EFFECT OF COMPOSITE EDIBLE COATINGS AND ABIOTIC STRESS ON POST HARVEST QUALITY OF FRUITS

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This thesis is dedicated to my Supervisor Dr Hosahalli S Ramaswamy

ABSTRACT

The post-harvest quality of perishable produce changes rapidly due to the accelerated rate of respiration, transpiration and ripening. The objective of this study was to determine the effect of pectin and alginate based composite coatings on the shelf life extension of cherry tomatoes and to study the effect of an abiotic stress on enhancing antioxidant properties of cranberries.

Two composite coating formulations were evaluated, a pectin based formulation with 3% pectin, 1.35% sorbitol, 1.2% bees wax and 0.36% monoglycerides, and an alginate based formulation with 2% alginate, 1.35% sorbitol, 1.2% bees wax and 0.36% monoglycerides. Whole cherry tomatoes were coated with one of the composite coatings and air dried at room temperature for a period of 3 h, transferred to preformed plastic containers and stored at selected temperatures (4, 12 and 24°C) along with a control. Alginate coated cherry tomatoes were additionally dipped in 2% $CaCl_2$ before drying to enhance film formation. Several quality parameters of the fruit - weight loss, respiration rate, color, texture, total soluble solids, titratable acidity, pH and total polyphenols were evaluated during selected time intervals. The control had a shelf life of 12, 15 and 18 days, at 24, 12 and 4°C, respectively, whereas both pectin and alginate coated samples had a respective shelf a life of 15, 18 and 21 days at 24, 12 and 4°C respectively demonstrating a 17-25% shelf-life enhancement. Overall, pectin coated cherry tomatoes had better quality in terms than alginate coated samples, both being better than control.

Cranberry fruits were subjected to abiotic stress in hot water at 40, 50 and 60°C for 1-10 min and evaluated for enhanced anti-oxidant properties. Treated fruits were removed from the hot water, drained, air dried, packed in plastic containers and stored at 4°C for 24 h allowing the fruits to relax/repair the stress damage. Total polyphenols, color and texture changes were evaluated. The hot water hormesis treatment at 60°C for 1 min resulted in an increment of the total polyphenols from 120 mg (control) to 258 mg and also showed the maximum color difference (ΔE) of 12.8 likely because of the accumulation of polyphenols. Samples treated for 1 min at 40°C also improved the firmness of the fruit. Hence hot water hormesis shows the potential to enhance the nutritive values of the fruits which can be applied at commercial level to improve the marketability of the fresh produce.

RESUMÉ

La qualité des produits post-récoltes change rapidement due à la vitesse accélérée de la respiration, de la transpiration, et de la maturation. Le but de ce travail était de déterminer l'effet d un enrobage , formé de pectine et d'alginate, sur l'extension de la durée de conservation des tomates cerises et d'étudier l'effet d'un stress abiotique sur l'amélioration des propriétés antioxydantes des canneberges.

Deux formulations d' enrobages ont été évaluées : une formulation à base de pectine avec 3% de pectine, 1,35% de sorbitol, 1,2% de cire d'abeille et 0,36% de monoglycérides, et une formulation à base d'alginate avec 2% d'alginate, 1,35% de sorbitol, 1,2% de cire d'abeille et 0,36% de monoglycérides. Des tomates cerises entières ont été enrobées avec une des enrobages composés et ont été séchées à l'aire à température ambiante pour un durée de 3 heures. Les tomates cerises enrobées d'alginate ont été trempées dans 2% $CaCl_2$ avant l'étape du séchage pour améliorer la formation de la pellicule. Ensuite, elles ont été transférées dans des contenants en plastiques et ont été conservées à des températures sélectionnées (4, 12 et 24°C) accompagnées d'un échantillon témoin. Plusieurs paramètres démontrant la qualité du fruit – la perte de poids, le taux de respiration, la couleur, la texture, la quantité totale des solides solubles, l'acidité titratable, le pH et la quantité de polyphénols - ont été evalués durant les intervalles sélectionnés. L'échantillon témoin avait une durée de conservation de 12, 15 et 18 jours, à 24, 12, et 4°C, respectivement, tandis que les échantillons enrobés de pectine et d'alginate avait une durée de conservation de 15, 18, et 21 jours à 24, 12 et 4°C respectivement. Ce qui démontre une amélioration de 17-25% en durée de conservation. En général, les tomates cerises enrobées de pectine avaient une meilleur qualité que les échantillons enrobés d'alginate, et les deux formes d'enrobages ont donnés de meilleurs résultats que les échantillon témoins.

Des canneberges fraiches ont été assujetties à un stress abiotique dans l'eau chaude à 40, 50 et 60 °C pour 1-10 minutes et ont été évaluées pour leurs propriétés antioxydantes. Les fruits qui ont été traités ont été enlevés du bain d'eau chaude, ont été séchés à l'air, ont été rangés dans des contenants plastiques et ont été conservés à 4°C pour une durée de 24 heures permettant aux fruits de relaxer/réparer les dommages dus au stresse. La quantité des polyphénols, et le changement de couleur et de texture ont été évalués. L'effet d'hormèse induit avec le traitement d'eau chaude à 60°C pour une minute a donné une augmentation de la quantité de polyphénols

de 120 mg (le control) à 258 mg et a aussi démontré un changement de couleur maximal (ΔE) de 12.8 qui est probablement causé par l'accumulation de polyphénols. Les échantillons traités pour une minute à 40°C ont été aussi améliorés en termes de la fermeté du fruit. Par conséquent, l'effet d'hormèse induit avec le traitement d'eau chaude démontre son potentiel pour améliorer les valeurs nutritives des fruits pouvant s'appliquer au niveau commercial pour augmenter la commercialisation des fruits et légumes frais.

CONTRIBUTIONS OF AUTHORS

Several presentations have been made based on the thesis research and manuscripts have been planned for publication. Hence the thesis is written in the manuscript style. There are two chapters highlighting the thesis research which can be suitably modified for publication. Two authors have been involved in these thesis manuscripts and their contributions to the various articles are as follows:

Phani Tej Raghav Narayanapurapu is the MSc candidate who planned and conducted all the experiments, in consultation with his supervisor, gathered and analyzed the results and drafted the thesis and the manuscripts for scientific presentations and publications.

Dr. Hosahalli S. Ramaswamy is the thesis supervisor, under whose guidance the research was carried out, and who guided and supervised the candidate in planning and conducting the research, as well as in correcting, reviewing and editing of the thesis and the manuscript drafts for publication.

LIST OF PUBLICATIONS AND PRESENTATIONS

Part of this thesis has been prepared as manuscripts for publications in refereed scientific journals:

Narayanapurapu, P.T.R. and Ramaswamy, H.S., 2012. Comparative evaluation of pectin and alginate based composite coatings on extending the shelf life of cherry tomatoes (to be submitted).

Narayanapurapu, P.T.R. and Ramaswamy, H.S., 2012. Effect of abiotic stress on the post-harvest quality of the cranberries (to be submitted).

Part of this thesis has been presented/ prepared for presentation at the following scientific conferences:

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Narayanapurapu, P.T.R. and Ramaswamy, H.S**.** Evaluation of alginate based composite coating on extending the shelf life of cherry tomatoes. Conference of Food Engineering (COFE), Leesburg, Virginia, USA, April 2012.

Narayanapurapu, P.T.R. and Ramaswamy, H. S. Evaluation of pectin based composite coating on extending the shelf life of cherry tomatoes. Institute of Food Technologists (IFT) Annual Meeting, Las Vegas, Nevada, USA, June 2012 (accepted).

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CHAPTER 1

INTRODUCTION

The issue of food losses is of high and global importance in the efforts to combat hunger, raise income and improve food security in countries of the world. Food losses have an impact on food security for poor people, on food quality and safety, on economic development and on the environment. The exact causes of food losses vary throughout the world and are very much dependent on the specific conditions and local situation in a given country. In broad terms, food losses will be influenced by crop production choices and patterns, internal infrastructure and capacity, marketing chains and channels for distribution, and consumer purchasing and food use practices. Irrespective of the level of economic development and maturity of systems in a country, food losses should be kept to a minimum. Food losses represent a waste of resources used in production such as land, water, energy and inputs. Producing food that will not be consumed leads to unnecessary and inefficient use of land and resources, increases in CO_2 emissions in addition to inflating the economic loss.

Economically avoidable food losses have a direct and negative impact on the income of both farmers and consumers. Given that many smallholders live on the margins of food insecurity, a reduction in food losses could have an immediate and significant impact on their livelihoods. Improving the efficiency of the food supply chain could help to bring down the cost of food to the consumer and thus increase access. Given the magnitude of food losses, making profitable investments in reducing losses could be one way of reducing the cost of food. But that would, of course, require that financial gains from reduced losses are not outweighed by their costs.

Food losses refer to the decrease in edible food mass throughout the part of the supply chain that specifically intended for human consumption. Food losses take place at production, post-harvest and processing stages in the food supply chain (Parfitt *et al.,* 2010). Food losses occurring at the end of the food chain (retail and final consumption) are rather called “food waste”, which relates to retailers’ and consumers’ behavior (Parfitt *et al.,* 2010). Food “waste” or “loss” is measured only for products that are directed to human consumption, excluding feed and parts of products which are not edible. Per definition, food losses or waste are the masses of food lost or wasted in the part of food chains leading to “edible products going to human consumption”.

Therefore food that was originally meant to human consumption and gets out the human food chain is considered as food loss or waste even if it is then directed to a non-food use (feed, bioenergy). There are several methods of reducing the food losses after the harvest. In post-harvest handling, fruits and vegetables are one of the important sector and eating fresh fruits and vegetables is a convenient way for consumers to receive nutritive, healthy, and tasty food products. When large percentages of the population suffer from obesity and nutrition related illnesses, people started searching for healthy food, replacing fast foods and unhealthy snack with nutritive fresh fruits in school and work which is a good way to improve quality of food intake. However, the reach of such approach is shortened due to the short shelf life and quality loss faced by fresh fruits during transport and storage, thus it is very important to find the best way to preserve the post-harvest produce (Olivas and Barbosa-Canovas, 2005).

Many storage techniques have been developed in order to extend the useful marketing distances and holding periods for fresh horticultural crops after harvest. Examples are maintaining the produce at low temperature and high RH, under controlled atmosphere storage, and/or modified atmosphere packaging. Each technique has its own advantages and disadvantages, with the later still a major factor in most cases. This is partly the reason why the maintenance of the quality of fresh produce is still a major challenge for the food industry (Kader and Saltveit, 2003).

The concept of using edible films or coatings to extend the shelf life of fresh fruits and minimally processed products has been receiving considerable attention in recent years. In general, an edible coating is defined as a thin layer of edible material formed on the surface of a food, whereas an edible film is a preformed thin layer of edible material placed on or between food components (Krochta and De Mulder-Johnston, 1997). In other words, edible coatings are applied and formed directly on the food by dipping, spraying, or brushing (Cuq *et al.,* 1995). On the other hand, edible films are preformed into solid sheets and then applied on or between food components. Edible coatings and films are natural polymers obtained from products or by-products of agricultural origin, such as animal and vegetable proteins, gums, lipids, and celluloses (Debeaufort *et al.,* 1998). By being edible and biodegradable, edible coatings represent a unique category of packaging materials that differ from other conventional packaging materials. The mechanism by which a semi-permeable coating preserves fruits and vegetables is by producing a modified atmosphere surrounding the product by isolating the coated product

from the environment. Several purposes can be served by this modified atmosphere, including reducing oxygen availability and increasing the fruit or vegetables internal carbon dioxide concentration (Smith *et al.*, 1987). During respiration, modified atmospheres created by coatings are produced by the physical trapping of carbon dioxide gas within the fruit tissues. The increased levels of carbon dioxide have been shown to lower respiration rates, and therefore delay senescence by decreasing the synthesis of ethylene, a hormone essential for ripening (Saltveit, 2003). The need for higher quality foods, the demand for new food processing and storage technologies, increased attention to environmental protection, and the discovery of the functionality of new materials have renewed researchers and industrial interests in edible films and coatings (Zhao and McDaniel, 2005).

In addition, coatings may have different levels of permeability to oxygen. Decreased oxygen permeability can also reduce respiration and increase shelf life. However, although reduction of gas transfer is desirable, extremely impermeable coatings may induce anaerobic conditions that can cause a decrease of the production of characteristic aroma volatile compounds in fruits and most importantly the development of off-flavors associated with anaerobic fermentation of the sugars (Mattheis and Fellman, 2000). In addition to reducing respiration rate, coatings prevent water loss from transpiration and act as hydrophobic barriers. For fruit and vegetable commodities such a feature is very useful. Water loss can lead to decreased turgor pressure which results in shriveling and wilting, both of which render produce unmarketable (Kester and Fennema, 1986). Slower softening, reduced texture changes, and increased color retention are other quality improvements reported for edible coatings. Edible films can also be used as protective coatings for food ingredients that are susceptible to oxidation. Polysaccharide coatings on nutmeats serve this purpose. To further increase food quality, stability, functionality, and safety, edible films and coatings can also carry functional ingredients such as antioxidants, antimicrobials, nutrients, and flavors (Krochta and De Mulder-Johnston, 1997).

Pectin and alginate based composite coatings were developed by Maftoonazad *et al.* (2007) and barrier, optical, and mechanical properties of such film were evaluated using response surface methodology. In addition, the adsorption behavior, thermal and thermo mechanical properties of the film were also evaluated as a function of moisture content and sorbitol (plasticizer) concentration. Finally, avocado fruits were coated with the pectin-based composite coating and

the associated quality changes were evaluated during storage. The results of the mentioned studies showed that this coating can hinder the ripening of the avocado fruits and improve all related quality parameters by lowering rate of loss of firmness, color changes, weight loss, and chemical changes (pH, titratable acidity, and total soluble solids) (Maftoonazad *et al.*,, 2006). The edible coatings like methyl cellulose was also used to extend the shelf life of peaches, the maximum acceptable shelf-life at 15°C for control samples was 15 days, the coated samples maintained their acceptability up to 21 and 24 days, respectively, with sodium alginate and methyl cellulose coating (Maftoonazad *et al.,* 2008). This was later extended to mango and cucumbers (Moalemiyan, 2010). This work explores to extend the post-harvest shelf life of cherry tomatoes using pectin and sodium alginate based composite coatings.

Importance of enhancing the nutritional content of fruits by abiotic stress: Fruits and vegetables are essential for a healthy and balanced diet. Consumption of fruits and vegetables as per the recommended level has many potential health benefits (WHO, 1991). The nutrient health benefits of fruits and vegetables have been proven epidemiologically (Block *et al.,* 1992). They are not only necessary for providing essential nutrients to the body but also contain bioactive compounds which help in preventing many diseases (Liu, 2003; Nicoli *et al.,* 1997). An important factor in the development of diseases like cancer, heart disease and stroke is poor diet (Doll, 1981; WHO 2003). Nutrition can be an important and essential tool for cancer deterrence (Roy *et al.,* 2002). A commonly accepted fact is that one third of the cancers are generally caused by the poor diet, while half of the cardiac diseases and hypertension are due to an inadequate diet (Goldberg, 1994). Infact, the consumption of fruits and vegetables is inversely proportional to the heart disease and various cancers (Beattie *et al.,* 2005). This character of disease prevention is mainly attributed to the presence of phytochemicals, natural anti-oxidant compounds, fibres and other bioactive compounds (Nicoli *et al.,* 1999). Fruits and vegetables contain a variety of antioxidant compounds (Wang *et al.,* 1996) and other nutrients. Generally, vitamins and pro-vitamin compounds are assumed to have significant health benefits (Vinson *et al.,* 1998).

Fruits and vegetables undergo different stages of handling, storage and processing which may affect the quality (Klein, 1987). The nutritional value of the fruits and vegetables gradually decreases after their harvest. Post-harvest processing, harvesting time, storage and preparation of

foods like cooking and peeling affect the nutritional content of fruits and vegetables (Goddard, 1979; Goldman *et al.*, 1999; Salunkhe *et al.*, 1973). Main nutritional compounds or protective agents like antioxidants decrease during food processing operations such as sterilization, pasteurization, dehydration, improper handling and cooking etc., (Jonsson, 1991; Nicoli *et al.*, 1999).

Among various fruits, berries are known for their antioxidant properties and are rich source of flavonoids and polyphenols (Heinonen *et al.*, 1989) which mainly include anthocyanins. Anthocyanins are the natural color pigments in many fruits and vegetables (Wang *et al.*, 1997) especially in edible berries (Francis and Markakis, 1989). These agents are responsible for the red violet, purple, blue and magenta colors in many plant species (Jackman *et al.*, 1987). Dietary consumption of anthocyanin has been shown to improve the overall antioxidant defence system against free radicals (Wang *et al.*, 1997). Cranberry (*Vaccinum Macropon*) is a wild berry predominantly found in North America and it belongs to *Aricaceae* family which has more than 30 different species. Fruits of *Vaccinium* sp. including cranberries are particularly rich in flavonoids and other phenolic compounds (Prior and Cao, 2000), (Wang and Stretch, 2001). Polyphenols play a major role in various natural defense mechanisms in plants as a response to environmental stress factors such as light, temperature, ultraviolet radiation and other internal factors like nutrients and hormones which all can potentially induce their synthesis. Polyphenols play a vital role in plant disease resistance (Nicholson and Hammerschmidt, 1992).

An abiotic stress is the stress caused by environmental conditions affecting the plants (extreme weather conditions, ultraviolet radiation, temperature and light). Plants produce or stimulate synthesis of various secondary metabolites when they are subjected to various abiotic stresses. Dixon and Paiva (1995) highlighted the stress induced phenylpropanoid mechanisms in plants resulting in the formation of stress metabolites which mainly include various polyphenols with reported health benefits. Researchers have explored the effect of ultraviolet light and pulsed light on the development of secondary metabolites in fruits and vegetables (Arakawa, 1993; Baka *et al.*, 1999; Murugesan, 2010) which has led to the use of abiotic stresses to enhance the nutritional content and storability of fruits and vegetables. To continue this research and explore more, it was decided to evaluate the effect of hot water treatment (hormesis) on cranberry fruits secondary metabolites especially total polyphenols.

