitosan in water, 1% acetic acid solution and tap water. Non-treated fruits served as a control treatment). Each fruit samples were carefully weighed (100 ± 1 g) after sorting fruit to be similar sized and free from injury on the surface of the fruit skin. Each group had a total of three replications. After treatments, each sample was placed in a sandwich bag (16.5cm x 14.9cm x 29.2μm) that had 16 6-mm holes punched into the bag to imitate commercial storage conditions. The sandwich bag was manufactured by Kroger, inc. and made out of polyethylene. The bags were then stored in single layers on a rack at 4 ± 1 °C with a relative humidity of 85 ± 5%. Every three days, each sample of each treatment from five different groups was tested for ethylene production, CO_2 production, skin color, firmness, and weight loss.

3.3. Dipping

This method was utilized for the four solutions (chitosan in acetic acid, water soluble chitosan, acetic acid, and tap water) to apply a uniform amount of coating material onto the surface of the fruit. All fruit samples (100g) were dipped for 30 seconds into 100 ml of solution and excess coating material was allowed to drain as the fruit was placed on a screen.

3.4. Drying, Packaging, and Storage

After the coating treatments were applied, each sample was air dried for 3 to 4 hrs using a medium setting of air-speed for a fan. Surface-dried samples were placed in prepared sandwich bags and stored at (4 ± 1 °C and 85 ± 5%

relative humidity) for up to 21 days.

3.5. Fruit Quality Analysis

3.5.1. GC Measurement for Ethylene and Carbon Dioxide

Weighed fruits (100 ± 1 g) from each replicate (three replicates per treatment) were sealed in 500 ml glass jars for 5 hrs at 25 °C, then 5 ml-samples were withdrawn from the headspace. Each sample was analyzed immediately for ethylene and CO_2 concentrations using an Agilent 6850 gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a flame ionization detector (for ethylene detection) or thermal conductivity detector (for CO_2 detection). The column was PLOT (porous layer open tubular) capillary column (50 m x 0.53 mm x 15.0 μm for ethylene detection and 30 m x 0.53 mm x 40 μm for CO_2 detection) and column flow was 10 ml/ min for both GC. The oven temperature was 150 ºC with inlet temperature of 60ºC and detector temperature of 265 ºC. Carrier gas was hydrogen with pressure of 50 psi. The measurements of ethylene and CO_2 production were performed every 3 days.

3.5.2. Measurement of Weight Loss

The samples from each of the test groups (100 ± 1 g) were weighed before placing into the glass jar to analyze the percentage weight loss from initial weight for each treated samples.

3.5.3. Measurement of Firmness of Fruits

Blueberries or grapes from each of the treatment groups were randomly selected to determine firmness as calculated as the maximum force to penetrate the surface of the fruit. Three replications were performed. Fruit firmness was measured by using TA.xt *plus* Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) equipped with 2 mm-diameter needle probes. The program was set at compression speed of 2 mm/s and the trigger force set at 5.0 g. The needles were pushed into the fruits flesh through the skin to the depth of the needles (15.0 mm). Eight blueberries and four grapes were used to evaluate the firmness of fruits per each treatment. Three replications were performed for each treatment samples in every 3 days.

3.5.4. Measurement of Color of Fruit Skin

Fruit skin colors were measured every three days by Hunter Miniscan XE plus colorimeter (Hunter Lab inc., Reston, VA). Three replications were performed. The ‘L-’, ‘a-’, and ‘b-’ values were reported as indexes of color changes from lightness to darkness, green to red, and blue to yellow. The colorimeter was calibrated with white and black tiles before the sample measurements. All samples were placed in a Petri plate. Approximately 10-15 blueberry samples were stacked in 2 to 3 rows and the surface color measured and 7 to 10 grape samples were stacked in 2 rows and the surface color measured. Three replicates were performed and the color was measured in a dark room to eliminate other light sources. Color for all treatment samples was

determined every 3 days.

3.6. Statistical Analysis

Analysis of Variance was performed using SAS software (SAS Institute, 1990). All experiments used completely randomized designs (CRD), and analyses included type of fruit, days of storage and replication as independent variables. Ethylene production, CO_2 content, color (L, a, and b), texture and moisture loss were dependent variables. The method of analysis of variance (ANOVA) was performed to the data obtained from each treatment to detect significance of differences at 5% level of significance ($p< 0.05$) and differences in mean values were determined using Tukey's procedures of statistical analysis system.

4. Results and Discussion

There were four dip treatments (1% water-soluble chitosan, 1% chitosan in 1% aqueous acetic acid, 1% aqueous acetic acid, and water) were performed in this study on blueberries and grapes. These treatments were compared to a control sample (no dip treatment). The treatments on the fruit using tap water and 1% acetic acid were not significantly different than no dip control ($P>0.05$) for ethylene production, respiration rate, firmness, and color tests. Therefore only chitosan treated and non-treated (control) fruit samples were included on the graphs below, however all data can be viewed in Appendices.

4.1. Gas Chromatography Analysis of Ethylene and Carbon Dioxide

4.1.1. Ethylene Production

The dip treatments with chitosan materials reduced ethylene production and respiration rates for the blueberries and grapes. The 100 g of blueberries produced much more ethylene than grape samples during the 5 hr collection period. These results are similar to Kader findings (1992). He reported the range of ethylene production for blueberries was 0.1 to 1.0 ppm and grapes were less than 0.1 ppm.

Figure 4 shows that blueberries with no treatment, the control fruit, had the highest ethylene production during the 21 d storage. The non-treated blueberries after the third day of storage produced approximately 0.11ppm per 5 mL headspace during the 5 hr period and gradually increased ethylene production during the next 18 d storage. The increase in ethylene production of

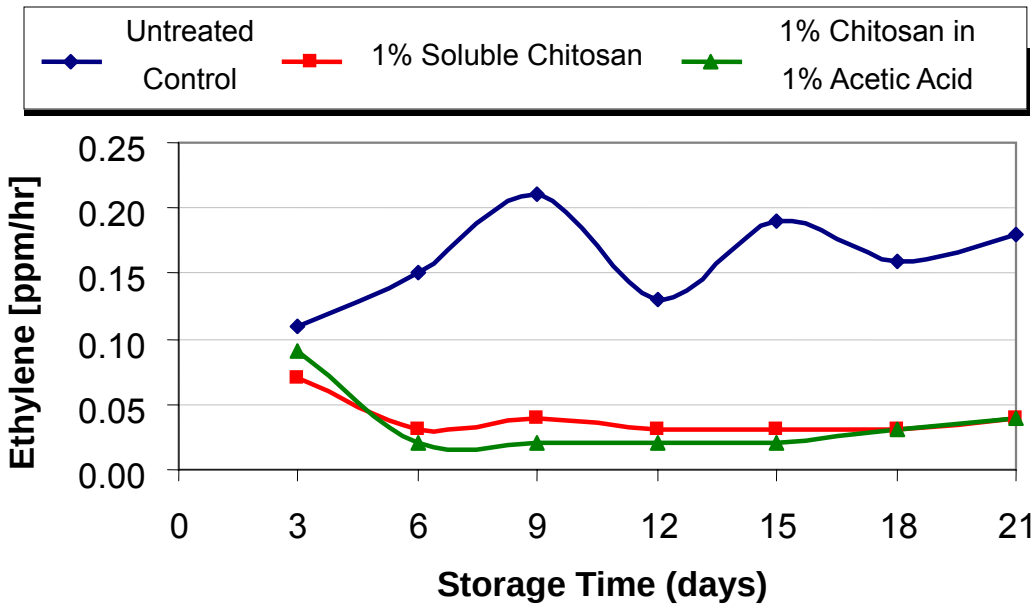

Figure 4: Measurement of ethylene production from blueberries for control, 1% water-soluble chitosan, 1% chitosan in 1% aqueous acetic acid treatments during storage at 2 °C. Each data point represents the average ethylene concentration from three jars of 100 ± 1 g blueberries ($p<0.05$).