The general objective of this thesis research to explore the effect of composite edible coatings and abiotic stress on post-harvest quality of fruits

With this background, the specific objectives of this thesis research were formulated as follows:

1) To evaluate pectin and alginate based composite coatings for their ability to extend the post-harvest shelf life of fresh cherry tomatoes at three different storage temperatures of 4°C, 12°C and 24°C.
2) To investigate the effect of a physical stress (hot water treatment) on the formation of secondary metabolites especially polyphenols in cranberry fruits. The hypothesis is subjecting cranberry fruits to temporary stress can enhance their polyphenolic activity by triggering the fruits' stress response mechanism.

CHAPTER 2

REVIEW OF LITERATURE

2.1 INTRODUCTION

It has been reported that about 25 to 80% of harvested fruits and vegetables are spoiled because of improper handling and preservation techniques before consumption. This results in heavy economic losses in developed countries and is even more devastating in many tropical regions of the world (Wills *et al.,* 1981). Spoilage occurs when fresh fruits and vegetables are treated as inert materials when they are in fact living tissues. Like members of the animal kingdom, fruits and vegetables need to breathe or otherwise they will go under certain anaerobic reactions and start to 'suffocate'. Also like animals, fruit and vegetable tissues 'perspire' or lose moisture to the air and are subject to attack by microorganisms. The rate at which fresh produce 'breathes' is called respiration and the rate of water loss is called transpiration. Temperature, gaseous makeup, and humidity of the environment surrounding the commodity affect both respiration and transpiration (Baldwin, 1994). Hence it is important to know the post-harvest physiology of fresh fruits, available post-harvest technologies for the produce and the problems faced by post-harvest produce. Then focus is driven on to how the problems were addressed by edible coatings, introduction of edible coatings, its components, functions, effect of edible coatings on the different quality parameters of the fruit.

2.2 POST HARVEST PHYSIOLOGY OF FRESH FRUITS AND VEGETABLES

Harvested fruits and vegetables continue to maintain physiological activities and sustain metabolic processes that were there before harvest. While attached to the plant, the losses from respiration and transpiration are replaced from the flow of sap, which contains water, photosynthates, and minerals (Wills *et al.,* 1998). However, after harvest, the product is dependent entirely on its own food reserves and water content. Losses of water and substrates used in respiration can no longer be replaced and deterioration of the product begins.

Fruit ripening involves many complex changes, including seed maturation, color changes, abscission from the parent plant, tissue softening, volatile production, wax development on skin, and changes in respiration rate, ethylene production, tissue permeability, carbohydrate

composition, organic acids and proteins (Wills *et al.,* 1998). Product respiration, transpiration, and ethylene production are major factors contributing to the deterioration of fresh fruits and vegetables. Reduction of these processes by technologies such as cooling and storage, enable the postharvest life of fresh produce to be prolonged.

2.2.1 Respiration: Every plant tissue requires energy to remain alive and to support developmental changes. The energy is generated by respiration, which is the oxidative catabolism of carbohydrates and other macromolecules. This process occurs in the mitochondria of living cells and mediates the release of energy and the formation of carbon skeletons necessary to the maintenance and synthetic reactions that occur after harvest (Kays, 1991).

Respiration can be considered a series of enzymatic reactions, involving three pathways: glycolysis (glucose → pyruvate), the tri-carboxylic acid or Kreb's cycle and oxidative phosphorylation. Oxygen (O_2) is consumed, whereas carbon dioxide (CO_2), water, some heat, and energy carriers are released. The process can be described by the simple equation:

$$C_6H_{12}O_6 + 6\ O_2 + 36\ ADP \longrightarrow 6\ CO_2 + 6\ H_2O + 36\ ATP \ldots\ldots\ldots\ldots \text{(Equation 1.1)}$$

Respiration Measurements: Respiratory gas exchange is often used as a general measure of the metabolic rate of tissues, since respiration has a central position in the overall metabolism of a plant produce. These data are used for several postharvest applications, such as the determination of optimum conditions for controlled atmosphere (CA) storage rooms or modified atmosphere (MA) packages.

There are various ways of measuring gas exchange, but the three methods used most often are:

1. ***Static method***: A specific gas composition is generated around an object and the gas flow is closed for a specific period of time. Gas composition is measured at the beginning and end of the period (Peppelenbos *et al.,* 1996).

2. ***Flow through method***: A specific gas composition is generated around an object and the gas composition of the inward and outward flow is measured (Boersig *et al.,* 1988).

3. ***MA method***: Using a package with film of known O_2 and CO_2 permeability, the equilibrium concentrations that develop are measured. Gas exchange rates can then be calculated (Beaudry *et al.,* 1992).

Factors effecting respiration: Pre-harvest and post-harvest respiration in fruits can be altered by many factors like temperature, oxygen and carbon-di-oxide concentrations, and other stresses experienced by the commodity.

- ***Temperature:*** Temperature influences the rate of respiration of fruits and vegetables and plays an important role in maintaining post-harvest quality of fresh fruits and vegetables. The velocity of a biological reaction may double or even triple for every 10°C increase according to Van Hoff's rule (Kader, 1987). This ratio of reaction rates at two temperatures that are 10°C apart has been called the temperature coefficient or Q_{10}. The respiration rate increases with increasing temperature. Lower temperatures can successfully reduce respiration. Some fruits and vegetables show sensitivity to lower temperatures and may experience chilling injury. Chilling injury refers to the damage that occurs when cold sensitive fruits are exposed to temperatures below their cold threshold level (Lyons and Breidenbach, 1987). Fruits and vegetables susceptible to chilling injury also show abnormally high respiration rates upon transfer from the chilling temperature to a higher temperature which can cause damage to tissues (Eaks, 1956; Wang, 1982).
- ***Atmospheric gas composition***: Gas composition of the atmosphere surrounding a fruit also affects the respiration rate. Obviously, aerobic respiration needs the presence of oxygen. The normal atmospheric concentration of oxygen, 21%, is optimal for respiratory processes with a minimum of 1-3% to maintain it under the aerobic state. However, when this level drops below the normal 21%, the respiration rate may be slowed (Greulach, 1973). High levels of carbon dioxide inhibit the decarboxylation reactions of normal respiration, and thus slow down the Kreb's cycle. However, if the concentration of carbon dioxide is too high (over 20%) anaerobic respiration will go on and result in damaged plant tissue (Kader, 1987).
- ***Ethylene and Stresses:*** The hormone ethylene is a normal physiological product of the fruit and a highly physiologically active compound even in trace amounts. The respiration rates have been shown to rise, when a fruit is exposed to this hormone. Upon

exposure to the hormone, climacteric fruits have increased respiration rates, which eventually decrease the time it takes for them to reach their climacteric respiration peak. Bruising or wounding a fruit can increase the respiration rate proportionally to the extent of the damage. Diseases 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).

2.2.2 Transpiration: 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-Yehosha, 1987). Transpiration is an effective way of keeping the plant tissue cool by lowering the surface temperature of the plant tissue. 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 surface. Loss of moisture therefore represents an economic loss. When fruit or vegetable is attached to the plant, this lost water is immediately replaced. However once harvested, no water replacement occurs, resulting in loss of saleable weight (Chakraverty *et al.*, 2003).

Transpiration obeys the basic Fick's law of diffusion:

$$J = AD (\Delta C/\Delta x)/ [RT] = AD [(Pi-Pa)/x]/RT \ldots\ldots\ldots\ldots \text{(Equation 1.2)}$$

J = gas flux expressed, A = area of the surface, D = diffusivity or diffusion coefficient , $\Delta C/\Delta x$ = concentration gradient which in this case is the gradient of water vapor pressure (Pi-Pa)/x with x representing the thickness of the surface layer offering resistance to moisture migration, R = Gas constant, T = Temperature (absolute).

Thus Fick's law states that the movement of any gas or vapor in or out of the plant tissue is directly proportional to the partial pressure gradient across the barrier surface, surface area of the barrier and diffusion coefficient of the surface and inversely proportional to the temperature and thickness of the surface. The water vapor pressure gradient Pi-Pa is the main driving force for moisture migration at a given temperature for a given product (Chakraverty *et al.*, 2003).

Factors influencing transpiration:

- ***Surface area***: The available surface area is determined by the shape and size of the produce. Produce with large surface to volume ratios would have very high 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 and narrower carrots as compared to their thicker counterparts and small apples lost more water than larger fruits (Phan *et al.*, 1975; Sastry *et al.*, 1978).
- ***Respiration:*** Respiration produces water and heat, both of which directly affect transpiration. The heat if not removed, can increase the produce temperature and hence its respiration and transpiration rates.
- ***Air currents:*** This plays an important role in storage chambers. On one hand, it assists in better heat transfer to effectively remove the heat of respiration. On the other hand, it also promotes the mass transfer in terms of removing the moisture transpired by the produce from its surrounding. This results in further transpiration. Both from the heat and mass transfer point of view, higher air velocities increase the rate of heat /mass transfer. In storage system a moderate speed of about 0.25-0.35 m/s is often employed (Chakraverty *et al.*, 2003).

2.2.3 Ethylene production: Ethylene is a gaseous plant hormone and biologically active at low concentrations (PPB to PPM) (Abeles *et al.*, 1992; Saltveit, 1999). Ethylene production occurs in all plant tissues at some minimal level. It is necessary for growth (usually thickening or lateral growth such as with etiolated pea seedlings), inhibits longitudinal growth, and promotes seed germination, de-greening, adventitious root formation, abscission, ripening, and senescence (Baldwin, 2004; Reid, 1985). Ethylene is considered autocatalytic when ethylene stimulates its own synthesis and auto-inhibitory when it turns off continued synthesis (Mattoo and White, 1991). Ethylene is thought to be synthesized from the amino acid L-methionine, which is converted to S-adenosyl methionine (SAM or adomet) by the enzyme methionine S-adenosyl transferase. S-adenosylmethionine is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS), which, in turn, is converted to ethylene by ACC oxidase (ACO) (Adams and Yang, 1979).

2.2.4 Fermentation: At low O_2 or high CO_2 atmospheres fermentation becomes increasingly important. The main fermentative metabolites found in plant tissues are ethanol, acetaldehyde, and lactic acid (Perata and Alpi, 1993; Richard *et al.*, 1994. Ethanol is the most abundant metabolite, especially under prolonged anoxia, resulting in additional CO_2 production (Pfister-Sieber and Brandle, 1994). Ethanol fermentation is the catabolism of pyruvate to ethanol: the combination of glycolysis and fermentation can be expressed as:

$$C_6H_{12}O_6 + 2\ ADP \rightarrow 2CO_2 + 2C2H5OH + 2\ ATP \ldots\ldots\ldots\ldots \text{(Equation 1.3)}$$

Physiological disorders of products stored in altered gas conditions are almost always found together with high concentrations of fermentative metabolites. Therefore, these metabolites are often considered to be the cause of storage disorders, such as necrotic or discolored tissues, off-flavors and off-odors (Kader *et al.*, 1989).An increase in fermentation helps the cell meet its ATP requirements (Good and Muench, 1993), whereas the main injuries related to anoxia may eventually be due to changes in energy metabolism (Pfister-Sieber and Brandle, 1994). There is no simple relation between survival and the rate of fermentation (Richard *et al.*, 1994). The total amount of energy produced during respiration and fermentation should cover maintenance requirements. Disorders such as necrotic tissues are expected to develop when the gas conditions supplied result in lower energy production than maintenance energy (Peppelenbos *et al.*, 1996).

2.3 POST HARVEST TECHNOLOGY OF FRUITS AND VEGETABLES

2.3.1 Storage at low temperatures and high relative humidity conditions: Pre-cooling, refrigeration, proper relative humidity, and optimal atmospheric composition in storage facilities and packages are essential to reduce postharvest losses of commodities. Providing short-term storage to balance the daily fluctuations of supply and demand and to provide long-term storage to extend the marketing season are the main objectives. Three factors involved in the deterioration of perishable commodities must be controlled to meet these objectives. The natural rate of respiration must be reduced as much as possible by controlling temperature and, for many commodities that respond positively to low O_2 and/or high CO_2 levels, the composition of the storage atmosphere. Moisture loss should also be minimized, and pathogenic microorganisms not be permitted to proliferate. Temperature, relative humidity and the atmospheric composition are

the factors influencing the storage of post-harvest produce at low temperatures. Pre-cooling, cooling and storage are the three stages in this process.

a. ***Pre-cooling methods*:** The distinction between pre-cooling and cooling is that pre-cooling is any method of removing field heat more rapidly than if the produce were simply placed in a storage chamber set at the desired temperature and allowed to cool. The sooner produce is cooled down, the better its chances at a relatively long storage life. Some fresh produce may deteriorate as much in 1 h at 26°C as in 1 week at 1°C, particularly if they have naturally high respiration rates. Pre-cooling may involve a technology separate from that of normal cooling in a refrigerated environment or may be the result of modifications to the refrigerated storage. The different types of pre-cooling methods employed are hydro cooling, contact icing, vacuum cooling and forced air cooling (Chakraverty *et al.*, 2003).

b. ***Cooling*** : Room cooling is one of the most widely used forms of cooling, primarily because the room in which the produce is cooled also serves for longer-term storage, thus reducing overall handling requirements and costs. It is not recommended for crops that have high respiration rates or crops harvested in the warmer months, since significant quality deterioration may take place during the cooling period. Other cooling techniques which are less employed were evaporative cooling, Carbon dioxide cooling, night time cooling, well water cooling, high altitude cooling, underground storage, thermo electric cooling by Peltier effect (Chakraverty *et al.*, 2003).

c. ***Refrigeration***: The vapor re-compression is most commonly used type of mechanical refrigeration used for the storage of the fresh produce which requires four important components like expansion valve, an evaporator, a compressor and a condenser. Capillary tubes and expansion valves are the two often used expansion valves. The two evaporator designs most often used in storage systems for fruits and vegetables are the bare-pipe and the finned-tube evaporators. The bare-pipe evaporators are the simplest form and are easy to clean and defrost. Compressor types that are most commonly used are reciprocating, rotary, and centrifugal compressors. There are three major types of condensers, water-cooled, air-cooled, and evaporative. The selection of a proper refrigerant is important for efficient use of a refrigeration

system. Factors to consider include the cost of the refrigerant, the latent heat of vaporization, the condensing pressure, and the toxicity. Other considerations include the environmental impact and the nature of the refrigerant with respect to its chemical properties (Chakraverty *et al.*, 2003).

d. ***Controlled atmosphere storage***: CA storage implies precise control of the gas concentrations of oxygen, carbon-di-oxide and ethylene inside the storage room. Modification of atmospheric gas levels may reduce the respiration rate of fresh produce, as well as control the level of ethylene (C_2H_4) and thus retard ripening. The gas concentrations of ambient air are 78.08% N_2, 20.95% O_2, and 0.03% CO_2. In most CA storage systems, the O_2 level is decreased and/or the CO_2 level is increased to decrease product respiration rate. Different types of produce respond differently to these two gases, and thus the proper atmosphere for a given commodity should be predetermined experimentally (Chakraverty *et al.*, 2003).

   The choice of CA system to use depends primarily on the gas composition that is desired and the rate at which it is to be achieved. The standard free volume (SFV) is the ratio of the volume of air to the volume of commodity. The SFV in typical warehouses ranges from 1.5 to 3.0 and is a function of the stacking arrangement, room geometrical characteristics, commodity shape and density, and method of packing, either in bulk or in crates. The controlled atmosphere storage has oxygen, carbon-dioxide and ethylene control systems which are used in order to monitor the concentrations of the gases in the chamber during the period of storage.

e. ***Modified atmosphere storage and modified atmosphere packaging***: The modified atmosphere systems use semi permeable membranes to regulate gas exchange between the modified atmosphere surroundings and the ambient air. The composition of the air in the package changes as a result of the respiratory action of the produce and permeability characteristics of the membrane. For example, silicone membranes are used to allow the gases to diffuse at different rates, which are determined by the chemical and physical characteristics of the gases. The important factors influencing the storage of the produce in modified atmosphere are temperature, membrane surface area, permeability of the membrane, gas partial pressure difference across the membrane. The modified atmosphere storage does not achieve the same degree of

atmospheric control as controlled atmospheric storage. It is a better approach for short term storage of small quantities of produce than controlled atmosphere storage and is often used in association with packaging.

2.3.2 Irradiation of fruits and vegetables: Irradiation is used for disinfection in case of dried fruits and vegetables which are used in soups and ingredients which have large number of the microorganisms responsible for spoilage (Wilkinson and Gould, 1996). A radiation dose of 5-10 kGy can reduce contamination. Yeasts and bacterial spores are more resistant to the radiation than the *Enterobacteriaceae*. Ionizing radiation of 0.15-0.75 kGy has been proposed for insect disinfection, however it was found that the dosage of 1.0 kGy may not sterilize some moth species (Tilton and Burditt, 1983). Irradiation is used to delay ripening, preserve the vitamin C content of the citrus fruits during the period of storage (Mahrouz *et al.*, 2002), bananas (Thomas, 1986a), papayas (Thomas, 1986a), apples (Thomas 1986 b), increase the shelf life of cherries (Salunkhe, 1961) and limited the fungal spoilage of figs (Wilkinson and Gould, 1996).

The concept of using edible coatings has been studied in the recent years to extend the shelf life of post-harvest produce. Need for high quality foods, demand for new food processing and storage technologies, increased attention to environmental protection and discovery of the functionality of new materials have led researchers and industries to focus on edible coatings.
The next section of this chapter gives a detailed introduction to edible films, functions, uses, requirements, components of edible coatings, properties of films, film forming and application techniques and various studies done on the application of novel edible films on foods for shelf life extension.

2.4 EDIBLE COATINGS

Edible coatings are defined as the thin layer of material which can be consumed and provides a barrier to oxygen, microbes from external source, moisture and solute movement for food. Edible coatings are gaining importance as an alternative to reduce the deleterious effects imposed by minimal processing on fresh fruits and vegetables and to increase the shelf life thereby catering the needs for the cut fruits in various preparations of salads by commercial airlines, catering agents etc., The semi permeable barrier provided by edible coatings is aimed to extend shelf life

by reducing moisture and solute migration, gas exchange, respiration and oxidative reaction rates, as well as suppress physiological disorders on fresh-cut fruits (Baldwin *et al.*, 1996; Park, 1999; Wong *et al.*, 1994).Controlling fresh quality and growth of spoilage and pathogenic bacteria is a challenging problem for the fresh-cut fruit industry hence edible coatings could be one of potential tool to this issue (Rojas-grau *et al.*, 2007a).

The principle functions of edible coatings are to restrict the loss of moisture from the fruit to the external environment and to lessen the absorption of the oxygen by the fruit. Coatings preserve the texture, aroma and flavor of the fruit by reducing the respiration rate and providing physical protection to the food product especially during handling and transport. Incorporation of plasticizers can improve the functional properties of edible coating like anti-microbial, anti-oxidant etc., and provide alternative to commercial packaging and can be a salvation from the pollution from plastics (Pranoto *et al.*, 2005).

Edible coatings serve as carriers of food additives such as anti-browning agents, anti-microbials, colorants, flavors, nutrients, and spices improving shelf life and food quality, as they are selective barriers to moisture transfer, oxygen uptake, lipid oxidation and the loss of volatile aromas and flavors (Pena and Torres, 1991; Wong *et al.*, 1994; Kester and Fennema, 1986). They are an alternative to modified atmosphere packaging (MAP) to improve the shelf-life of fresh-cut fruits and reduce the deleterious effects concomitant with minimal processing, not solely retarding food deterioration and enhancing its quality, but also improving its safety because of their natural biocide activity or by incorporating antimicrobial compounds (Petersen *et al.*, 1999).

The potential uses of edible coatings are to produce a modified atmosphere in the fruit thereby reducing decay, water loss, aroma loss and exchange of humidity between fruits. They delay ripening, color changes and improve appearance. They serve as carriers of antioxidants and texture enhancers and nutraceuticals (Undurraga *et al.*, 1995; Rojas-Grau *et al.*, 2008). The edible coatings should be stable under high relative humidity, generally recognized as safe. They should be colorless, tasteless and should have good water barrier, gas barrier and mechanical properties (Undurraga *et al.*, 1995).

Freshly harvested fruits and vegetables like mango, strawberries, peaches, tomatoes, papaya which are subjected to faster deterioration and foods which are susceptible to oxidation are good candidates for edible coatings. Foods are also coated in order to reduce the migration of fats and oils in the confectionery industry, improve the mechanical and maintain the structural integrity of the food product and to secure the components in place in case of foods composed with discrete particles (Ali *et al.*, 2011; Murray and Luft, 1973).

2.4.1 Different components of the edible coatings:

Edible film components are divided into three categories

a. Hydrocolloids
b. Lipids
c. Composites
d. Plasticizers

Hydrocolloids include proteins, cellulose and its derivatives like alginates, pectins, starches and other polysaccharides. Lipids include waxes, acyl glycerols and fatty acids. Composite films constitute both lipid and hydrocolloid films.

a. ***Hydrocolloids:*** These are used where control of water vapour migration is not the prime objective, but it is for the resistant barrier properties towards the oxygen and carbon dioxide. These films are used mainly for improving the structural integrity of the packaging material of foods because of their good mechanical properties. These films under the action of heat will dissolve thereby not altering the sensory properties of the food. They are again classified into film forming carbohydrates and the film forming proteins. Film forming carbohydrates include starches, plant gums, chemically modified starches and film forming proteins include casein, soy protein, whey protein, wheat gluten and zein.

b. ***Lipids:*** The lipid films are mainly used for improving the resistance of the foods against water vapour. Their other applications include adding gloss to confectionery products. Their application as pure films is advised due to their lack of structural integrity and

durability. Lipid films are generally used for coating the fruits and vegetables to retard respiration, and reduce the loss of moisture.

c. ***Composite films:*** They are combination of both the hydro colloid films and also the lipid films. They have the advantages of both films, i.e. having both mechanical properties as well as increasing the barrier properties to water vapour and other gases.

d. ***Plasticizers:*** Addition of plasticizers in minor amounts to the edible film would alter the properties of the edible film like functional, organoleptic, nutritional, and mechanical properties of the film. Addition of plasticizers such as glycerol, acetylated monoglyceride, polythelene glycol and sucrose will improve the mechanical properties of the film. Other categories of plasticizers include antimicrobial agents, vitamins, antioxidants, flavors and pigments.

2.4.2 Film forming techniques:

a. ***Coacervation:*** Coacervation involves the separation of the polymeric coating film from the solution by the heating, altering pH, adding solvents and altering charge on the polymer involved. It is separated into two types aqueous and non-aqueous type based on the type of phase separation used. Aqueous phase separation needs a hydrophilic coating such as gelatin or gum acacia which is deposited on water - insoluble core particle. Non aqueous phase separation involves a hydrophobic coating deposited on the water soluble or water insoluble (Dziezak, 1988).
b. ***Solvent removal:*** Materials which are available dispersed in aqueous solutions are separated from by the solvent removal technique. Rate and temperature of drying will affect the crystalline and mechanical properties of cellulosic films (Greener, 1992).
c. ***Solidification of melt:*** Solidification of the melt by cooling is a common technique for the separation and preparation of lipid films. The rate of cooling influences the predominant polymorphic state and degree of recrystallization.

2.4.3 Film application techniques:

a. ***Dipping:*** This method is used for foods which require a uniform coating on an irregular surface. After dipping the excess coating material is allowed to drain from the product

and then dried or allowed to solidify. This method has been used to apply films of acetylated monoglycerides to meats, fish and poultry and to apply coatings of wax to fruits and vegetables.

b. ***Spraying:*** This method is used for applying a thin and uniform application of films on foods. This method is more suitable for applying a film to only one side of the food, for example when pizza crust is exposed to a moist sauce.

c. ***Casting:*** This method can be accomplished by controlled thickness spreading or by pouring. Controlled thickness spreading requires a spreader, product reservoir and an adjustable gate.

2.4.4 Properties of films:

A. ***Permeability:*** It is defined as the ability of the substance to transmit other substances that flow through it. The steady state permeability is better explained by using both Henry's law of solubility and Fick's law of diffusion. Fick's law of diffusion states that the permeate flux (J) is dependent on the permeate diffusivity (D), the differential of concentration (dC) in the film, and the differential of thickness (dX) which is expressed as

$$J = -D\,(dC/dX) \quad \text{(Equation 1.4)}$$

The negative sign indicates that the migration occurs in the direction of the lower concentration. Henry's law states that the concentration of the permeate in the film is equal to the product of the solubility coefficient (S) and the permeate partial pressure in the adjacent air (P).

B. ***Permeance:*** It is the performance evaluation factor, and not an inherent property of the film. Permeance is often used when the film is heterogeneous of unknown composition or of unknown thickness.

C. ***Transmission rate:*** It is defined as the amount of permeate flowing through the film per unit area of the film in unit time. Transmission rate is considered appropriate for describing the permeabilities of the films when they are tested at single relative humidity gradient. Transmission should not be used to compare the films in terms of the barrier

properties of the film of different compositions or thickness unless in situations where the pressure gradient and the film thickness are unknown.

D. ***Resistance:*** The property of the film which is generally used to describe the ability of the film material to serve as the barrier to the permeate. Resistance is the inverse of the permeance when expressed in terms of the concentration as the driving force. It is generally represented using the following equation

$$\text{Resistance} = (W_i - W_a)/J \ \ s\ m^{-1} \quad \text{(Equation 1.5)}$$

W_i is saturated water vapour concentration at the surface temperature of the film in $g.m^{-3}$, W_a is saturated water vapour concentration of the test solution in $g.m^{-3}$, J is flux of the water vapour in $g.\ m^{-2}.\ s^{-1}$

E. ***L Number:*** Hong *et al.*, (1990) used a dimensionless number to describe the moisture barrier properties of the film and food products. It is the ratio of the moisture permeance of the food to the moisture permeance of the coating material. L number less than 0.04 indicate that they are more resistant to the moisture migration in the food. L numbers of greater than 4 indicate the strength of the film.

Extensive study has been done in various areas to explore the application of edible coatings such as the development of novel edible coatings from plant, animal sources, and application of coatings against microbes in various foods. Studies have also been done on improving the mechanical properties of the films by incorporating various plasticizers and their performance was evaluated.

2.4.5.1 Edible coatings from animal sources: Protein based films have been prepared from red tilapia (*Oreochromis niloticus*) from washed and unwashed mince solubilised at pH 3 and 11. The properties like tensile strength, water vapour permeability, film solubility were characterised during a storage period of 20 days. It was reported that the yellow discoloration of the film was due to the involvement of the carbonyl group in the Maillard reaction and oxidation of the lipid (Tongnuanchan *et al.*, 2011). Chitin and chitosan, which are major components of the shells of crustaceans are known to possess multiple functional properties like hypo cholesterolemic, anti microbial, immune stimulating, anti-tumour, anti-cancer, accelerating calcium and iron

absorption, anti-inflammatory and anti oxidant, enabling their potential use as edible coatings (Li and Xia, 2010). The study on semi-hard cheese to evaluate the effects of the application of chitosan coating containing natamycin on the physicochemical and microbial properties using three cheese groups viz., samples without coating, samples coated with chitosan and with chitosan containing 0.50 mg/ml of natamycin, reported that chitosan-based coating/films can be used as a controlled release system containing natamycin to create an additional hurdle for moulds/yeasts in cheese thus contributing to extend its shelf-life (Fajardo *et al.,* 2010).

2.4.5.2 Study of Edible coatings against microbes: Nanocomposite films from the methyl cellulose, carvacol, and montmorillonite were tested against the *Escherichia coli and Staphylococcus aureus* and it was reported that these organisms were completely inhibited when they have the concentration of 11.1±0.2 mg of carvacol. The amount of carvacol release from developed antimicrobial films can be controlled by montmorillonite concentration within the film matrix and by the storage temperature of film (Tune and Duman, 2010). Incorporation of kim wood extract as a natural antibacterial agent in the hydroxypropyl methyl cellulose exhibited more effective impact on the growth reduction of the *L. Monocytogenes* than *Staphylococcus aureus* and *E.coli* at 5 fold minimum bactericidal concentration of kim wood extract. New hydroxypropyl methyl cellulose-lipid edible composite films containing parabens and their mixtures inhibited *Penicillium digitatum* and *Penicillium italicum* than film containing organic acid salts like potassium sorbate and potassium benzoate (Valencia-Chamorro *et al.,* 2008). The incorporation of ethanolic extract of propolis in hydroxypropyl methyl cellulose showed anti-fungal activity and improved the water vapour permeability giving rise to more rigid, less flexible, deformable, more opaque and colored films with lower gloss and transparancy (Pastor *et al.,* 2010).

2.4.5.3 Studies of edible coatings on various foods: The quality of the blueberries coated with Semperfresh^{TM} (Agricoat Industries Ltd., UK), acid soluble chitosan, water soluble chitosan, calcium caseinate, and sodium alginate were studied. It was reported that calcium caseinate coated fruit delayed fruit ripening which was evidenced by higher acidity, lower pH, and greater firmness when compared with the control (Duan *et al.,* 2011). The edible coating of chitosan on Eksotika II papaya (*Carica papaya)* showed effective control in reducing weight loss, maintaining firmness, delayed changes in peel color and soluble solids concentration during a 5

week period of storage at 12±1°C and 85-90 % relative humidity (Ali *et al.*,2011). Coatings of sucrose fatty acid esters reportedly are effective moisture barriers for maintaining crispness of snack foods (Kester *et al.*, 1990) and for extending the shelf life of apples (Drake *et al.*, 1987). Limonene and peppermint oil were incorporated into the modified chitosan to create bioactive edible coatings and were tested for the ability of the film to extend the shelf life of fresh strawberries during storage. Formulations based on modified chitosan containing LIM and Tween 80 were shown to perform better than other formulations (Vu *et al.*, 2011). Chitosan-based edible coatings were used to extend the shelf-life and enhance the nutritional value of strawberries (*Fragaria ananassa*) and red raspberries (*Rubus ideaus*) stored at either 2°C and 88% relative humidity (RH) for 3 weeks or −23°C up to 6 months using three chitosan-based coatings (chitosan, chitosan containing 5% gluconal CAL, and chitosan containing 0.2% DL-α-tocopheryl acetate). Results indicated that adding high concentrations of calcium or Vitamin E into chitosan-based coatings did not alter their anti-fungal and moisture barrier functions but significantly decreased decay incidence and weight loss, and delayed the change in color, pH and titratable acidity of strawberries and red raspberries during cold storage (Han *et al.*, 2004). Edible coatings of carrageenan and whey protein concentrate on the cut apple slices effectively extended the shelf life of the minimally processed apple slices by 2 week when stored in the packed trays at 3°C. Addition of 1% $CaCl_2$ inhibited the loss of firmness significantly ($p<0.05$) (Lee *et al.*, 2003). A chitosan coating on sliced mango placed in plastic trays and over wrapped with PVDC film and then stored at 6°C, retarded water loss and drop in sensory quality, increasing the soluble solid content, titratable acidity, and ascorbic acid content and inhibited the growth of microorganisms (Chien *et al.*, 2005). Tomatoes at breaker and pink stage of maturities were coated with corn-zein film. Color, weight and firmness changes and sensory quality were compared with non-coated tomatoes during storage at 21°C. Corn-zein film delayed color change and loss of firmness and weight during storage. Shelf life was extended by 6 days with film coatings as determined by sensory evaluation (Park *et al.*, 1994). The application of chitosan coating partially inhibited decay of litchi during storage, however increasing the concentration of chitosan coating did not increase the beneficial effects of the chitosan on browning and decay of fruit, when it was treated with aqueous solutions of 1.0 or 2.0 % chitosan coating for 1 h after dipping in 0.1% thiabendazole (TBZ) and then stored at 4°C and 90% RH (Zhang and Quantick, 1997). Borage containing film on the horse mackerel patties showed protective effect on the lipid

oxidation of patties during the storage period of 240 days. The patties were evaluated in terms of peroxide value, thio barbituric acid reactive substances and polyene ratio (Giminez *et al.,* 2011). Cinnamon (*Cinnamomum zeylanicum*) and nisin in alginate calcium coating were used to maintain the quality of the northern snake head fish fillets at refrigeration temperature of 4±1°C, the fishes were treated with different compositions of cinnamon and alginate and it was reported that coating had better effect on inhibiting bacterial growth, maintaining values of pH, total volatile base nitrogen and thiobarbituric acid (TBA) (Lu *et al.,* 2010).

2.4.5.4 Mechanical properties of film using plasticizers: Sorbitol, glycerol and combination of sorbitol and glycerol in ratio of (1:1, 1:3 and 3:1) were used as plasticizers for sago starch based films and it was reported that all were heat sealable, and highest heat seal strength was obtained at combination of sorbitol/glycerol in ratio of 3:1 (Abdorreza *et al.,* 2011). Biodegradable films can be prepared using rice flour, cellulose fibers with glycerol or sorbitol as plasticizers. The study reported that films incorporated with fibers improved mechanical strength and exhibited high tensile strength but it did not influence the deformation capacity (Dias *et al.,* 2011). Sodium caseinate films are incorporated with Tung oil in three different levels in order to reduce the water affinity of the protein based films. It was reported that the oil affects the mechanical response of the film, increasing tensile strength and elastic modulus but decreasing deformability. However, Tung oil incorporation into the caesinate matrix did not affect significantly the water vapour permeability probably due to pore formation (Pereda *et al.,* 2009). Gennadios *et al.,* (1997) reported that when high water vapour-transmitting hydrophilic edible films are measured with the cup method, the resistance of the stagnant air layer can be eloquent and, if neglected, can lead to underestimation of water vapour transmission rates. For both a methylcellulose and a corn zein film, water vapour permeabilities measured with air gaps of 1.0 and 1.5 cm were statistically significantly ($\alpha = 0.05$) different.

2.4.5.5 Performance and formation of new edible films from different sources: Composite films made from gelatin and low methoxyl pectin from reversible and permanent polyion – complex hydrogels improved the mechanical performance and water resistance but it did not alter the thermal stability of the film (Farris *et al.,* 2009). Shellac coatings when formed as supporting matrix to provide effective barrier properties to gases and water vapour (Hagenmaier and Shaw, 1992). Lipids existing in a liquid state or having a large proportion of liquid

components offer less resistance to gas and vapour transmission than those in a solid state (Kamper and Fenemma, 1984; Kester and Fennema, 1989a, 1989b), indicating their molecular mobility of lipids detracts from their barrier properties. Rao *et al.* (2010) prepared film using chitosan and guar gum, with different proportions of guar gum varying from 0-50% (v/v) and evaluated the properties like transparency, opacity, color, water vapour transmission rate, oxygen permeability, mechanical and antibacterial properties. Films containing 15% (v/v) showed very low oxygen permeability, good tensile strength and puncture strength. Gontard *et al.* (1994) studied the water vapour permeability, dispersion in water, opacity and mechanical properties of composite films made up of wheat gluten and various concentrations of lipid fractions. It was reported that Beeswax, a solid and highly hydrophobic lipid, was the most effective lipid for improving moisture barrier properties of films; but these films were opaque, weak and disintegrated easily in water. Combining wheat gluten proteins with a diacetyl tartaric ester of monoglycerides reduced water vapour permeability, increased strength and maintained transparency. Maftoonazad *et al.* (2006) evaluated the effect of pectin, beeswax and sorbitol concentration on the opacity, mechanical properties and water vapour permeability using the response surface methodology. It was reported that increasing the amount of pectin and decreasing the sorbitol concentration increased tensile strength (TS) and modulus of elasticity (EM), while elongation at break (EB) increased by increasing both pectin and sorbitol concentration. Methoxy poly(ethylene glycol)-grafted-chitosan (mPEG-g-CS) was attempted to increase the water solubility of Chitosan(CS), and it was applied as wall material to microcapsulate algal oil. Algal oil contained within microcapsules was protected from UV-light and heat compared with free algal oil. Release rate of algal oil was initially rapid and then followed by controlled release with the erosion mechanism playing a dominant role. The mPEG-g-CS proved to be a promising wall material with excellent solubility and film forming ability, and therefore supplied great potential applications in food industry (Peng *et al.,* 2010). An increase in the glycerol content in the hydroxypropyl methyl cellulose and bees wax composite film increased the film flexibility and water vapour permeability, whereas the increase in the mannitol enhanced the film brittleness without effecting the water vapour permeability for the quality and shelf life studies on Angeleno plums (Tarazaga *et al.,* 2008). The melting point of the composite film made from the wheat protein isolate and gelatin increased by 9°C compared to

the single component films but led to the decline of the puncture strength of the film. Inturn the water vapour permeability and deformation increased with gelatine content (Jiang *et al.,* 2010).