fruit could be related to ripening (Jiang and Li, 2001). Ethylene production for both 1% chitosan in 1% acetic acid and 1% water-soluble chitosan treated blueberry samples were similar as shown in Figure 4. Blueberries from both treatments started near 0.10 ppm and ethylene production into the headspace decreased during the next 18 d storage. The results showed either chitosan treatment significantly reduced the ethylene production for blueberries.

The ethylene production by grapes was less than half the production by blueberries as shown in Figure 5. The ethylene production as measured in the headspace decreased for all treatments but was significantly less for the chitosan treated blueberries compared to the control samples during the 21 days storage periods.

Researchers have reported that chitosan coatings can delay the ripening of tomatoes, cucumbers and bell peppers (El Ghaouth and others, 1992), and apples (Hu and Zou 1998) by slowing down the production of ethylene. In this study it was also shown that fruit coated with chitosan had significantly ($p<0.05$) lower rates of ethylene production than control fruit during the whole period of storage (Figure 4 and 5).

Ethylene is a plant hormone and is an important part of the mechanisms controlling plant growth and development. For ethylene biosynthesis, the amino acid methionine is considered to be the starting point of ethylene production. As shown in Appendix D, methionine is converted to SAM (S-adenosylmethionine), and SAM converts to ACC (1-aminocyclopropane-1-carboxylic acid). ACC is the main control of ethylene production (Kader, 1992). Chitosan coating are consider

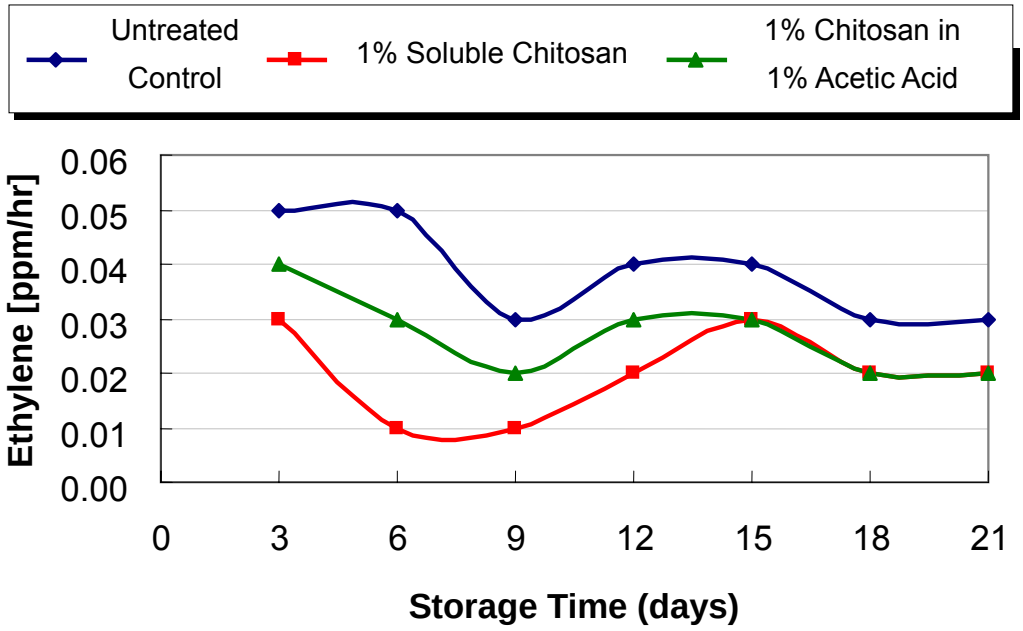

Figure 5: Measurement of ethylene production from grapes for control, 1% water-soluble chitosan, 1% chitosan in 1% aqueous acetic acid treatments during storage at 2 °C. Each data point represents the average ethylene concentration from three jars of 100 ± 1 g grapes ($p<0.05$).

to be a good barrier on the surface of fresh fruits. Probably chitosan coating can trap the ethylene production inside of the fruits by reducing the ethylene synthesizing enzyme, ACC oxidase and ACC synthase. According to Abeles and others (1992) report, ethylene biosynthesis is dependent on the presence of O_2. In this case, chitosan coating is helped to reduce the oxygen entering into the fruits. Low O_2 entering into fruit causes the less ethylene production by slowing down the fruit metabolism. However, Larrigaudiere and others (1997) reported that ethylene is sensitive to low temperature and ethylene production is lowered at low temperature due to slowing the ethylene synthesizing enzymes reactions. The benefit of producing ethylene on quality of fresh fruits could be the promotion of red color development, degreening and stimulation of ripening. But the disadvantage of ethylene production could be the acceleration of senescence, stimulation of chlorophyll loss and excessive softening (Saltveit, 1999).

4.1.2. Carbon Dioxide Production

The CO_2 production which can be used as an indication of respiration rate was significantly decreased for both blueberries and grapes when fruits were treated with either 1% chitosan in 1% acetic acid or 1% water-soluble chitosan ($p<0.05$). For blueberry samples, the control had the highest CO_2 production as measured as percent CO_2 in the headspace during the 21 d storage as shown in Figure 6.

The CO_2 production rate in the headspace of grape samples was much lower than blueberry samples. For grapes in Figure 7 show that the control had a