Laminar structures improved the moisture barrier properties but resulted in the more brittle, less stretchable, more opaque and less glossy films depending on the particle size, when the edible films of hydroxypropyl methyl cellulose based coatings were added with lauric acid, palmitic acid, stearic acid, and oleic acid in the ratio of 1:0.15 of polymer to lipid ratio (Jimenez *et al.*, 2010). The rate of the weight loss and the changes in the chemical structure and texture of the peaches were lower with the beeswax-coconut oil emulsion coated peaches than the uncoated peaches, while the minimum acceptable shelf-life for uncoated samples was 10 days, the samples coated with this emulsion maintained their acceptability for 14 days. The coating of the peach surfaces with this emulsion decreased the water vapour and oxygen gas transfer, resulting in the diminished respiration rate thus increased the shelf-life of the fruit (Erbil and Muftugil, 1986). A study was performed on oranges on their ability to retain flavor components at 21°C on comparison with uncoated fruits. The coated fruits showed increase in components considered important to fresh orange flavor. The use of bees waxes emulsion and TAL Pro-long alone or in combination was the most effective coating in retaining or increasing the volatile compounds (Nisperos-Cariedo *et al.,* 1990). Use of Nature seal ^{TM} 1020, a cellulose based edible coating as carrier of anti-oxidants, acidulants, and preservatives prolonged the shelf life of cut apple and potato by about 1 week when stored in over wrapped trays at 4°C, whereas the storage of the same in vacuum packed trays reduced weight loss and browning compared to over wrapped tray storage. It is reported that the addition of soy protein to the original cellulose based coating reduced the permeability to oxygen and water vapour (Baldwin *et al.,* 1996). Films made from the high amylase starch showed lower water vapour vapor permeability and gas permeability than regular corn starch films. Permeabilities of films with sorbitol were lower than the glycerol as plasticizer. Differential scanning calorimetry experiments demonstrated that the films with plasticizer and lipid showed lower crystalline amorphous ratio compared to films without additives (Garcia *et al.,* 2000). Polysaccharide based and carnauba wax applied on mango fruits separately reported that both films created modified atmospheres, reduced decay and improved appearance by imparting a subtle shine, but only the polysaccharide coating delayed the ripening and increased the concentrations of flavor volatiles and carnauba wax coating significantly reduced water loss compared to uncoated and polysaccharide coating

treatments ($p<0.05$) (Baldwin *et al.*, 1999). There is reduction in the water vapour permeability of the wheat gluten films with increasing curing temperature and exposure time. Hunter L (whiteness) decreased whereas the a(redness) and b(yellowness) values increased with increasing heat treatment temperature and exposure time, when physical properties of the film were studied for effect of thermal treatments on a wheat gluten film (Ali *et al.*, 1997).

Since no study was done on the extension the shelf life of cherry tomatoes, it was decided to study the effect of a formulated pectin and alginate based composite coatings on extending the shelf life of whole cherry tomatoes. This study is presented in chapter 3.

From the conclusions of the introduction chapter, it is understood that the second objective of this study was to evaluate the effect of a hot water hormesis treatment on enhancing the nutritive components, color and firmness of fruits. Hence a literature review introduction on the importance of antioxidants in food and human nutrition followed by the stress induced enhancement of antioxidants in various studies on the production of stress metabolites under abiotic stress, is presented in in the next section of this chapter.

2.5 Importance of antioxidants in food and human nutrition: An antioxidant is defined as a molecule which has the ability to inhibit the oxidation of other molecules. When a food is oxidized, it transfers electrons from the food to the oxidising agent, thereby forming the free radicals. Antioxidants prevent the formation of the free radicals by themselves undergoing oxidation. Hence they are also called as reducing agents.

Auto-oxidation of lipids and generation of the free radicals are natural phenomenon in biological and food systems. In biological systems various biochemical defense mechanisms involving enzymes, trace minerals, antioxidant and vitamins protect the cellular components from oxidative damage. In food systems, naturally occurring antioxidants impart a certain amount of protection against oxidation. In food, antioxidants retard the onset of the lipid oxidation in food products.

These antioxidants can be used for controlling degenerative oxidation reactions caused by reactive oxygen and free radical species in living tissues and the inhibition of lipid peroxidation in foods caused by processing and during storage (Halliwell, 1994).

In biological systems, the formation of the reactive organic free radicals is mediated by a number of factors like high oxygen tension, radiation and xenobiotic mechanism. The free radicals formed are highly reactive with molecular oxygen forming peroxyradicals, hydro peroxides and thus initiating a chain reaction. Pro-oxidant states causes cellular lesions in all major organs by damaging cellular components like polyunsaturated fatty acids, phospholipids, free cholesterol, DNA and proteins. In foods, lipid oxidation will deliberately cause many problems during handling, processing and storage. Oxidation of the unsaturated lipids in the food systems is catalysed by light, heat, ionizing radiation, trace metals, metalloproteins, and enzymatically by lipoxygenase. Lipid oxidation is one of the prime factor for the development of the off flavor compounds, rancidity and other reactions that reduce the shelf life and nutritive value of foods. Amino acids, carnosine, citric acid, Eugenol, lecithin, phytic acids, proteins, saponins, tartaric acids, turmeric, vitamin E, β carotene, carnosol, curcumin, flavonoids, lignans, phenolic acids, sterols, uric acid, vitamin C and vanillin are examples of naturally occurring anti-oxidants. The primary biological role of anti-oxidants is in preventing the damage that the reactive free radicals can cause to cells and cellular components. In fact almost all the nutrient constituents identified in the dietary survey thus far, as having a protective effect against specific diseases, seem to have some kind of antioxidant properties.

In human metabolic activity, free radicals cause damage to lipids, protein and nucleic acid by oxidation which may lead to disease development (Bagchi *et al.,* 2004). Antioxidants can neutralize free radicals to prevent oxidative damage in the prevention of many diseases (Wang *et al.,* 1996). Fruits and vegetables are a good source of antioxidants, including mainly polyphenols and flavonoids. The normal intake of polyphenols in an American diet is estimated to be around 1g or more per day (Kühnau, 1976).

The biological role of flavonoids and polyphenols has not fully been explained through research; however it has been proven that consumption of these compounds by dietary means has long term health benefits to consumers (Miller *et al.,* 1995). Polyphenols play a major role in various natural defence mechanisms in plants as a response to environmental stress factors such as light, temperature, ultraviolet radiation and other internal factors like nutrients and hormones which all can potentially induce their synthesis. Polyphenols play a vital role in plant disease resistance (Nicholson and Hammerschmidt, 1992). Within the nutraceutical category, these antioxidants,

such as vitamins C and E, carotenoids, and phenolic compounds, have preventive roles against certain cancers and cardiovascular diseases (Scheerens, 2001).

2.6 Stress induced enhancing of antioxidants: Plants when exposed to the unfavourable environments like water deficit, chilling, heat stress, oxygen deficiency and air pollution will result in the expression of its immunity towards these factors genetically in its progeny; hence stress provided on the post-harvest produce could be classified into two categories as biotic stress and abiotic stress. The stress which is provided by the living organisms like microbes, insects, weeds etc., is called as the biotic stress. The stress which provided by the nonliving factors such as light, wind, ultra violet radiation, pulsed light, pulsed electric field, high pressure is called as the abiotic stress.

An abiotic stress is the stress caused by environmental conditions to plants (i.e. extreme weather conditions, ultraviolet radiation, light, etc.). Plants produce or stimulate synthesis of various secondary metabolites when they are subjected to various abiotic stresses. Various stresses can induce polyphenols' synthesis in plants, especially in fruits and vegetables. Dixon and Paiva (1995) reported stress induced phenyl propanoid mechanisms in plants. These metabolites are produced to defend the plant against abiotic stresses.

The secondary metabolites mainly include various antioxidants which have reported health benefits. Researchers have explored the effect of ultraviolet light on the development of secondary metabolites in fruit and vegetables (Arakawa, 1993; Baka *et al.*, 1999), which has led to the use of abiotic stresses to enhance the nutritional content and storability of fruits and vegetables. Some researchers have explored the use of pulsed ultraviolet light to induce vitamin D2 content in mushrooms (Kalaras and Beelman, 2010). Abiotic stresses will affect the pathways involved in the biosynthesis of the 3 principal groups of secondary metabolites: terpenes, phenolics, and nitrogen-containing compounds (Scheerens, 2001). Due to their biological activity, secondary metabolites are used commercially as insecticides, fungicides, pharmaceuticals, fragrances, flavorings, medicinal drugs, and industrial materials (Gershenzon, 1998).

2.7 Production of stress metabolites: Improving the health benefit properties of fresh produce will add value and create new opportunities for growers and processors by reaching these health-

oriented markets to meet the standards of the consumers whose focus is shifting towards the health promoting foods. To achieve this goal, there is a need to provide technologies that can ensure the delivery of high quality products with high levels of the desired nutraceuticals. Hormesis is the application of potentially harmful agents at low doses to living organisms in order to induce stress responses. When fruit are exposed to low doses of UV a number of changes are induced including the production of anti-fungal compounds and delays in ripening. Both of these responses could be exploited by the horticultural sector to reduce postharvest losses (Shama and Alderson, 2005). Plants when exposed to the unfavourable environments like water deficit, chilling, heat stress, oxygen deficiency and air pollution will result in the expression of its immunity towards these factors genetically in its progeny. Hence stress provided on the post-harvest produce could be classified into two categories as biotic stress and abiotic stress. The stress which is provided by the living organisms like microbes, insects, weeds etc., is called as the biotic stress. The stress which provided by the non-living factors such as light, wind, ultra violet radiation, pulsed light, pulsed electric field, high pressure is called as the abiotic stress. Abiotic stresses will affect the pathways in fruits and vegetables when exposed in synthesis of three principal groups of secondary metabolites like terpenes, phenolics, and nitrogen-containing compounds. (Cisneros-Zevallos, 2003). These compounds serve the plants as the defences against the herbivores, pathogens, and act as aid for attracting the pollinators and seed dispersal animals. These compounds gained attention due to their wide application commercially as pharmaceuticals, fragrances, flavorings, medicinal drugs, and industrial materials (Gershenzon, 1998). Post-harvest exposure of the Napoleon table grapes to ultraviolet radiation B and C, induced a large increase of the resveratrol derivatives which means the serving of 200 g of the grapes would provide approximately 1mg of the resveratrol which is supplied by one glass of red wine. It was reported that the refrigerated storage and UV exposure of the grapes can be beneficial in terms of the increase in its content of health promoting substances (Cantos *et al.*, 2002). The higher the intensity of light during the growing season, the greater is the vitamin C content in plant tissues. Temperature management after harvest is the important factor to maintain vitamin C of fruits and vegetables; losses are accelerated at higher temperatures and with longer storage durations. Conditions favourable to water loss after harvest result in a rapid loss of vitamin C especially in leafy vegetables (Lee and Kader, 2000). It has been observed that various abiotic stresses can be used to induce polyphenol accumulation in

purple flesh potato (Reyes and Cisneros-Zevallos, 2003). Ethylene, methyl jasmonate, temperature and light were not found to increase the polyphenolic content and antioxidant activity significantly ($p<0.05$). It was found that wounding increased the polyphenols content to about 60% and antioxidant activity to about 85%. The phenol accumulation caused by wound stress, was not same in the flesh as compared to the peel. The polyphenols in the flesh increased by about 61% but in the peel it only increased by around 41 % as compared to the control (Reyes and Cisneros-Zevallos, 2003). Lowering the storage temperature was found to increase the total flavonoid content in potatoes when stored at a temperature of 4°C. Potatoes showed an increase in flavonoids and anthocyanins concentrations. Anthocyanin increased from 350 to 430 µg /g (fresh weight) and phenolic acid increased from 2500 to 2800 µg/g (fresh weight) but there was no significant increase of flavonoids and total phenolic acid at higher temperature ($p>0.05$) (Lewis *et al.,* 1999). The maximum anthocyanin accumulation of 13.8 CV (color value)/g fresh cell weight occurred on 7th day when jasmonic acid was added to the cultures at a final concentration of 20µm on day 0. This represented an 8.5-fold increase compared with the control culture in the dark (Zhang *et al.,* 2002).

During cold storage at temperature of 4°C, the anthocyanin concentration in colored tubers increased, whereas tubers stored at higher temperatures did not show this increase. The increased color in cold-stored tubers is reported to be due to cold sweetening and the increased concentration of sugars in cold-stored tubers (Lewis *et al.,* 1999). Irradiation of *Monastrell* grapes with UV-C radiation of light was proposed as a potential method to produce stilbene-enriched red wine with resveratrol and piceatannol, which are molecules with reported health-beneficial activities. The final wine made from UV-C irradiated grapes was enriched about 2-1.5-fold resveratrol and piceatannol, respectively, when compared to the control wine. In addition, no difference was detected regarding the standard enological parameters (color, acidity, etc.) (Cantos *et al.,* 2003).

An increase in the resveratrol content was reported in UV treated Napoleon table grapes to nearly 11 times greater than non-treated control samples. This was achieved at power level of 510 W for the illumination duration of 30 sec and storage period of 3 days at a distance of 40 cm from the source. Furthermore, there were no change in total phenolics content, firmness, color, size and mass of the treated fruits (Cantos *et al.,* 2001). Estrada *et al.,* (1999) determined the

effect of water on biological parameters of the phenyl prepanoid metabolism, it was reported that the amount of capsaicinoids (capsaicin and dihydrocapsaicin) in Padron pepper fruits of water-stressed plants, was higher than that in control plants, especially with low water treatment and it was suggested that the environmental conditions, such as water stress, have a strong effect upon the accumulation of capsaicinoids in Padron pepper fruits.

Wu *et al.* (2010) studied the effect of refrigerated storage on the bioactive compounds and anti-oxidant capacities of *maron* and *evergreen* varieties of blackberries at 2°C and 95% RH. It was reported that during storage, the bioactive compounds fluctuated in *maron* variety but declined in the *evergreen* variety of the blackberries. The 6-month room storage had little effect on the bioactive compounds of frozen and freeze dried samples, but reduced anthocyanin contents in hot-air dried, canned, and jam samples, and antioxidant capacities of all samples. Measuring the accumulation of the anthocyanins and quercetin glycosides after irradiation with UV-B visible light in the skin of the apple fruit of varieties Gala and royal gala at temperatures of 10°C and 20°C for five harvests upto 2 weeks after the start of the harvest reported that the lower temperature of 10°C prevented the accumulation of the anthocyanins but not quercetin. The fruits skin's previous exposure to the light and temperature are the major modifying factors in the accumulation of anthocyanins in Gala and Royal gala (Reay and Lancaster, 2001). The effect of light on anthocyanin production in apple (*Malus pumila* Mill. cv. Jonathan) skin disks was investigated, with prolonged irradiation from different light sources, and it was reported that when UV-B light, provided by a fluorescent lamp which had an emission peak at 312 nm, was combined with the white light, anthocyanin production was synergistically stimulated and increased up to the highest fluence rates of white light tested (44 W m^{-2}). This UV-B light was more effective than red and blue light provided from fluorescent lamps, but anthocyanin production became saturated at about 1.7 W m^{-2}(Bakshi and Arakawa, 2006). Kalt *et al.* (1999) investigated the effect of storage temperature on small fruits, i.e. fresh strawberries (*Fragaria ananassa Duch*.), raspberries (*Rubusidaeus Michx*.), 15 highbush blueberries (*Vaccinium corymbosum* L.), and lowbush blueberries (*Vaccinium angustifolium Aiton*). They stored the fruits at different temperatures i.e. 0, 10, 20 and 30°C for 8 days. They observed that the anthocyanin and antioxidant content of all the fruits stored at different temperatures were affected positively. All fruits showed increased amount of anthocyanin and antioxidant activity for temperatures above and below the 20°C control temperature. Anthocyanin increased in

strawberry to about 1.7 folds at 0°C while the increase was higher at higher temperature, for example the anthocyanin increased in strawberry 6.8 fold at 30°C. In raspberry kept at 30°C, the anthocyanin increased 1.5 times more than the control kept at 20°C. Strawberries treated with UVC dosage of 0.25 kJ/m2 maintained good quality with good texture, higher titratable acidity, and higher anthocyanin content, while increasing the storage period by 3 to 4 days as compared to untreated fruits (Baka *et al.,* 1999).UV radiation can increase the anthocyanin pigment in cherries while maintaining the quality for the fruit (Arakawa 1993). Pepper quality was investigated following a UV-C treatment. It was reported that when UV-C (7 kJ/m^2) treated pepper experienced a delay in decay and a reduced susceptibility to chilling injury. No significant difference was found in color and firmness between treated and control peppers which were stored at 10°C ($p>0.05$), but antioxidant 22 capacity of pepper increased significantly when compared to control samples ($p<0.05$) (Vicente *et al.,* 2005).

Literature showed no research on the effect of hormesis on enhancing the nutritive components like antioxidants and polyphenols of cranberries. Hence cranberries are chosen to study the effect of abiotic hormesis by temperature on enhancing the polyphenols of cranberries by stimulating their synthesis.

The following sections give detailed information on cranberries from harvesting to storage, their antioxidant properties and health benefits.

2.8.1 ***Harvest Practices*****:** The growing environment strongly affects fruit quality. Factors such as soil fertility and pH, water availability, fruit pollination, pruning, plant age and vigor, and the presence of abiotic and biotic stresses can effect fruit growth, composition, and quality. For example, in blueberries, rates and application methods of fertilizers can affect fruit firmness (Pavlis, 2006). Augmenting nitrogen fertility in cranberries can reduce fruit storage-life by increasing storage rots (Davenport, 1996). Proper application and timing of fungicides, particularly around the time of bloom, can reduce latent infections and reduce fruit decay during storage in blueberries and cranberries (Bristow and Patten, 1995).

2.8.2 ***Maturity*****:** Cranberry fruit maturity is based primarily on color. Fruits develop color as a response to environmental temperatures and light. Therefore fruit in the upper canopy of the plant that receive the most light develop better color and ripen first, while fruit buried deep in the

canopy are slow to develop color and some fruit may remain white. Several studies have shown that better colored fruits store longer than white or poorly colored fruits. Ceponis and Stretch (1983) found that "*Early Black*" fruit that were highly colored had less physiological breakdown than less colored fruit within a harvest. Ozgen *et al.* (2002) also observed that dark-red '*Stevens*' fruits were more marketable fruit than light red, blush, or white fruit. They also noted that dark-red fruit had thicker cuticles and lower respiration rates than lighter color fruits. However, if fruits are left on the plant too long, they will be subjected to greater physiological breakdown during storage. When '*Early Black*' fruit were harvested early, middle, or late during the ripening period, greater physiological breakdown were reported to occur during storage of the late harvested fruit, but rates of decay were not affected by harvest time (Ceponis and Stretch, 1983).

2.8.3 ***Harvest method*****:** Cranberries are harvested by wet or dry methods. The most common method, wet-harvesting, involves scooping or raking of the fruit in flooded bogs with a motorized water reel (Norton, 1982). Dry harvesting involves hand picking, hand scooping, and machine scooping (Eck, 1990). Recently, a dry-harvester that works in a fashion similar to a vacuum cleaner has been developed in upstate New York (Bailey, 2000). Harvest methods influence storage life through the different degrees of bruising and stress imposed on the fruits (Davis and Shawa, 1983). The method of harvest and postharvest handling can have a large impact on fruit quality and storage life. The water reel beats the fruit off the plant resulting in the fruit floating in the water where it is collected. Wet rake harvesters remove fruit from the plant using a comb-like device and convey them out of the water into holding containers. Fruit for the fresh market may be harvested dry, which is done with dry raking machines or by hand raking. Swanson and Weckel (1975) found that water reel harvested '*McFarlin*' cranberries had several fold greater rates of spoilage during storage than fruit harvested by wet or dry raking. Similarly, fruit losses during storage of 6 cultivars harvested with a water reel harvester were 2 to 4.6 fold greater than with handpicked fruit (Stretch and Ceponis, 1986). Decay and physiological breakdown during storage increase as a result of prolonged water immersion (Ceponis and Stretch, 1981).

2.8.4 ***Post-Harvest Environment:*** Proper temperature management is the most critical factor in the postharvest handling of fruit. The recommended storage temperature for fresh cranberries in

various handbooks ranges from 2 to 7°C. These variable recommendations are based on reported chilling sensitivity of cranberry fruit (Levine *et al.,* 1941; Wright *et al.,* 1937) and variable responses of fruit to temperature among different studies. However, in a recent re-evaluation of the effects of storage temperature, no evidence of chilling sensitivity was found (Forney, 2008). When fruit were evaluated immediately upon removal from storage, the loss of marketable fruit was greatest in fruit stored at 10°C and least in fruit stored at 0, 3 or 5°C, among which there was no significant difference. In addition, after 2 months of storage the marketable fruit stored at 7°C was no longer different from the fruit stored at 0, 3 or 5°C. Differences in marketable fruits stored at 7 and 10°C were the result of greater amounts of physiological breakdown. No differences were observed for rates of decay among fruit stored at 0 to 10°C throughout the 6 month storage period. (Forney, 2008) Surprisingly, the relative humidity (RH) of the storage environment had a greater effect on fruit storage life than temperature, with the greatest losses of marketable fruit occurring in high RH of ~98% (Forney, 2008). After storage for 2 months or longer, fruits held in high RH were decayed and developed greater physiological breakdown than fruit held in medium (82% to 88%) or low (75%) RH regardless of storage temperature. After 5 months, the high and medium RH storages had 71% and 31% fewer marketable fruit than the low RH storage. Even after fruit was removed from the storage chambers and held an additional week at 20°C, fruit previously held in high RH continued to have the highest loss of marketable fruit. Others have also reported that storage RH of about 70% maintained better fruit quality than storage in 90% to 95% RH (Stark *et al.,* 1974; Wright *et al.,* 1937). Similarly, Hruschka (1970) reported that fruit stored in polyethylene bags that maintained a high RH had more decay and physiological breakdown than fruit stored in boxes, well-ventilated bags, or unlined cartons. Interestingly, the higher rates of weight loss observed in low RH does not affect fruit firmness (Forney, 2008). In addition to the effect of temperature on breakdown and decay, temperature also affects fruit color. At 2°C and above color tends to darken. Therefore, color can be improved in early harvested fruit, which tend to be pale in color by storing at 7 to 10°C for several weeks (Levine *et al.,* 1941). Pre-cooling cranberries can be performed using cold air (forced-air) or water (hydro-cooling). If significant field heat is present at the time of harvest, fruit may benefit from its rapid removal (Kaufman *et al.,* 1958). However, since cranberries are harvested late in the year when field temperatures are normally low, pre-cooling is normally not

done. If good air circulation is maintained through and around the fruit, room cooling can cool fruit to room temperature in 24 to 48h.

2.8.5 ***Storage:*** Cultivar and fruit maturity can also affect storage quality (Stretch and Ceponis, 1986). Optimum storage temperatures were reported to be 1.7 to 4.4°C (Swanson and Weckel, 1975). No consistent benefits of CA storage have been reported for cranberry fruit. In CA storage trials conducted with '*Stevens*' fruit, no extension of storage life was observed in fruit stored in 0, 5, 10 or 15 kPa CO_2 and 1 or 15 kPa O_2 at 5°C. Similarly, '*Howes*' cranberries, stored in all combinations of 0, 5, and 10 kPa CO_2 with 3, 10 and 21 kPa O_2 at 0 or 3°C had greater losses than the 3°C air control (Anderson *et al.,* 1963). Stark *et al.* (1969) found that cranberries stored at 22°C for 3 weeks in atmospheres of 5 or 10 kPa CO_2 with 3 kPa O_2 had the same levels of rot as air stored fruit, while fruit held in 100 kPa N_2 became dull and water soaked in appearance and had a fermented odor. Attempts to use more extreme atmospheres have also had limited success. Concentrations of 70 kPa O_2 had no beneficial effect in reducing decay or physiological breakdown and induced production of acetaldehyde, ethanol, and ethyl acetate (Gunes *et al.,* 2002). However, Gunes *et al.* (2002) did find that atmospheres of 30 kPa CO_2 and 21 kPa O_2 reduced decay and breakdown of '*Pilgrim*' and '*Stevens*' fruit when stored for 2 months at 3°C, but after 4 months these benefits were no longer apparent due to extensive breakdown (>97%) in all atmospheres tested. High relative humidity normally associated with controlled atmosphere storage appears to be detrimental to cranberry fruit storage life. When RH was lowered in CA storage chambers, fruit breakdown and decay in some atmosphere combinations maintained fruit quality similar to the air controls (Anderson *et al.*, 1963). Ozgen *et al.* (2002) studied the relationship between postharvest life of cranberry (*Vaccinium macrocarpon Ait. cv. Stevens)* fruit and ripeness stage at harvest. Wet harvested, mature fruit were sorted and rated for quality after 4 and 7 weeks of cold storage at 3°C. In addition, CO_2 and ethylene production as well as anthocyanin content were measured. It was reported that the color changed for each grade and the ethylene production was nearly the same for all the ripeness stages. Studies suggest that red fruit have longer postharvest life, possibly because of lower respiration rates, thicker cuticle and wax accumulation (especially at the calyx end) on these fruit may retard the entry of microorganisms into the fruit during wet harvest and may mitigate mechanical injury by harvesting equipment (Ozgen *et al.,* 2002). Initial treatments with N_2 gas have been shown to improve cranberry storage life by reducing fungal decay (Lockhart *et al.,*

1971). These studies were carried out before the advent of rapid and commercial CA storage technology in which CA regimes are imposed within a few days of harvest, and in addition, further research is needed to identify potential benefits and limits of tolerance of cranberries to CO_2 (Kader, 1997). Wszelaki and Mitcham (2000) found that super atmospheric O_2 reduced the decay of strawberries; no studies on the effect of this technology are available for cranberries. Although information about the effects of air storage on antioxidant capacity and associated antioxidant compounds of strawberries, raspberries, and blueberries is available (Kalt *et al.*, 1999), little is known about the effects of postharvest treatments, especially CA storage, on the antioxidant and phenolics contents of fruit. Wang and Stretch (2001) found that the antioxidant activities and anthocyanin and phenolic contents of cranberries increased during storage, with maximum increases occurring at 15°C compared with 0, 5, 10, and 20°C. Cranberries can be stored for 2-3 months with adequate cooling and ventilation, but quality losses can be high because of shrinkage due to moisture loss, physiological breakdown, end rot, and fungal diseases (Stretch and Ceponis, 1986). When a selection of Wisconsin-grown cultivars were stored at 4°C, '*Stevens*' and '*McFarlin*' had the greatest storage-life compared with '*Howes*', '*Searles*', '*Black Veil*', and '*Metallic Bell*' (Swanson and Weckel, 1975). Similarly, when New Jersey-grown cranberries were stored for 12 weeks at 3°C followed by 4 days at 21°C, '*Franklin*' and '*Pilgrim*' were superior to '*Early Black*', while '*Ben Lear*','*Wilcox*', and '*Stevens*' were intermediate (Stretch and Ceponis, 1986).

2.8.6 ***Postharvest Treatments:*** Exposure of harvested fruit to 0.1 to 10 µL/L ethylene increased color formation in poorly colored fruit. This effect of ethylene was greatly enhanced when fruit were exposed to light (Craker, 1971). Heat treatments using hot water or air can reduce decay and spoilage during storage by killing pathogens or altering the physiology of the product. Hot water treatments of 60°C for 15 or 30 sec extended the storage life of *'Burlington'* blueberry fruit (Fan *et al.,* 2008). After 4 weeks of storage, 92% of the hot water treated fruit were marketable compared to 76% in the untreated controls. In addition to controlling decay, heat treatments reduced shriveling, splitting, and weight loss of fruit. Hot water treatments also reduced fruit titratable acidity, soluble solids content, and the waxy bloom on the fruit, while inducing an increase in ethanol. Hot water treatments of '*Stevens*' cranberries were conducted over 2 seasons. Treatments consisting of 50°C for up to 180 sec reduced decay and physiological breakdown in fruit following 2 to 6 months of storage at 3 or 7°C and 86% RH. Decay was reduced by about

20% by the 180 sec treatment, while physiological breakdown was reduced by 6% to 36% by the 90 s treatment, depending on the season and storage duration. Steam treatment of 50°C for 90 s also was effective in reducing decay 34% and physiological breakdown 21% of 'Stevens' cranberry fruit following storage for 4 months at 7°C. Anderson and Smith (1971) reported that hot water treatments of 49°C for 150 or 300 s and 52°C for 150 s had some benefits in prolonging cranberry storage life. However, treatments were less effective on late harvested fruit, where heat treatments increased physiological breakdown.

Exposure of blueberry fruit to ultraviolet-C (UV-C) radiation showed slight effect on postharvest decay and phenolic metabolism. '*Collins*' and '*Bluecrop*' blueberry fruit exposed to 1, 2, or 4 $kJ.m^{-2}$ UV-C light had a 10% reduction in decay primarily caused by ripe rot (*Colletotrichum acutatum*) after storage for 7 days at 5°C followed by 2 days at 20°C (Perkins-Veazie *et al.*, 2008). Anthocyanin content was increased 10% by 2 and 4 kJ m^{-2} UV-C light treatments in '*Bluecrop*', but was reduced by the 4 kJ m^{-2} UV-C light treatments in '*Collins*'. Ferric reducing antioxidant power (FRAP) values were decreased by the 2 and 4 kJ m^{-2} UV-C light treatments in 'Collins' but increased by all UV-C treatments in 'Bluecrop'. The UV-C treatments had no effect on weight loss, firmness, titratable acidity, or soluble solids content of the fruit. In cranberry fruit, there was no significant effects of 0.5 to 2.0 KJ m^{-2} of UV-C light on decay or physiological breakdown of 'Stevens' cranberries following storage at 7°C for 3 or 6 months (Forney 2008). Cranberries (*Vaccinium macrocarpon Aiton*) were treated with high voltage electric fields (HVEF) of 2, 5 or 8$kVcm^{-1}$in strength for 30, 60 or 120 min in a parallel plate electrode system. and stored at ambient conditions (23°C and 65% RH) for three weeks to study the effect of treatments on their respiration rate, physiological loss of mass, color, total soluble solids (TSS) and skin puncture strength (Palanimuthu *et al.*, 2009).

2.8.7 ***Studies on antioxidant and polyphenolic properties of cranberry fruit:*** Peonidin-3-glucoside (41.9%) and cyanidin-3-glucoside (38.3%) were the main anthocyanins isolated from fruits of *Vaccinium oxycoccus* L. (small cranberry) (Andersen, 1989). Smaller amounts of the 3-monogalactosides and 3- monoarabinosides of peonidin and cyanidin were found in addition to the 3-monoglucosides of delphinidin, petunidin and malvidin. The total anthocyanin content in the fruit averaged 78 mg/100g fresh fruit. This anthocyanin pattern is different from that of the American cranberry (*Vaccinium macrocarpon* L.) (Andersen, 1989). The

major anthocyanins in cranberry are galactosides and arabinosides of cyanidin and peonidin (Fuleki and Francis, 1968). Vaccinium fruits are among the most plentiful food sources of anthocyanin. Content varied widely among cranberry cultivars, averaging 25–65 mg/100 g of ripe fruit at harvest (Wang and Stretch, 2001), with reports of anthocyanin content as high as 100 mg/100 g fresh fruit weight (Vvedenskaya and Vorsa, 2004). Fruit of the Early Black cultivar was significantly higher in anthocyanins and proanthocyanidins than most other cranberry cultivars (Vorsa *et al.,* 2003). The effects of light and abscisic acid (ABA) on the postharvest ripening of late-harvested white cranberries (*Vaccinium macrocarpon Ait*.) were determined. Storing cranberries in light resulted in visibly more red fruit with a 4-fold higher concentration of anthocyanins than fruit held in the dark. Fruit held in light also had 28% more phenolics and 24% higher antioxidant capacity than fruit held in the dark (Forney and Walt 2009).

2.8.8 ***Health benefits****:* Cranberry ranks high among fruit in both antioxidant quality and quantity (Vinson *et al.,* 2001) because of its substantial flavonoid content and a wealth of phenolic acids. Cranberry extracts rich in these compounds reportedly inhibit oxidative processes including oxidation of low-density lipoproteins. The antioxidant properties of the phenolic compounds in cranberry fruit may contribute to the observed antitumor activities of cranberry extracts, but recent studies suggest that cranberry's anticancer activity may involve a variety of mechanisms. In 2002, a University of Illinois study revealed that extracts of whole cranberry containing pro-anthocyanidins and other flavonoids inhibited Ornithine Decarboxylase (ODC) activity in mouse epithelial (ME-308) cells (Kandil *et al.,* 2002). Characterization of an active subfraction revealed the presence of dimers and oligomers of catechin epicatechin, monomeric catechins, and quercetin glycosides. ODC has an important role in the biosynthesis of polyamines involved in cellular proliferation. A UCLA study showed that water soluble cranberry phenolic extracts prepared from commercial cranberry powder effectively inhibited proliferation of several human tumor cell lines (Sreeram *et al.,* 2004). A study by Canadian researchers reporting that cranberry juice inhibited breast tumor growth appeared in 2000 (Guthrie, 2000) and was followed by a more detailed study showing that an extract of cranberry press cake inhibited proliferation of MCF-7 and MDA-MB-435 breast cancer cells (Ferguson *et al.,* 2004). In fact, cranberry phenols have been shown to protect against ascorbic acid destruction (Larson, 1988). Flavonoids such as anthocyanins, flavonols, and proanthocyanidins have been found to significantly reduce mortality rates due to coronary heart disease (Armstrong *et al.,* 1975; Heinonen *et al.,* 1989;

Verlangieri *et al.,* 1985; Wang *et al.,* 1996) and to have anticancer properties (Ames 1983; Ames *et al.,* 1993; Bomser *et al.,* 1996; Dragsted *et al.,* 1993; Willet, 1994). Members of the *Vaccinium* genus, such as blueberries, contain high amounts of anthocyanins, as can be seen by their deep violet pigmentation (Kalt and Dufour, 1997). Anthocyanins have been used for several therapeutic purposes including the treatment of diabetic retinopathy, fibrocystic disease, and vision disorders (Leonardi *et al.,* 1993; Politzer, 1977; Timberlake and Henry, 1988). Anthocyanins also have the potential to serve as radiation protective agents, vasotonic agents, and chemo protective agents (Wang *et al.,* 1997). In addition to its antioxidant effects, blueberry anthocyanins can act against carbon tetrachloride-induced lipoperoxidation (Morazzoni and Bombardelli, 1996).