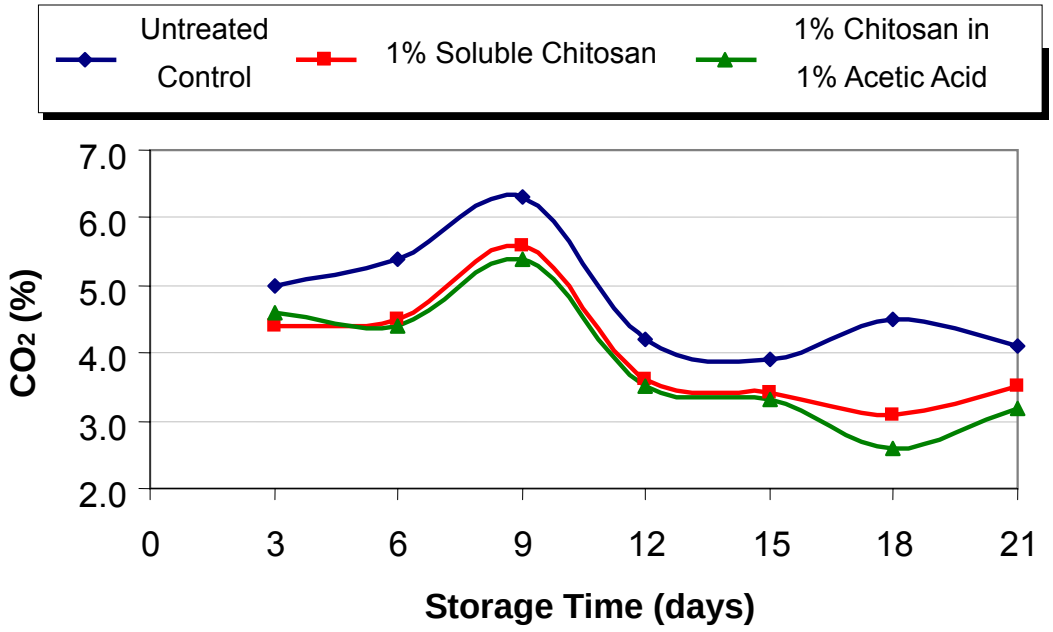

Figure 6: Measurement of respiration rate from blueberries for control, 1% water-soluble chitosan, 1% chitosan in 1% aqueous acetic acid treatments during storage at 2 °C. Each data point represents the average carbon dioxide concentration from three jars of 100 ± 1 g blueberries ($p<0.05$).

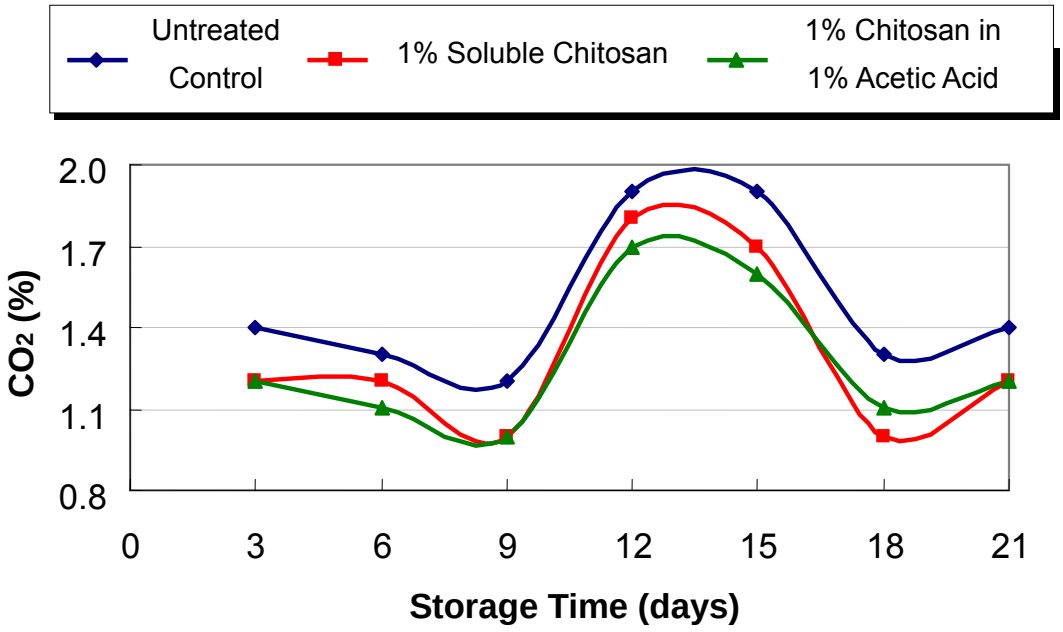

Figure 7: Measurement of respiration rate from grapes for control, 1% water-soluble chitosan, 1% chitosan in 1% aqueous acetic acid treatments during storage at 2 °C. Each data point represents the average carbon dioxide concentration from three jars of 100 ± 1 g grapes ($p<0.05$).

higher CO_2 level than both chitosan treated samples. Chitosan coating fruits have been shown to delay ripening by modifying CO_2 and O_2 level and reducing the respiration rate (El Ghaouth and others, 1991). This implies that chitosan coating may form a protective barrier on the surface of the fruit and reduce the availability of oxygen, which reduces respiration rate, and delays ripening (Du and others, 1997; El Ghaouth and others, 1991; Jiang and Li, 2001). However, control (non-coated) blueberry samples on day 18 showed slight increase in CO_2 production. This reaction possibly indicated increased CO_2 due to fungal infection on the fruits. Both chitosan treated samples maintained a low CO_2 production, which indicated chitosan has ability to inhibit the fungal growth. The fungal inhibition result agreed Sams and others (2004) study.

As shown in Appendix C, respiration is the process of stored organic materials, such as carbohydrates, proteins, and fats, are broken down into simple end products with release of energy. Oxygen (O_2) is used in this process and carbon dioxide (CO_2) is produced. The primary gas exchange of O_2 and CO_2 was through the openings on the surface of fruits. The cuticle is also permeable to O_2 and CO_2 and may allow transmission of these gases (Cameron and Yang 1982). Chitosan coating provides protective surface barrier on the surface of fruit which can be reduced internal O_2 levels and produce low CO_2 level. Thus, low O_2 has important effects on metabolism on respiration and also ethylene production which can have significant effect on quality and extend the shelf life of the fruits.

4.2. Effect of Firmness of Fruit

Firmness is a good indicator of desirable fruit quality in fresh fruit. Maintaining the flesh firmness indicates that a slow rate of softening is occurring during storage. In this study, there were no significant differences in firmness between the control and both chitosan treated samples for blueberries and grapes ($p>0.05$). As shown in Figure 8, the highest firmness of blueberry samples was the control. It gradually increased 1.5 kg to 1.9 kg force to penetrate the fruit skin. The least firm sample was 1% water-soluble chitosan treated blueberry sample. It gradually increased 1.1 to 1.8 kg force. The 1% chitosan in 1% acetic acid treated blueberry sample was not consistently firm. The firmness started at 1.6kg and suddenly increased to 2.0kg and decreased to 1.9 kg on day 21. Inconsistency firmness also occurred for water and 1% acetic acid treated samples during 21 d periods.

For grapes as shown in Figure 9, the highest firmness sample was 1% chitosan in 1% acetic acid treated sample (1.9 kg) and lowest was 1% water-soluble chitosan treated sample (1.4 kg). On day 12, the firmest sample was the 1% water-soluble chitosan treated sample (2.1 kg) and lowest was the control (1.5 kg). At day 21, all samples were near 1.8kg firmness. This result showed there was no significant difference on treated and non-treated samples ($p>0.05$). Similar results are also reported by Garcia and others (1996) and Zhang and Quantick (1998) that indicate coating did not significantly control the loss of firmness.