Based on the background material presented so far, the thesis research was focussed on evaluating the effect of pectin and alginate composite coatings on extending the shelf life of cherry tomatoes (chapter 3) and effect of abiotic hormesis on enhancing the post-harvest quality of cranberries (chapter 4) followed by general summary, conclusions and future research recommendations (chapter 5).

CONNECTING STATEMENT

TO CHAPTER 3

Chapter 3, which follows, highlights the evaluation of formulations based on pectin and alginate based composite coatings, found successful in previous studies in our laboratory, for cherry tomatoes for their shelf life extension and quality loss reduction. The fruits were coated with pectin and alginate coatings and stored at three different temperatures of 4, 12 and 24°C for comparative evaluation. The quality parameters were evaluated at selected intervals and compared to the control. The coatings worked to extend the shelf life and retard the rate of change of quality factors and physiological activities like respiration, transpiration and ripening.

Part of the results of this study has been prepared for publication: **Narayanapurapu, P.T.R.** and Ramaswamy, H.S., 2012. Comparitive evaluation of pectin and alginate based composite coatings on extending the shelf life of cherry tomatoes (to be submitted).

The experimental results of the alginate coated samples were presented at Conference of Food Engineering, Leesburg, VA, USA, April 2012, and the results of pectin coated cherry tomatoes were accepted for IFT Annual meeting, June, 2012.

Experimental work and data analysis were carried out by the candidate under the supervision of Dr. HS Ramaswamy.

CHAPTER 3

COMPARITIVE EVALUATION OF PECTIN AND ALGINATE BASED COMPOSITE COATINGS ON EXTENDING THE SHELF LIFE OF CHERRY TOMATOES

Abstract

The post-harvest produce perishes due the accelerated rate of respiration, transpiration and ripening. The objective of this study was to determine the effect of pectin and alginate based composite coatings on the shelf life extension of cherry tomatoes. Composite coatings contained 3% pectin, 2% alginate, 1.35% sorbitol, 1.2% bees wax and 0.36% monoglycerides. Whole cherry tomatoes were coated with the composite coating and air dried at room temperature for a period of 3 h, transferred to polyethylene tetra phthalate preformed containers and stored at selected temperatures (4, 12 and 24°C) along with a control. Several quality parameters of the fruit - weight loss, respiration rate, color, texture, titratable acidity, total soluble solids, pH and total polyphenols were evaluated during selected intervals. The coating had significant effect ($p \leq 0.05$) on extending the shelf life of the fruits. The control had a shelf life of 12, 15 and 18 days, at 24, 12 and 4°C, respectively, whereas the treated samples had shelf life of 15, 18 and 21 days demonstrating a 17-25% enhancement. The reduced respiration and transpiration rates as a result of coatings were considered responsible for maintaining the quality and increasing the shelf-life of cherry tomatoes.

3.1 Introduction

Fresh fruits and vegetables are rich sources of antioxidants. As such, a high intake of fresh fruits and vegetables has been demonstrated to be protective against both heart disease and certain types of cancer (Giovannucci, 1999). Tomato (*Lycopersicon esculentum Mill.*) is an important fruit worldwide, both for the fresh and the processed markets. It is available all year round and is rich in nutritional components including vitamin C, flavonoids and carotenoids, which are believed to be beneficial to human health (Wold *et al.,* 2004). Lycopene and beta-carotene comprise about 78 and 7%, respectively, of the total carotenoid content of tomatoes (Rao *et al.,* 1998). It is believed that carotenoids offer protection against some forms of cancer, possibly because of their antioxidant properties. In addition, beta-carotene may also promote health through its pro-vitamin A activity. Tomato commonly used in the Mediterranean diet, is a major

source of antioxidants. Tomatoes are consumed fresh or as processed products (canned tomatoes, sauces, juice, ketchup, soup). The consumption of fresh tomatoes and tomato products has been inversely related to the development of some types of cancer and to plasma lipid peroxidation (Parfitt *et al.,* 1994; Balestrieri *et al.*, 2004).

Tomato fruits are harvested at different ripening stages (colors) from mature green to red depending on the market and consumer requirements. According to Wills and Ku (2002), tomato fruits are often harvested at a mature green stage to minimize damage during transport to market and may later be treated with ethylene before shipment to retailers. However, when fruits are harvested mature green, they may not have developed the ability to produce much flavour when fully ripe. On the other hand if fruits were harvested after coloration, they would have a very short postharvest life because of the advanced ripening stage.

Tomato fruits have a relatively short postharvest life and, during the fruit ripening, various physiological activities influence the fruit quality. The post-harvest loss due to spoilage of fruits and vegetables is very large and this means that a method to control ripening would be of great economic importance (Hoeberichts *et al.,* 2002). Delaying the fruit ripening process would allow producers more time for shipment and increase the shelf life of the fruit for consumers. Klee *et al.* (1993) suggested that, if the ripening process could be slowed down sufficiently, a tomato fruit could be preserved for long enough to develop a superior taste and still be sold to the consumer before it spoiled.

Lenucci *et al. (*2006) carried out a study to highlight variations in the nutritional value of 14 different cultivars of fresh uniformly ripe cherry tomatoes (red-ripe stage) and 4 industrial tomato cultivars. The fruits were characterized by a high lycopene content and cherry tomatoes cv. LS203 was considered to be the most suitable cultivar to enhance carotenoid and tocopherol contents, whereas cv. Corbus was the best choice for breeding for hydrophilic antioxidant content.

Tomatoes are the most highly consumed vegetable in Italy, with the highest average consumption among European countries (NETTOX, 1998). In a study on the Italian food consumption patterns in the 1990s, Turrini *et al. (*2001) estimated the per capita consumption of tomatoes (both for salad and ripe) to be of 75.5 g/day. As a matter of fact, tomatoes have been

assessed to be the second most important source of vitamin C in the Italian diet (La Vecchia, 1998). Moreover, in a recent study on dietary sources of vitamin C, vitamin E and specific carotenoid in Spain (Garcia-Closas *et al.,* 2004), tomatoes ranked first as a source of lycopene (71.6%), second as a source of vitamin C (12.0%), pro-vitamin A carotenoids (14.6%) and β-carotene (17.2%), and third as a source of vitamin E (6.0%). Recent intervention studies have also demonstrated that regular intake of small amounts of tomato products can increase cell protection from DNA damage induced by oxidant species (Riso *et al.,* 2004). This protective action is typically attributed to antioxidant components like lycopene and other carotenoids, ascorbic acid, flavonoids and vitamin E.

In Italy, cherry tomatoes are largely used for fresh consumption (more than 25% of the market), and their commercial importance is continuously increasing (Leonardi *et al.,* 2000 b). In Sicily, as well as in other regions of the Mediterranean basin, cherry tomatoes are grown year round in unheated greenhouses, which have no climate control systems and are covered with plastic film; consequently, development and ripening of fruits occur under varying climatic conditions. Temperature and light intensity exert a direct influence on the quality attributes of tomato fruit, such as appearance, firmness, texture, dry matter and sensory properties (Dorais *et al.,* 2001). On the other hand, environmental factors can also affect the antioxidant content of tomatoes: Dumas *et al.* (2003) have recently reviewed the available knowledge about the effects of climatic and other pre-harvest factors on the antioxidant contents of tomatoes. At present, a thorough understanding of the influence of environmental factors and their interactions with agronomic practices on the accumulation of antioxidants during the fruiting period is still lacking.

Light exposure is favourable to vitamin C accumulation (Dumas *et al.,* 2003; Lee and Kader, 2000). Several studies have reported on the effect of shading (by leaves or artificial covers) in decreasing the ascorbic acid content (El-Gizawi *et al.,* 1993), whereas greenhouse-grown tomatoes were usually found to have lower vitamin C levels than those grown outdoors, chiefly because of the lower light intensity (Lopez-Andreu *et al.,* 1986). On the contrary, lycopene synthesis is severely inhibited by exposure to intense solar radiation, and it has been suggested that radiation injury to tomato fruit might be due to the general effects of overheating on irradiated tissues (Adegoroye and Jolliffe, 1987; Dumas *et al.,* 2003). In fact, the formation of lycopene depends on the temperature range and seems to occur between 12 and 32°C, whereas

higher temperatures specifically inhibit its accumulation (Hamauzu *et al.,* 1998; Leoni, 1992; Robertson *et al.,* 1995). With regards to phenolic compounds, although genetic control represents the main factor in determining their accumulation in vegetable foods, external factors may also have a significant effect on this (Macheix *et al.,* 1990). In many plant species the flavonol content may be enhanced in response to elevated light levels, in particular to increased UV-B radiation (Brandt *et al.,* 1995). As a matter of fact, it has been reported that cherry tomato plants grown in greenhouse under high light accumulated an approximately two-fold greater soluble phenols content (rutin and chlorogenic acid) than low-light plants (Wilkens *et al.,* 1996). A few studies have investigated on seasonal fluctuations of nutrients, and in particular the phytonutrient content of tomatoes. Vanderslice *et al. (*1990) reported on seasonal differences in ascorbic acid content of tomato, with higher levels in summer than in spring. Seasonal variations in vitamin C content were observed in greenhouse-grown tomatoes at the mature-green stage, and were directly correlated with temperature variations (Liptay *et al.,* 1986). With regards to phytonutrients, marked variations have been observed in the level of quercetin in cherry tomatoes grown at different times of the year, but no definite seasonal trends have been brought into evidence (Crozier *et al.,* 1997; Stewart *et al.,* 2000). Only very little information is available on the seasonal variations of carotenoid content in the tomato fruit (Heinonen *et al.,* 1989). Additionally, cherry tomatoes of the same cultivar, greenhouse-grown under similar conditions in the same geographical area, and harvested at similar stage of ripeness but at different times of the year showed significant differences in the antioxidant content (Raffo *et al.,* 2006).

Pectin belongs to a class of complex water-soluble polysaccharides used to form coatings. It is a purified carbohydrate product obtained by aqueous extraction of some edible plant material, usually citrus fruits or apples. Under certain circumstances, pectin forms gels; this property has made them a very important additive in jellies, jams, marmalades, and confectionaries, as well as edible coatings. Pectin is a high-volume and potentially important food ingredient available in high percentages in agricultural wastes. In addition, its nutritional benefits for human health and its pharmaceutical activities make it interesting to use in a variety of food products. Several studies have been performed on pectin films, dating mostly from the 1930s into 1950s (Henglein and Schneider, 1936; Maclay and Owens, 1947; Swenson *et al.,* 1953). Generally these studies involved derived pectins and the use of polyvalent cations such as calcium.

Pectin coatings have been also studied for their ability to retard lipid migration and moisture loss, and to improve appearance and handling of foods. Zaleska *et al.* (2000) and Mariniello *et al.* (2003) used the complex of apple pectin with whey protein isolate and whole soy flour respectively as raw material for producing hydrocolloid edible films; pectinate coatings, however, are poor moisture barriers; they can reduce water loss from the food by acting as a sacrificing agent. Maftoonazad and Ramaswamy (2005) used a pectin-based composite coating on avocados and evaluated the extent of quality changes under different storage temperatures, as well as some kinetic parameters (reaction rate constant and activation energy) for predicting the quality loss in stored avocados. Their results showed that pectin-based composite coatings significantly reduced the rate of physical, chemical, and physiological changes in avocados during storage, extending the shelf life to over a month at 10°C.

Alginate, derived from marine brown algae (Phaeophyceae), is a common polysaccharide used as gelling agents in food industry. This polysaccharide is of interest as a potential coating component because of its unique colloidal properties and gel forming properties mainly due to their capacity to form strong gels or insoluble polymers in the presence of multivalent metal cations like calcium (Mancini and McHugh, 2000; Rhim, 2004; Yang and Paulson, 2000). The gelling mechanism involves interactions between calcium ions and carboxylic groups, forming a three-dimensional cross-linked network. That interaction is produced by mixing the components and casting them as films, or by pouring the cationic solution onto a previously cast and dried film (Rhim, 2004). Polysaccharide-based coatings are expected to be a good oxygen barrier due to their tightly packed, ordered hydrogen bonded network structure although they do not behave well as moisture barriers because of their hydrophilic nature (Nisperos-Carriedo, 1994; Yang and Paulson, 2000). Plasticizers like sorbitol, added to increase coating flexibility by reducing the internal hydrogen bonds between polymers chains and increasing intermolecular spacing, generally increase film permeability to oxygen and moisture transmission (Rojas-Grau *et al.*, 2007a). Therefore, lipid incorporation, in small quantities, may be necessary to improve water vapor barrier properties of hydrophilic nature coatings. The addition of sunflower oil with essential fatty acids was shown to improve the barrier properties of alginate and gellan-based edible coatings for fresh-cut 'Fuji' apples (Rojas-Grau *et al.*, 2007b). The addition of a lipid to coating formulations for fresh-cut apples, based on apple puree and pectin, also remarkably diminished the gas permeation through the edible matrix (McHugh and Senesi, 2000). The

respiration rate, moisture loss and changes in quality parameters were much lower with sodium alginate and methyl cellulose coated peaches as compared with the control. While the maximum acceptable shelf-life at 15°C for control samples was 15 days, the coated samples maintained their acceptability up to 21 and 24 days, respectively, with sodium alginate and methyl cellulose coating (Maftoonazad *et al.*, 2008).

To our knowledge, there have been no published data about the use of pectin and alginate composite coatings for maintaining quality and extending shelf life of cherry tomatoes. Consequently, the objective of this study was to evaluate the effect of edible coatings based on pectin and alginate composite coatings with beeswax, monoglycerides and Sorbitol on the quality indices and shelf-life extension of cherry tomatoes.

3.2 Materials and methods

Fruits: Cherry tomatoes fruits were obtained from the local market. The fruits were carefully selected to be uniform in appearance (size and color). Fruits were surface cleaned and washed with water and air-dried. The fruits were then divided into three replicate lots. The first lot constituted the controls which were stored without coating. The second lot was coated with pectin and the third lot were coated with the alginate solution.

3.2.1 Preparation and application of the pectin and alginate coating

3.2.1.1 Pectin coating: Pectin (15 g) (HM rapid set powder, TIC GUMS, Belcamp, MD) was rehydrated in distilled water (500 mL) for 18 h at 20°C, Sorbitol as plasticizer (6.75g) (Sigma, Oakville, ON) was added to the pectin solution and thoroughly mixed with a magnetic stirred. Melted bees wax (6 g) and monoglycerides (1.8 g) (Sigma, Oakville, ON) were added as emulsifiers to this mixture and emulsified using a homogeniser (PowerGen 700, Fisher Scientific, Pittsburgh, PA) at 14000G for 4 min and cooled to room temperature.

The fruits were dipped in the pectin solution for 2 min and then they were transferred to a netted container in order to drain off the excess coating. The coated fruits were air dried for 3 h at room temperature. They were transferred to the polyethylene tetra-phthalate containers and stored at refrigerated temperatures of 4, 12 and 24° C along with an uncoated control.

3.2.1.2 Alginate coating: Sodium Alginate (10 g) (Sigma, Oakville, ON) was rehydrated in distilled water (500 mL) for 18 h at 20°C, sorbitol as plasticizer (6.75 g) (Sigma, Oakville, ON) was added to the Alginate solution and thoroughly mixed with a magnetic stirred. Melted bees wax (6 g) and monoglycerides (1.8g) (Sigma, Oakville, ON) were added as emulsifiers to this mixture and emulsified using a homogeniser (PowerGen 700, Fisher Scientific, Pitsburg, PA) at 14000 G for 4 min and cooled to room temperature.

The fruits were dipped in the alginate solution for 2 min and then they were transferred to a netted container in order to drain off the excess coating. The coated fruits were transferred in a calcium chloride solution (2%) to help set the coating for 2 min. The fruits were then air dried for 3 h at room temperature, packed and stored at the three temperatures (4, 12 and 24°C) along with the untreated control like before.

The following evaluations were made out to monitor quality changes in test samples every 3 days until fruits showed signs of spoilage:

Weight loss: The weight loss of the cherry tomatoes due to transpiration was determined periodically by weighing the samples with a digital balance (OHAUS, Model TS4KD, Florham Park, NJ, USA) and was reported as percent loss in weight of samples based on the initial weight.

Titratable acidity: Fifty gram samples were blended with 150 ml distilled water and titratable acidity was determined by the AOAC (1990) titrimetric method and results were expressed in % citric acid.

pH: Fifty gram samples were blended with 150 ml distilled water and pH was measured using a standard calibrated pH meter (ACCUMET Model 15, Guelph, ON, Canada).

Texture: The firmness of the cherry tomatoes was estimated by a compression test using a TA. XT2 plus texture analyzer (TA-XT plus Texture Analyser, Texture Technologies Corp, USA). The samples were compressed to 30% deformation in a single compression decompression cycle at a steady speed of 3 $mm.min^{-1}$ using a 50 mm diameter circular flat plate. Maximum force needed to penetrate the fruit (grams) was used as an index of firmness (Tangwongchai *et al.*, 2000).

Respiration rate: Known quantities of intact whole tomatoes were placed in an airtight Plexi-glass chamber (18 cm ×12 cm ×27 cm). A CO_2 sensor (ACR Systems Inc, St-Laurent, Quebec, Canada), connected to a data acquisition system (Smart Reader plus 7,Data Logger Analysis Software, Version 1.0 for Windows; ACR Systems Inc), was placed inside the chamber to monitor CO2 concentration. The data logger was programmed to collect on-line data of CO_2 concentration at 1 min interval over a 2 h period. Respiration rate was obtained from the regression slope of CO_2 concentration vs. time data and evaluated as ml.CO_2 kg^{-1} h^{-1}. (Maftoonazad *et al.*, 2008)

Soluble solids: Soluble solids in the cherry tomatoes were measured with a Refractometer (ATAGO N1, Kirkland, DC, USA).

Color: The color characteristics were assessed using a tristimulus Minolta Chroma meter (Minolta Corp, Ramsey, NJ, USA) to determine L value (lightness or brightness), a* value (redness or greenness) and b* value (yellowness or blueness) of cherry tomato samples. The colorimeter was warmed up for 20min before calibration. Measurements were taken for four samples and the average of L, a* and b* values were obtained. Color of fruit was determined on six different locations on the surface of the fruit. The instrument was calibrated with a white standard tile: L=95.87, a = 0.86 and b=2.47. (Maftoonazad *et al.*, 2008)

Total polyphenol content: The amount of total phenolic compounds in tomatoes was determined according to the Folin Ciocalteu procedure (Singleton *et al.*, 1999) with some modifications. A sample of 50 g was grounded and centrifuged at 22,100g for 15 min at 4°C (Thermal IEC, IEC Multi RF, Model 120, USA) and then, filtered through a Whatman No 1 filter. An aliquot of 0.5 ml of the supernatant was added to 0.5 ml of Folin Ciocalteu solution. After 3 min, 10 ml of saturated sodium carbonate solution was added and brought up to 25 ml with distilled water. The absorbance of the blue color that developed was read at 725 nm after 0.5 h in darkness conditions. Phenolic concentrations were determined by comparing the absorbance of the samples with standards. Results were expressed as milligrams of Gallic acid in 100 g of fresh tomatoes.

Statistical Analysis: The statistical Analysis was performed using MINITAB 16. The Analysis of variance was performed using two-way ANOVA at 95% level of confidence and 5% level of

significance. The effect of coatings and temperature on the quality parameters was evaluated by two-way ANOVA.

3.2.2 Properties of the film

Water Solubility and Swelling Ratio

The water solubility (WS) and swelling ratio (SR) of pectin and alginate films were determined following the method of Gontard *et al.* (1992) and Rhim (2004). Six samples were chosen at random from each type of film; 3 of them were dried at 105°C for 24 h to obtain the initial dry matter. The other 3 samples were individually placed in 50-mL beakers containing 30ml of distilled water; the beakers were sealed with parafilm and placed in a drying cabinet at 25°C for 24 h, and stirred occasionally. Films were then removed, rinsed gently with distilled water, placed in an oven at 105°C for 24 h and the remaining (unsolubilized) dry matter was determined. The water solubility of the films was calculated as follows:

$$WS = (So - S)/So = \text{g soluble solids/g total solids},$$

where So is the initial dry matter and S is the final insoluble dry matter.

The swelling ratio of films was also determined, according to Lee *et al.*, (2004) and Rhim (2004). Pre-weighed triplicate film samples were immersed in water at 25°C for 10min, gently blotted with paper for 1min to remove surface water, and the final weight of the swollen samples was then measured. Swelling Ratio was expressed as a fraction of water gained (g) against total solids in the film (g).

3.3 Results and Discussion

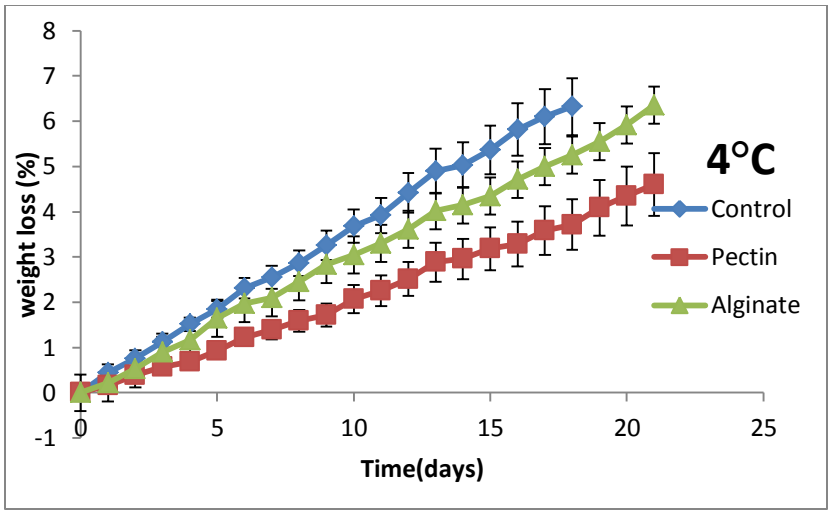
3.3.1 Weight loss: The weight losses in test samples during storage are shown in Figure 3.1. At all three storage temperatures, the weight loss of the control was found to be more pronounced as compared to the pectin and alginate coated tomatoes. The weight loss of the samples at 4°C after 7 days was 2.55% for the control whereas the weight losses for the pectin and alginate samples were 1.38% and 2.09 %. The weight loss then progressively increased to 5.02%, 2.95 % and 4.14% for control, pectin and alginate samples respectively by the 14^{th} day. The control samples had a shelf life of 18 days where as the pectin and alginate samples had a shelf life of 21 days.

The pectin and alginate samples had weight loss of 4.60 and 6.35% at the end of 21 days of storage life (Figure 3.1A).

The weight loss of the samples at 12°C after 7 days was 3.77% for the control whereas for the pectin and alginate samples they were 2.07% and 2.72 %. The weight loss then increased to 6.51%, 4.18 % and 5.09% for control, pectin and alginate samples respectively on 14th day. The control samples had shelf life of 15 days where as the pectin and alginate samples had shelf life of 18 days. The pectin and alginate samples had weight loss of 5.01 and 6.01% at the end of 18 days of storage life (Figure 3.1B).

The weight loss of the samples at 24°C after 7 days was 6.84% for the control whereas the weight losses for the pectin and alginate samples were 5.14% and 6.13 %. The weight loss then increased to 13.87%, 8.91 % and 9.87 % for control, pectin and alginate samples respectively on 12th day. The control samples had shelf life of 12 days where as the pectin and alginate samples had shelf life of 15 days. The pectin and alginate samples had weight loss of 10.91 and 12.04% at the end of 15 days of storage life as shown in Figure 3.1C.

These results confirm that weight loss of the pectin coated samples was less when compared to the alginate samples and control. The decrease in moisture loss of fruits coated with pectin compared with sodium alginate is obviously because of the higher water vapour barrier properties associated with the coatings. Vapour-phase diffusion driven by a gradient of water vapour pressure between the fruit and the surrounding air is the primary mechanism of moisture loss from fresh fruits and vegetables (Maftoonazad *et al.*, 2008). Temperature and relative humidity of the environment are important due to the effects on vapor pressure difference between fruit and atmosphere. Slower rates of moisture loss in coated fruits can be attributed to the barrier properties for gas diffusion of stomata, the organelles that regulate the transpiration process and gas exchange between the fruit and the environment (Salunke *et al.*, 1991). Retarding moisture loss through the use of chitosan on litchi (Dong *et al.*, 2004), methyl cellulose on avocado (Maftoonazad and Ramaswamy, 2005) and Semperfresh^{TM} on cherries



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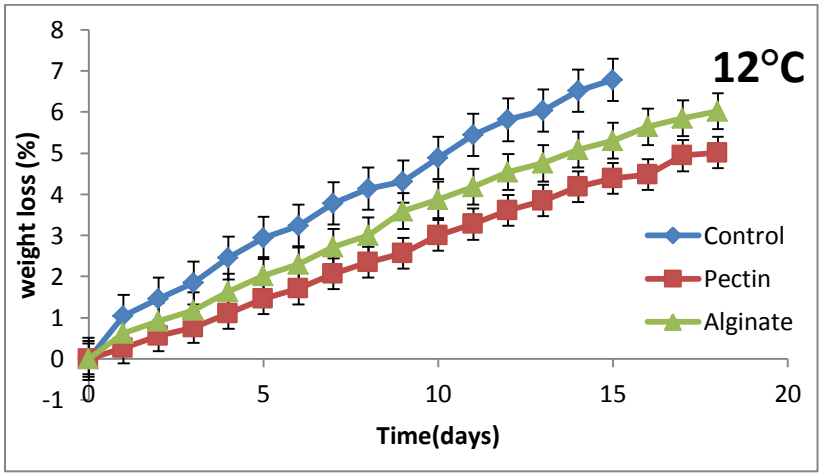

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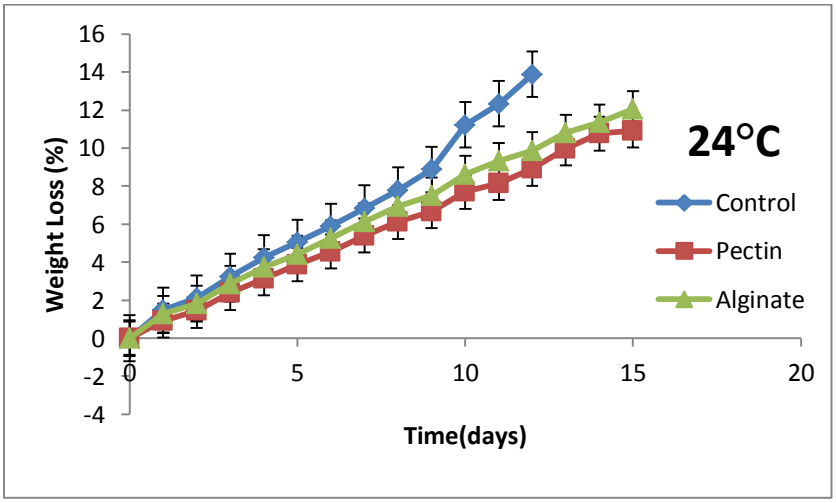


C

Figure 3.1 Weight Loss (vs) time for control, pectin and alginate coated samples at 4°C (A), 12°C (B) and 24°C (C)

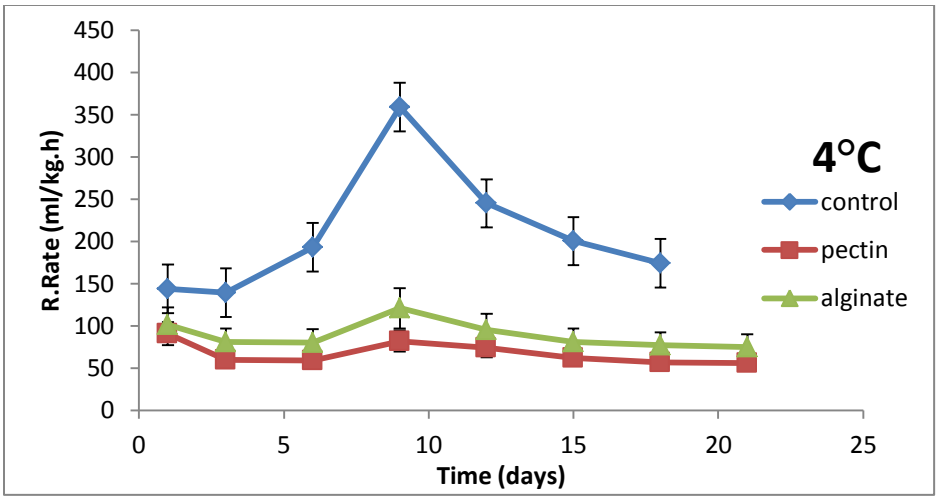
(Yaman and Bayoindirli, 2002) have been reported. Nussinovitch and Hershko (1996) showed that alginate coatings served as a barrier to moisture loss in garlic.

3.3.2 Respiration Rate: Figure 3.2 shows the respiration rates of the fruits for both coatings along with control at all the storage temperatures of 4, 12 and 24°C respectively with time. In this research the edible coatings were used to provide a physical barrier to gas exchange. The barrier effect generates modified atmosphere condition within the fruit which in turn controls the rate of respiration as measured by the amount of carbon dioxide produced. The Respiration rate of the cherry tomatoes at 4°C reached climacteric peak on day 4 for all the coatings and control, where the respiration rate of the fruits were 358.9, 120.8 and 82 ml/kg.h for control, pectin and alginate coated fruits respectively (Figure 3.2A). Similarly climacteric peaks were observed on day 3 for all the coated samples and the control at 12°C, the respiration rates reached peak value of 438, 355 and 158 ml/kg.h for control, pectin and alginate coated fruits respectively and reduced to 176, 144.15 and 125.2 ml/kg.h on day 15 and 18 for control and coated fruits respectively (Figure 3.2B). The respiration rates of the fruits stored at 24°C showed climacteric peaks on day 6 and day 3 for control and coated samples respectively with values of 432.4, 300.14 and 340.28 ml/kg.h for control, pectin and alginate samples (Figure 3.2C).

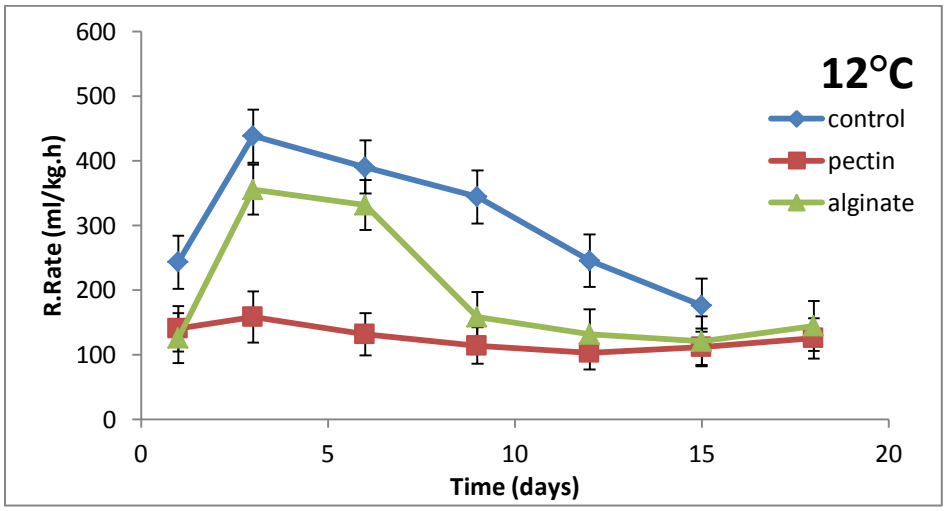
Overall observation showed that the respiration rate of the pectin coated samples was less when compared to the alginate coated samples at all the storage temperatures with time. Even though the coated samples were shelf stable for same period of time, the alginate coated cherry tomatoes had higher respiration rate when compared to the pectin, from which it can be understood that the sodium alginate films has higher permeability to water, O_2 and CO_2 than the pectin films.

Li and Yu (2001) reported a significantly lower respiration rate in chitosan coated peaches than the control fruit during the whole period of storage. Reduction of the respiration rate as a result of coating with such edible films has been reported for banana (Banks, 1984), pear (Meheriuk and Lau, 1988), tomato (Nisperos-Carriedo and Baldwin, 1988) and kiwifruit (Xu *et al.*, 2001).

Respiration activity has also been considered to contribute to weight loss in the produce because of the breakdown of sugars such as glucose to CO_2 and water (Pan and Bhowmik, 1992), although that should be relatively insignificant relative to transpiration losses. However, the heat



A



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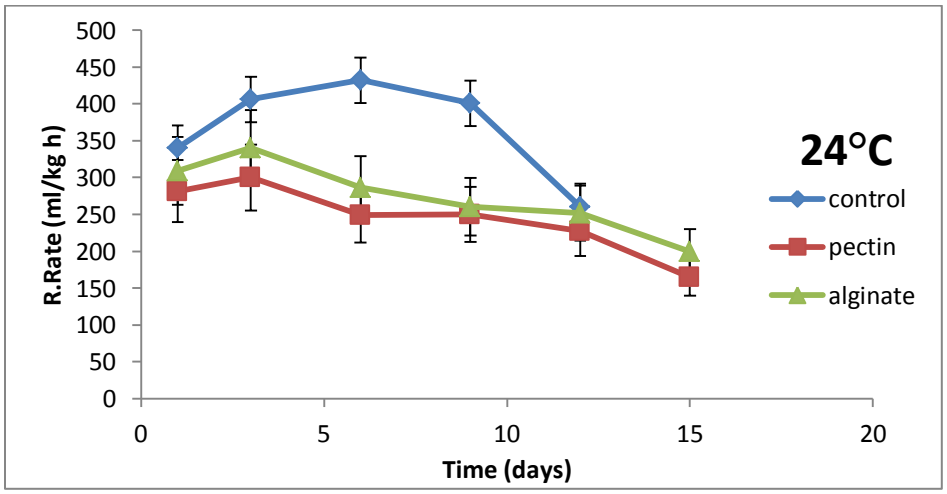


C

Figure 3.2 Respiration rate (vs) time for control, pectin and alginate coated samples at 4°C (A), 12°C (B) and 24°C (C)

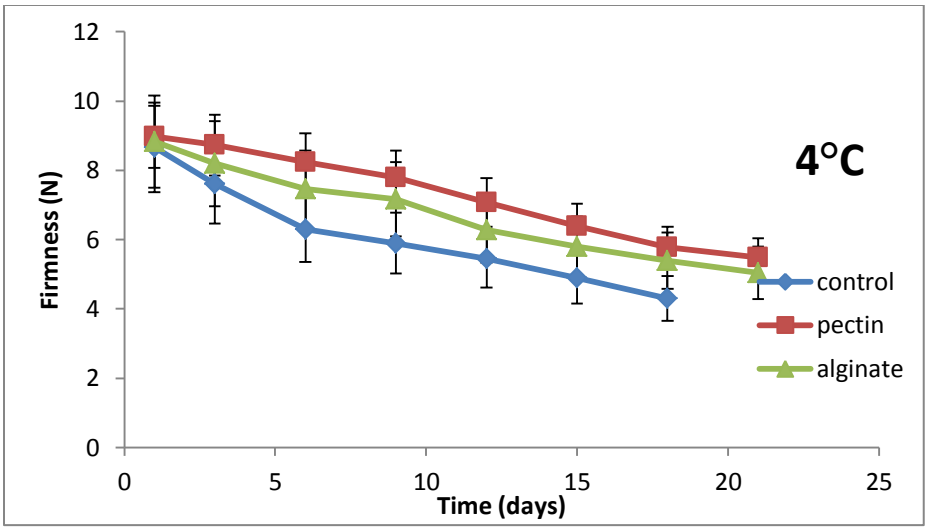
produced by respiration if not removed effectively, can cause more significant damage because of local temperature rise which enhances both respiration and transpiration losses.