As shown in Table A3 and B3 at the Appendixes, the firmness of water

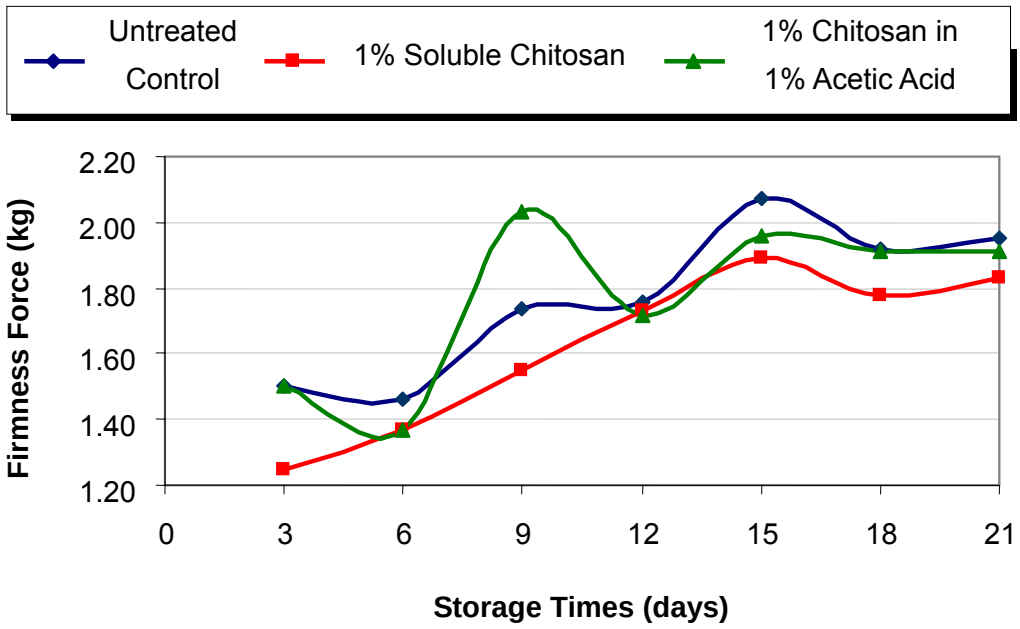

Figure 8: Measurement of firmness of fruit from blueberries for control, 1% water-soluble chitosan, 1% chitosan in 1% aqueous acetic acid treatments during storage at 2 °C. Each data point represents the average firmness force for eight blueberries per each test ($p>0.05$).

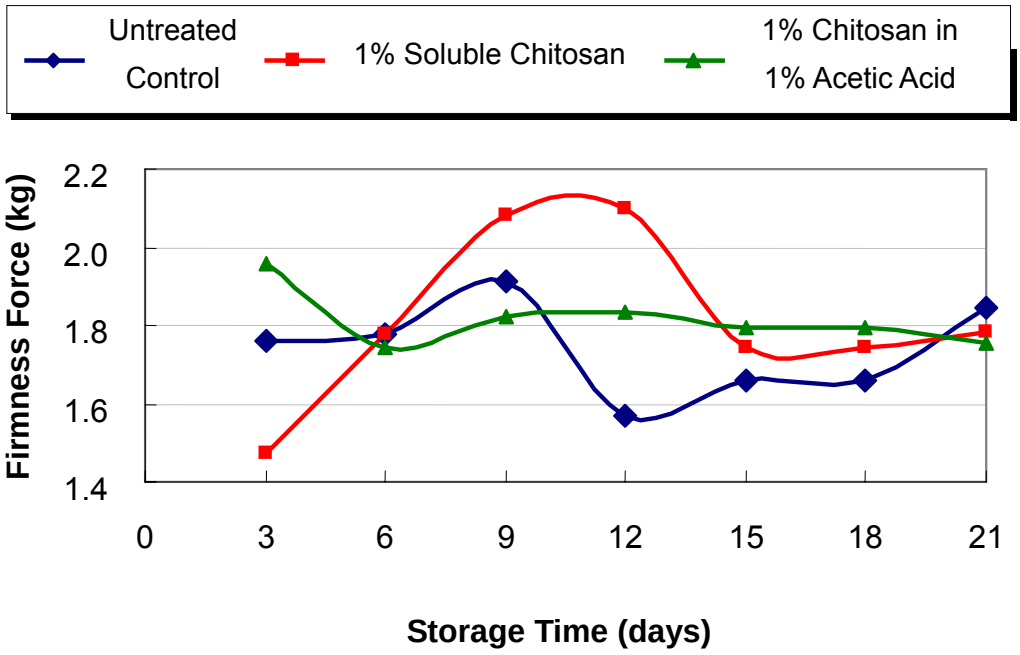

Figure 9: Measurement of firmness of fruit from grapes for control, 1% water-soluble chitosan, 1% chitosan in 1% aqueous acetic acid treatments during storage at 2 °C. Each data point represents the average firmness force for four grapes per each test ($p>0.05$).

and 1% acetic acid treated samples were slightly softer than the control or chitosan treated samples for both grapes and blueberries during 21 d periods. Therefore the results showed that use of either chitosan treatment will help to maintain similar texture and mouth-feel as non-treated (control) samples after 21 d storage life.

4.3. Effect of Color of Fruit Skin

Color change of fruits were measured in Hunter 'L-', 'a-', and 'b-' values, as indexes of color changes from lightness to darkness, green to red, and blue to yellow. There was no significant differences of Hunter color value for L- and a- between control (see Appendix B) and both chitosan treated samples for blueberry and grape samples ($p>0.05$), but color of b-value was significantly different than the control ($p<0.05$) and both chitosan treated samples. The control appeared slightly less blue color than others for blueberry and grape samples. For the reference for sample color appearances, blueberry samples are shown in Appendix E and Appendix F shows for the grape samples.

Figure 10 shows the Hunter color b-value for blueberry. Control appeared slightly more blue color than other samples. As b-value decreased, more blue color appeared. On day 3, control started at -1.8 and gradually increases to -1.1 then decreased to -1.5 on day 21. Both chitosan treated samples began with -1.0 b-values. Samples treated with 1% chitosan in 1% acetic acid increased to -0.4 until day 9 and decreased to -1.0 until day 21.

For grape (shown in Figure 11), the control also showed lower b-value

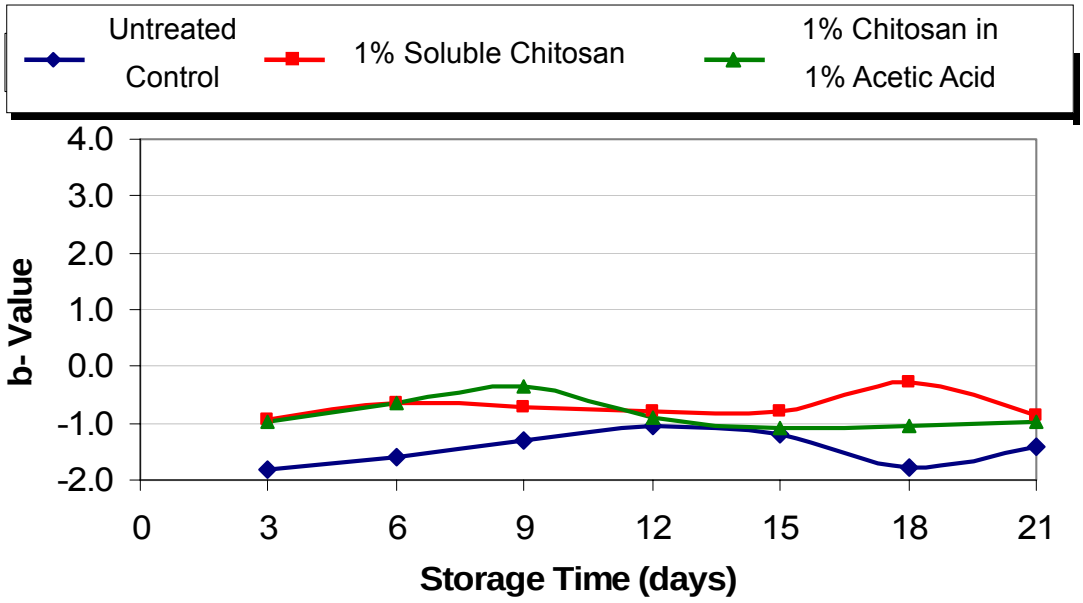

Figure 10: Measurement of fruit skin color from blueberries for control, 1% water-soluble chitosan, 1% chitosan in 1% aqueous acetic acid treatments during storage at 2 °C. Each data point represents the average b-value color measurements for three blueberries ($p<0.05$).

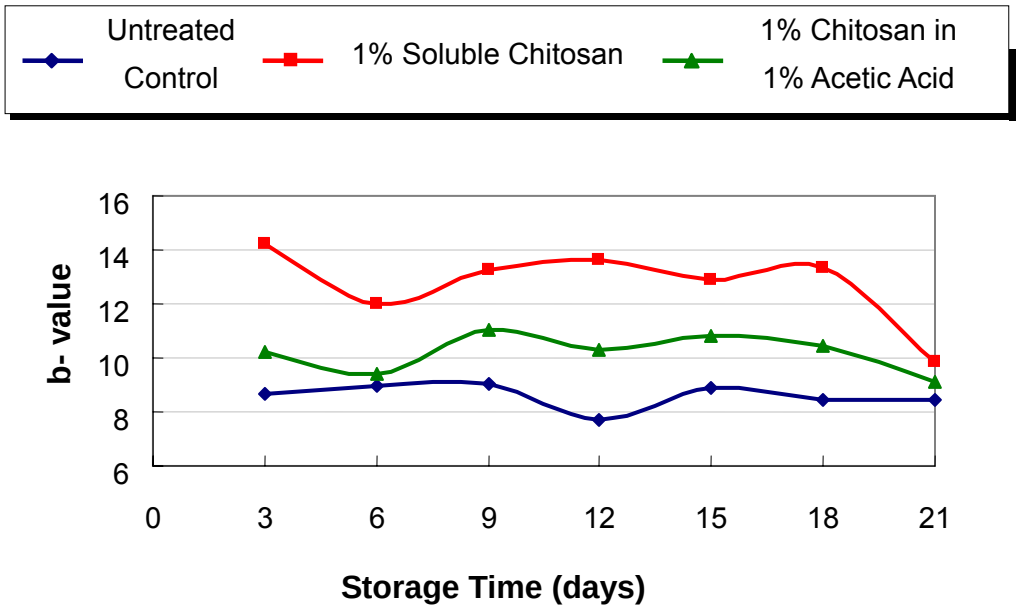

Figure 11: Measurement of fruit skin color from grapes for control, 1% water-soluble chitosan, 1% chitosan in 1% aqueous acetic acid treatments during storage at 2 °C. Each data point represents the average b-value color measurements for three grapes ($p<0.05$).

than chitosan treated samples. It was significantly different than the control ($p<0.05$) and chitosan treated samples. The color of b-value of control was 9 on day 3, stayed consistent, and ended with a b-value of 8 on day 21. The highest b-value level was 1% water-soluble chitosan. It started at b-value of 14 on day 3 and very slowly decreased to b-value of 13 on day 18 then dropped to 10 on day 21. Grape with 1% chitosan in 1% acetic acid started b-value of 10 on day 3 and stayed a similar level until day 21.

Fruit color change can be involved combinations of chlorophyll breakdown and the synthesis and degradation of carotenoids and phenolic pigments such as anthocyanins (Lancaster and others, 1997). Saltveit (1997) reported low O_2 reduces rate of degreening due to chlorophyll loss and prevents softening. Hunter b-value was significantly different between control and chitosan treated samples was probably due to low O_2 entering into fruit. It also can be the coating itself interferes the color sensor of the Hunter colorimeter due to slightly a glossy or matte fruit skin, which causes slightly reflect during the measurements. However, further researches are needed to understand the mechanisms of color appearances.

4.4. Effect of Weight Loss

Once harvested, fruit constantly lose water to the environment. Since this water cannot be replaced by the tree or plant, weight loss occurs. The rate of water loss is controlled by the vapor pressure difference between the fruit and the surrounding air, which is affected by temperature and relative humidity. In this

study (shown in Figure 12 and 13), there were no significant differences among all four treatments and control for blueberry and grape samples ($p>0.05$). El Ghaouth and others (1991) used edible chitosan coating to reduce water loss from cucumbers and bell peppers. They reported chitosan coating had significant effect to reducing the weight loss, but our results showed no significant effect for blueberries and grapes. All four treated blueberry samples and control were started with 0.2% weight loss on day 3 and gradually increased to near 5.5% until day 21, except 1% acetic acid treated samples (4.5%). It showed a lower weight loss percentage than other samples at day 21.

Weight loss measurements for grape samples also occurred with a similar pattern to the blueberry samples. All treated samples and control started near 0.5% weight loss on day 3 and gradually increased weight loss to 4% to 4.4% on day 21. Control and water, however, treated samples had the lowest weight loss (4%) compared to other treated samples (4.4%). Both blueberry and grape samples for all treatments increased in weight loss as storage occurs. Most weight loss was probably caused by water evaporation but also carbohydrates in fruits could be possibly involved in weight loss. The equation of respiration reaction in plants (shown in Appendix C) describes when carbohydrates and oxygen are present the plant metabolism starts and produce CO_2 and water. This indicates when CO_2 production is reduced there is less sugar loss so the increase of weight loss of fruit samples is most likely due to just moisture loss.

Water loss is the cause of deterioration because it results not only in weight reduction but also in losses in appearance by wilting and shriveling,

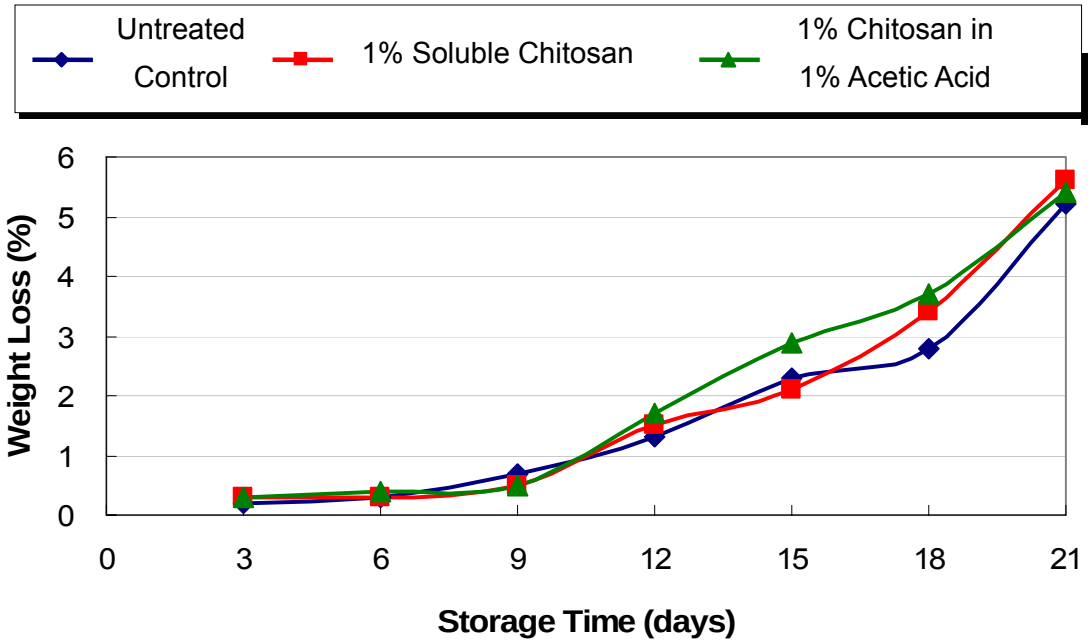

Figure 12: Measurement of weight loss from blueberries for control, 1% water-soluble chitosan, 1% chitosan in 1% aqueous acetic acid treatments during storage at 2 °C and 85% RH. Each data point represents the average weight loss measurements for three blueberries ($p>0.10$).

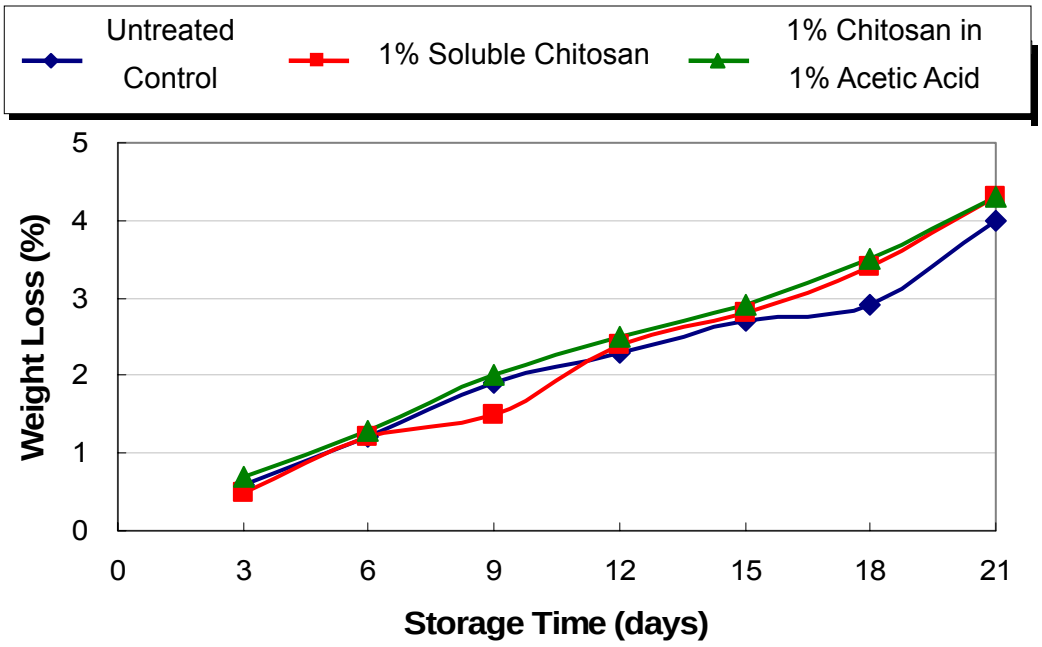

Figure 13: Measurement of weight loss from grapes for control, 1% water-soluble chitosan, 1% chitosan in 1% aqueous acetic acid treatments during storage at 2 °C and 85% RH . Each data point represents the average weight loss measurements for three grapes ($p>0.10$).

textural quality by softening, limpness, and loss of crispness, juiciness, and nutritional quality. The chitosan coating is formed on surface of the fruit delayed migration of moisture from the fruit into the environment to reduce the weight loss during storage. Chitosan coating provides outer protective covering which can be controlled the evaporation of water from the fruit tissues and reducing the juice leaking. El Ghaouth and others (1992) used edible chitosan coatings to reduce water loss from cucumber and bell pepper. Kader (1992) suggested that in order to prevent the weight loss as much as possible, fruits shall be stored at low temperature and relative humidity should be kept between 90 to 95%.

In this study, the weight loss of both blueberry and grape samples were a similar pattern during 21 d storage periods. According to El Ghaouth and others (1992), chitosan coating helps to reduce water loss, but our study indicated it was not any more effective to use to prevent the weight loss for blueberries and grapes then use of non-coating (control). This result was different probably due to lower relative humidity in storage in this study compare to El Ghaouth and others (1991) studies. There is no significant difference between control and all other treated samples. However, further study is needed, especially longer storage periods in high relative humidity level.

5. Conclusions

Both chitosan coatings decreased ethylene and CO_2 production in blueberries and grapes. The coatings also provided similar firmness to the firmness of non-coated (control) berries, which indicates the treatments helped maintain the flesh firmness in the fruit during the storage. Chitosan coatings are not significantly preventing the weight loss of berries but it appeared to be similar weight loss occurred as non-coated berries during the storage. There is no significant difference for the appearance of color changes on all berries; however, it has slightly more blue than non-coated berries.

The chitosan-based coatings are proved to decreased respiration rates, and delaying of ripening to the reduction of ethylene and carbon dioxide production. The reduction of respiration rate and ethylene production as a result of chitosan coating has also been reported for apples (Hu and Zou, 1998), tomatoes, cucumbers and bell peppers (El Ghaouth and others 1991). The results suggest that chitosan, as a semi-permeable coating, can maintain the qualities of the treated fruit and prolong its storage life. It could be considered that chitosan coating slows down the aging process of small fruits by decreasing the respiration rate and ethylene production. It also helps to maintain the flesh firmness and appearance as days go by. Thus, chitosan coatings have a potential to be used on small fruits, especially for blueberries and grapes, to maintain quality, improving storability, and extend shelf-life.

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APPENDICES

Appendix A. Data of Blueberries

Table A1. Measurement of Ethylene Production [ppm]

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	0.11±.10	0.15±.05	0.21±.10	0.13±.05	0.19±.01	0.16±.10	0.18±.05
Water	0.09±.05	0.05±.01	0.05±.05	0.04±.01	0.07±.01	0.07±.01	0.06±.01
1% Soluble Chitosan	0.07±.05	0.03±.01	0.04±.01	0.03±.01	0.03±.01	0.03±.01	0.04±.01
1% Acetic Acid	0.08±.05	0.03±.01	0.03±.01	0.03±.01	0.04±.01	0.04±.01	0.05±.01
1% Chitosan in 1% Acetic Acid	0.09±.05	0.02±.01	0.02±.01	0.02±.01	0.02±.01	0.03±.01	0.04±.01

Table A2. Measurement of Carbon Dioxide (%)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	5.0±.5	5.4±.1	6.3±.2	4.2±.1	3.9±.1	4.5±.2	4.0±.1
Water	5.1±.1	5.2±.1	6.6±.05	4.1±.1	3.7±.2	3.3±.1	3.9±.1
1% Soluble Chitosan	4.4±.1	4.5±.2	5.6±.1	3.6±.1	3.4±.1	3.1±.1	3.7±.1
1% Acetic Acid	4.7±.1	4.7±.2	6.0±.1	3.7±.1	3.4±.1	2.5±.2	3.7±.1
1% Chitosan in 1% Acetic Acid	4.6±.1	4.4±.1	5.4±.05	3.5±.1	3.3±.2	2.6±.2	3.6±.1

Table A3. Measurement of Firmness of Fruit Skin (kg)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	1.50±.2	1.46±.1	1.74±.2	1.76±.2	2.07±.2	1.92±.3	1.95±.3
Water	1.27±.1	1.29±.1	1.58±.1	1.69±.2	1.39±.2	1.62±.2	1.58±.3
1% Soluble Chitosan	1.25±.1	1.37±.1	1.55±.1	1.73±.2	1.89±.2	1.78±.2	1.83±.3
1% Acetic Acid	1.14±.1	1.36±.2	1.22±.2	1.61±.2	1.51±.2	1.56±.2	1.73±.3
1% Chitosan in 1% Acetic Acid	1.50±.1	1.37±.2	2.03±.1	1.72±.1	1.96±.2	1.91±.3	1.91±.3

Table A4. Measurement of Color of Fruit Skin (L-value)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	13.85±.2	15.31±.1	14.45±.3	15.17±.2	15.59±.2	15.74±.3	17.03±.2
Water	12.51±.1	14.48±.2	14.48±.3	15.13±.2	14.57±.2	15.39±.2	15.48±.2
1% Soluble Chitosan	14.31±.2	14.11±.1	14.17±.2	15.55±.1	13.76±.1	15.14±.3	15.63±.1
1% Acetic Acid	15.05±.3	14.56±.1	14.73±.2	14.40±.1	15.61±.1	14.38±.4	14.62±.2
1% Chitosan in 1% Acetic Acid	14.18±.1	13.85±.1	14.87±.2	15.50±.2	15.36±.1	16.05±.3	14.87±.2

Table A5. Measurement of Color of Fruit Skin (a-value)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	0.43±.1	0.18±.1	0.34±.2	0.33±.2	0.50±.2	0.25±.1	0.27±.1
Water	0.93±.2	0.30±.3	0.44±.1	0.41±.1	0.31±.1	0.52±.2	0.49±.1
1% Soluble Chitosan	0.81±.1	0.16±.1	0.50±.2	0.77±.1	0.89±.2	0.49±.2	0.38±.1
1% Acetic Acid	0.48±.2	0.57±.1	0.79±.2	0.17±.1	0.50±.3	0.34±.1	0.85±.2
1% Chitosan in 1% Acetic Acid	0.59±.2	0.28±.1	0.41±.2	0.10±.1	0.59±.2	0.72±.2	0.35±.2

Table A6. Measurement of Color of Fruit Skin (b-value)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	-1.81±.1	-1.58±.1	-1.29±.2	-0.61±.2	-1.20±.2	-1.79±.1	-1.40±.2
Water	-1.22±.1	-1.10±.1	-1.15±.1	-1.37±.2	-1.53±.2	-1.00±.2	-1.12±.1
1% Soluble Chitosan	-0.95±.1	-0.63±.1	-0.73±.2	-0.79±.1	-0.81±.2	-0.29±.2	-0.87±.2
1% Acetic Acid	-1.55±.2	-0.22±.2	-0.50±.3	-0.88±.2	-1.75±.2	-0.78±.2	-0.78±.2
1% Chitosan in 1% Acetic Acid	-0.98±.1	-0.64±.2	-0.36±.2	-0.91±.2	-1.27±.2	-1.04±.2	-0.99±.1

Table A7. Measurement of Weight Loss (%)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	0.2±.1	0.3±.1	0.7±.1	1.3±.1	2.3±.1	2.8±.2	5.2±.2
Water	0.3±.1	0.3±.1	0.6±.1	1.5±.1	2.5±.1	3.0±.2	5.8±.2
1% Soluble Chitosan	0.3±.1	0.3±.1	0.5±.1	1.5±.1	2.1±.1	3.4±.2	5.6±.2
1% Acetic Acid	0.2±.1	0.3±.1	0.4±.1	1.8±.2	3.0±.1	3.1±.2	4.5±.2
1% Chitosan in 1% Acetic Acid	0.3±.1	0.4±.1	0.5±.1	1.7±.1	2.9±.2	3.7±.2	5.4±.2

Appendix B. Data of Grapes

Table B1. Measurement of Ethylene Production for Grapes [ppm]

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	0.05±.01	0.05±.02	0.03±.0	0.04±.0	0.04±.0	0.03±.01	0.03±.01
Water	0.06±.01	0.03±.0	0.02±.0	0.04±.0	0.03±.0	0.02±.01	0.02±.01
1% Soluble Chitosan	0.03±.01	0.01±.0	0.01±.0	0.02±.0	0.03±.0	0.02±.01	0.02±.01
1% Acetic Acid	0.04±.01	0.04±.0	0.03±.0	0.05±.0	0.02±.0	0.02±.01	0.03±.0
1% Chitosan in 1% Acetic Acid	0.04±.01	0.03±.0	0.02±.0	0.03±.0	0.03±.0	0.02±.01	0.02±.0

Table B2. Measurement of Carbon Dioxide for Grapes (%)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	1.4±.2	1.3±.1	1.2±.03	1.9±.03	1.9±.1	1.3±.02	1.4±.05
Water	1.4±.1	1.2±.2	1.0±.03	1.8±.01	1.8±.1	1.1±.03	1.3±.02
1% Soluble Chitosan	1.2±.1	1.2±.05	1.0±.03	1.9±.05	1.7±.05	1.0±.02	1.2±.02
1% Acetic Acid	1.5±.1	1.5±.05	1.2±.03	2.0±.03	2.0±.01	1.2±.02	1.4±.05
1% Chitosan in 1% Acetic Acid	1.2±.1	1.1±.05	1.0±.1	1.8±.02	1.7±.03	1.1±.05	1.2±.02

Table B3. Measurement of Firmness of Fruit Skin for Grapes (kg)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	1.75±.05	1.77±.1	1.91±.1	1.57±.05	1.65±.1	1.65±.05	1.84±.05
Water	1.04±.05	1.22±.05	1.69±.1	2.10±.1	1.35±.1	1.35±.1	1.20±.05
1% Soluble Chitosan	1.47±.05	1.77±.1	2.17±.1	2.10±.1	1.72±.1	1.74±.05	1.78±.05
1% Acetic Acid	1.50±.05	1.47±.1	1.71±.1	1.28±.1	1.29±.1	1.28±.1	1.34±.1
1% Chitosan in 1% Acetic Acid	1.96±.1	1.74±.1	1.82±.1	1.83±.1	1.79±.1	1.79±.05	1.52±.05

Table B4. Measurement of Color of Fruit Skin for Grapes (L-value)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	34.02±.1	33.72±.2	33.95±.1	31.52±.1	31.23±.1	30.98±.1	31.68±.1
Water	33.39±.1	31.87±.1	31.45±.1	32.05±.1	31.12±.1	31.89±.1	29.32±.1
1% Soluble Chitosan	32.67±.1	33.33±.1	32.63±.1	32.05±.1	30.88±.1	33.71±.2	28.23±.1
1% Acetic Acid	33.86±.2	29.91±.1	34.32±.2	29.95±.1	28.98±.1	32.87±.2	29.67±.1
1% Chitosan in 1% Acetic Acid	30.24±.1	30.54±.1	34.39±.2	31.19±.1	32.02±.1	32.61±.2	30.38±.1

Table B5. Measurement of Color of Fruit Skin for Grapes (a-value)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	13.30±.1	14.08±.2	15.78±.2	14.79±.2	14.52±.2	15.01±.2	14.62±.1
Water	18.89±.2	15.18±.2	17.97±.2	14.94±.2	18.33±.1	20.56±.2	19.41±.2
1% Soluble Chitosan	16.26±.2	19.01±.1	17.15±.1	14.94±.1	15.23±.2	17.82±.2	17.23±.1
1% Acetic Acid	17.96±.1	19.62±.1	17.74±.2	19.93±.2	20.15±.2	16.67±.1	16.89±.1
1% Chitosan in 1% Acetic Acid	18.42±.2	19.45±.2	16.14±.1	20.13±.2	19.32±.2	16.09±.2	13.93±.1

Table B6. Measurement of Color of Fruit Skin for Grapes (b-value)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	8.63±.1	8.93±.2	9.07±.2	7.71±.2	8.89±.2	8.41±.2	8.44±.2
Water	13.27±.1	9.45±.2	13.43±.1	13.64±.1	10.56±.1	10.75±.1	10.45±.2
1% Soluble Chitosan	14.25±.1	12.02±.1	13.26±.2	13.64±.1	12.86±.2	13.31±.2	9.36±.1
1% Acetic Acid	10.86±.1	6.66±.2	11.44±.1	8.91±.2	8.97±.2	7.79±.1	8.43±.2
1% Chitosan in 1% Acetic Acid	10.23±.1	8.38±.1	11.01±.2	10.33±.2	10.78±.2	10.48±.2	7.83±.1

Table B7. Measurement of Weight Loss for Grapes (%)

Treatments	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	0.6±.02	1.2±.05	1.9±.1	2.3±.1	2.7±.2	2.9±.3	4.0±.2
Water	0.7±.01	1.3±.05	1.6±.1	2.3±.2	2.4±.1	2.7±.2	3.9±.2
1% Soluble Chitosan	0.5±.01	1.2±.05	1.5±.1	2.4±.1	2.8±.1	3.4±.2	4.3±.05
1% Acetic Acid	0.6±.02	1.3±.1	2.1±.2	2.2±.2	2.7±.2	3.4±.2	4.3±.05
1% Chitosan in 1% Acetic Acid	0.7±.02	1.3±.1	2.0±.1	2.5±.1	2.9±.1	3.5±.3	4.3±.1

Appendix C. Equation of Respiration Reaction in Plants

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy}$$

The equation is in moles which indicate the oxidation of glucose to CO_2 and Water.

Appendix D. Ethylene Biosynthetic Pathway

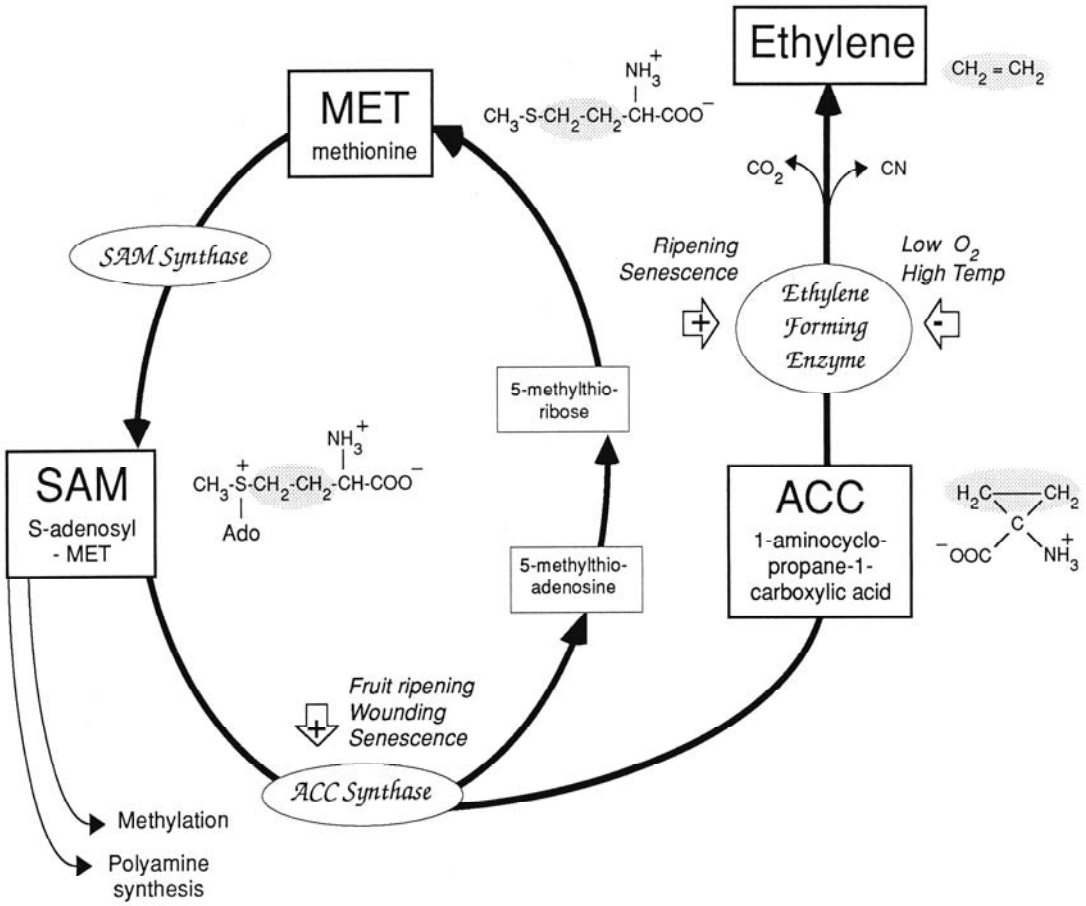

Diagram is adapted from Yang, 1987.

Appendix E. Appearance of Blueberry Sample Comparison between day 0 and day 21

Day 0, Control

Control

Water

1% Soluble Chitosan

1% Chitosan in 1% acetic acid

1% Acetic Acid

Appendix F. Appearance of Grape Sample Comparison between day 0 and day 21

Day 0, Control

Control

Water

1% Soluble Chitosan

1% Chitosan in 1% acetic acid

1% Acetic Acid

VITA

Jason Noh was born in Seoul, Korea. He moved to Los Angeles, California when he was in high school years. Jason graduated from the University of Tennessee, Knoxville, where he earned his Bachelors of Science Degree in Animal Science with minor in Chemistry in December 2000. In August 2003, he decided to pursue his Master's Degree in the Department of Food Science and Technology at the University of Tennessee. His research was focused on effects of chitosan on quality and shelf life of small fruits. Jason graduated with his Masters of Science degree in December of 2005. After completing his Masters, Jason obtained a challenging career in a leading food industry as a food scientist.