Jeong *et al.* (2003) reported that CO_2 production of avocados treated with 1-methyl-cyclo propene with or without wax reached the maximum of 146 and 151ml/(kg h) after 8 and 9 days of storage respectively. Alginate and gellan coatings significantly reduced ethylene production of fresh cut Fuji apples however, no significant effect of coatings on respiration rates was observed probably due to the plastic wrap of moderate oxygen permeability used that did not allow accumulation of O_2 and CO_2 in the head space for sampling and detection (Rojas-grau *et al.,* 2007a)

When fresh cut persimmons were edible coated with soy protein isolate, citric acid and calcium chloride there was equilibrium between the film permeability and the O_2 consumption and CO_2 production of the fresh cut persimmons (Ghidelli *et al.,* 2010). Similar trends in respiration rates were observed when avacadoes were treated with methyl cellulose based composite edible coatings (Maftoonazad and Ramaswamy, 2005), peaches with methylcellulose and sodium alginate (Maftoonazad *et al.,* 2008), mangoes with pectin (Moalemiyan *et al.,* 2011).

3.3.3 Texture: Firmness values of the cherry tomatoes decreased demonstrating texture softening as the length of storage progressed for both coated and control fruits at different temperatures (Figure 3.3). However, coating of fruits showed a significant beneficial effect on firmness retention, with pectin demonstrating a better effect than sodium alginate at all storage temperatures. The firmness of the pectin coated fruits reduced from 8.97N on day 1 to 8.23 N on day 6 and finally reduced to 5.49 N on day 21 showing no sign of spoilage, similarly the firmness of the alginate coated sample reduced from 8.82 N on day 1 to 7.45 N on day 6 and finally reduced to 5.04 N on day 21 showing no sign of spoilage in comparison to the control where the firmness reduced from 8.65 N to 6.29 on day 6 and finally to 4.30N on day 18 at 4°C (Figure 3.3A).

The firmness of the pectin coated ones reduced from 9.07 N on day 1 to 3.79 N on day 18 showing no sign of spoilage, similarly the firmness of the alginate coated sample reduced from 8.92 N on day 1 to 3.10 N on day 18. The firmness of the control reduced from 8.69 N to 3.04 N on day 18 at 12°C (Figure 3.3B).



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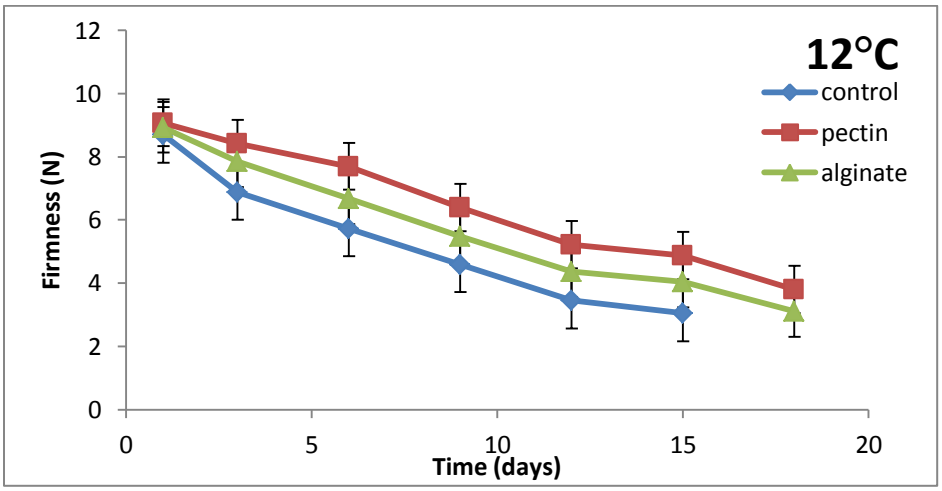

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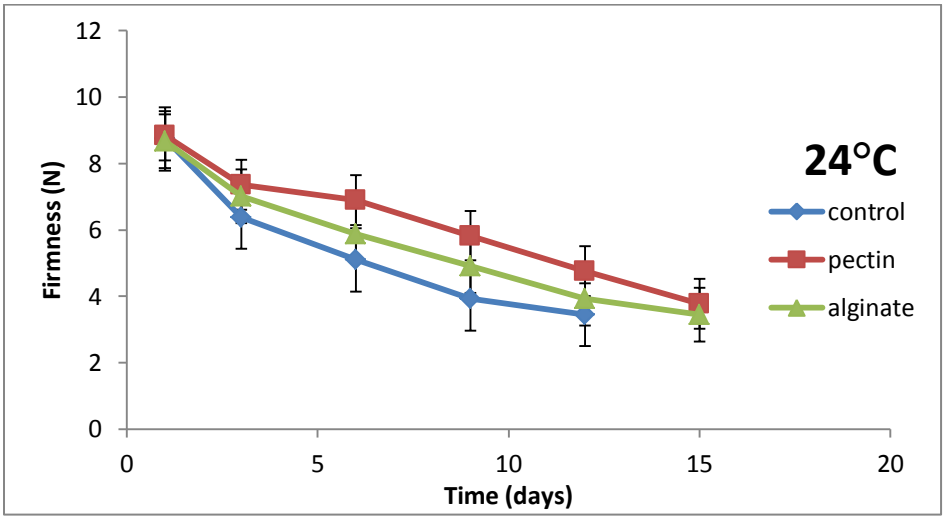


C

Figure 3.3 Firmness (vs) time for time for control, pectin and alginate coated samples at 4°C (A), 12°C (B) and 24°C (C)

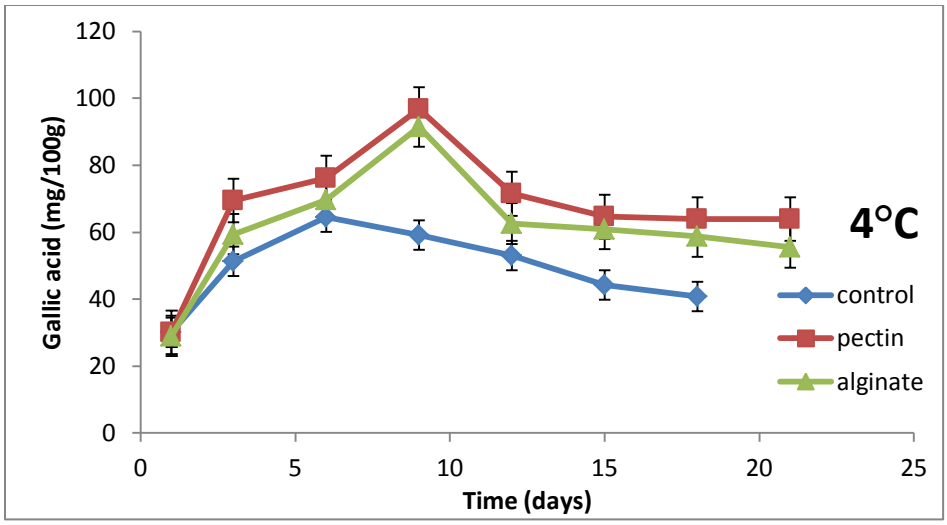
The firmness of the pectin and alginate coated fruits reduced from 8.83 N to 3.77 N and 8.67 N to 3.44 N from day 1 to day 15 showing no sign of spoilage in comparison to the control where the firmness reduced from 8.74 N to 3.44 N on day 12 at 24°C (Figure 3.3C)

Maftoonazad and Ramaswamy (2005) and Xu *et al.* (2001) confirmed retarding of softening in coated avocado and kiwi respectively. Retention of firmness can be explained by retarded degradation of components responsible for the structural rigidity of the fruit, primarily the insoluble pectin and proto-pectin. During fruit ripening, de-polymerisation or shortening of pectin and other pectic substances occurs with an increase in pectin-esterase and poly-galactronase activities (Maftoonazad and Ramaswamy, 2005). Low oxygen and high carbon-dioxide concentrations in the environment potentially reduce the activities of these enzymes and allows retention of the firmness of fruits and vegetables during storage (Salunkhe *et al.*, 1991). The coating of fruits can be expected to modify the internal gas composition of fruits, especially reducing the oxygen concentrations and elevating carbon dioxide concentration which might explain the slower textural changes in the coated fruits (Maftoonazad *et al.*, 2008).

The reported treatment with 1-methylcyclopropene delayed fruit softening at all fruit maturity stages and treated fruits was always firmer than control fruits, even 13 days after treatment, treated mature green fruits were still significantly firmer when compared with all other treatments (Opiyo and Ying, 2005) however firmness results of alginate, gellan and pectin with N-acetyl cysteine and glutathione show that texture of uncoated fresh-cut pears was retained over the storage period and thus, the use of calcium chloride seem not to be necessary to maintain firmness of fruit wedges (Oms-Oliu *et al.*, 2008a). Various post-harvest treatments such as modified-atmosphere packaging and modified atmosphere-coating, as well as storage at low temperatures, slowed the softening and resulted in corresponding retardation of both polygalactouronase and pectinesterase activity (Lazan *et al.*, 1990; Lazan and Ali, 1993).

3.3.4 Total polyphenols: The total polyphenols expressed as mg of gallic acid equivalents per 100 g of fruit sample showed an increasing trend to a peak value and then reduced in all the coatings at all storage temperatures (Figure 3.4).

The total polyphenols at 4°C in pectin coated cherry tomaotes has increased from 30.1 mg on day 1, increased to peak value of 96.9 mg on day 9 after which it reduced to 63.9 mg on day 21.



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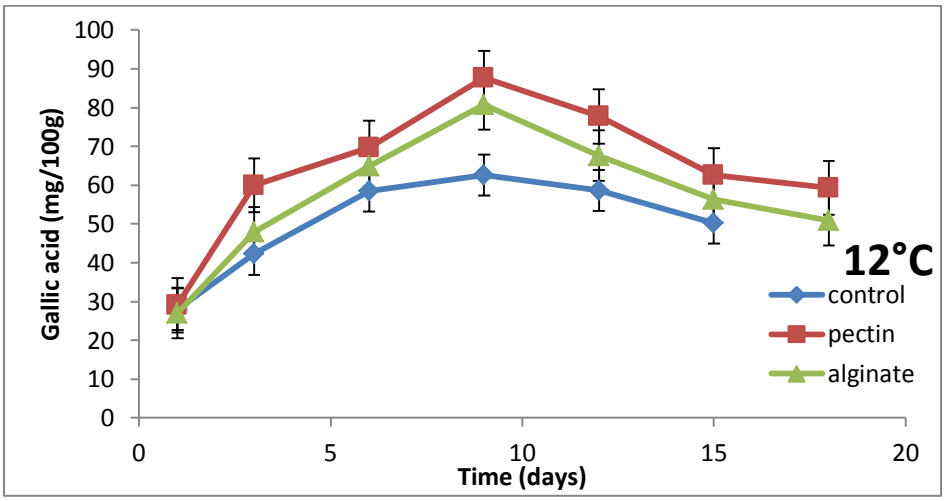

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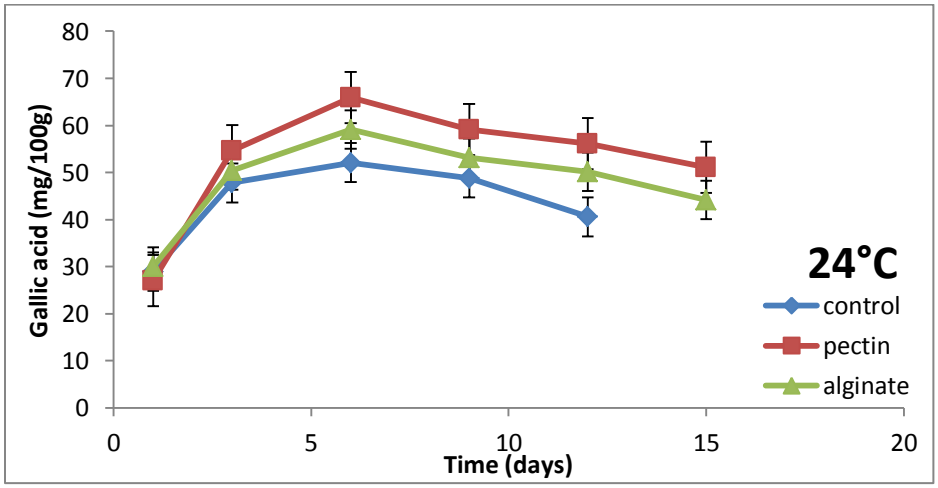


C

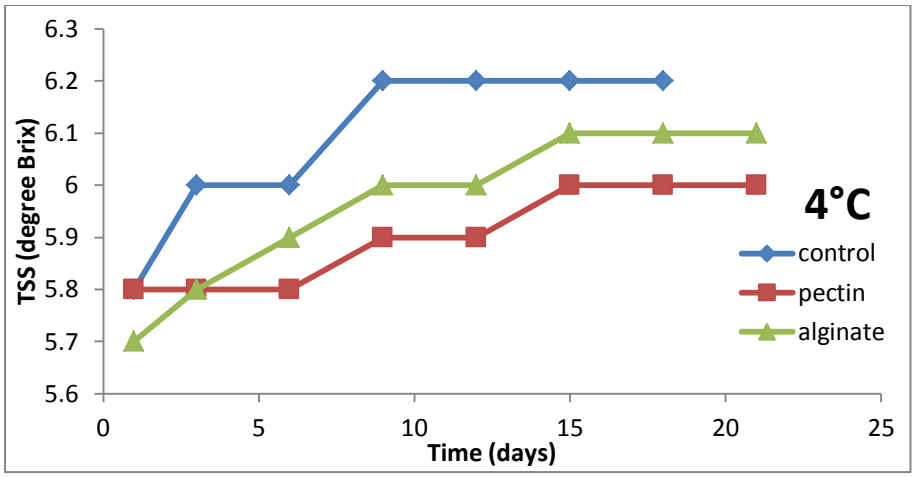
Figure 3.4 Total Polyphenols (vs) time for control, pectin and alginate coated samples at 4°C (A), 12°C (B) and 24°C (C)

Similarly the total polyphenols of alginate samples had increased from 29.0 mg on day 1 to 91.6 mg a peak value on day 9 after which the polyphenolic activity had reduced to 55.6 mg on day 21, where as the phenolic activity of control had increased from 30.2 mg on day1 to peak value of 61.0 mg on day 6 and finally reduced to 40.2 mg on day 18. (Figure 3.4A.)

The edible coatings acted as a potential abiotic stress to the fruits thereby resulting in the production of sceondary metabolites like phenols etc., In coated samples, an increase has also been observed in the control at all temperatures of 4, 12 and 24°C due to the eventual ripening of the fruit. Similar peak trends are noted at 12°C (Figure 3.4B) and 24°C (Figure 3.4C) for coated and control samples. The polyphenolic activity of the pectin samples was higher than alginate and control samples at all the temperatures.

The accumulation of the phenolic compounds in coated and uncoated samples of *piel de sapo* water melon was noticed when it was coated with alginate, gellan and pectin respectively, which may be due to phenylalanine ammonia-lyase (PAL) activity (Oms-Oliu *et al.*, 2008b). Puschmann *et al*, (2007) observed an increase in PAL activity during storage of both starch chitosan-coated baby carrots and uncoated fruit. PAL activation has been observed in response to several stresses including CO_2 treatment (Ke and Salveit, 1989) in water melons. Previous studies showed that a 2.5 kPa O_2+ 7 kPa CO_2 active modified atmosphere induced a greater production of phenolic compounds during storage of fresh-cut 'Piel de Sapo' melon than atmospheres with a higher O_2 content, which was related to oxidative stress induced by too-low and high CO_2 concentrations inside packages (Oms-Oliu *et al.*, 2008b). Therefore, the enhanced phenolic accumulation in melon pieces coated with gellan may be due to the substantial modification of both fruit and package headspace atmospheres.

3.3.5 Total Soluble Solids: Total soluble solid (°Brix) is an important maturity index for fruits, and edible coatings are effective in lowering TSS, or, in other words, lowering ripening rates. The TSS increased during storage time for coated and non coated samples at all temperatures. The TSS of the control increased slowly from 5.8 to 6.0 on day 3, and maintained 6.2 till day 18 for control at 4°C, where as the TSS for the pectin samples increased from 5.8 to 6 slowly and maintained till day 21. The TSS for the alginate samples increased from 5.7 to 6.1 slowly and maintained till day 21 (Figure 3.5A).



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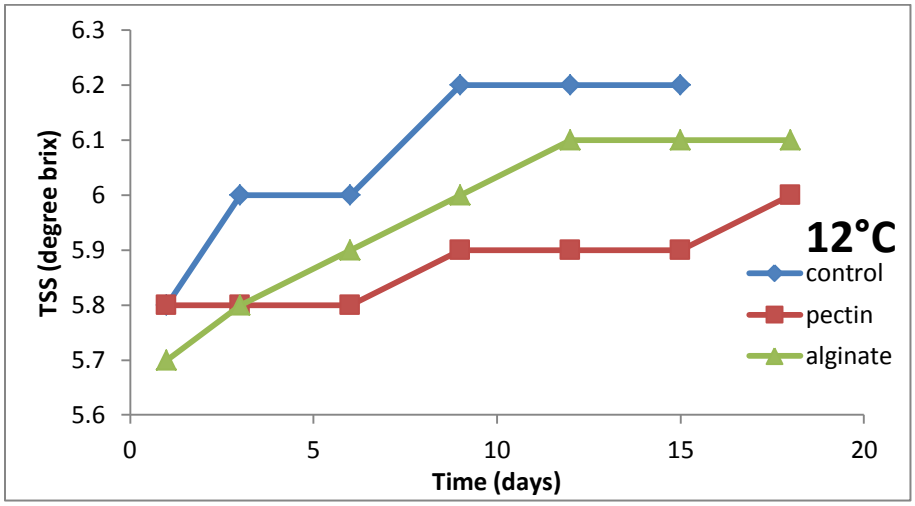

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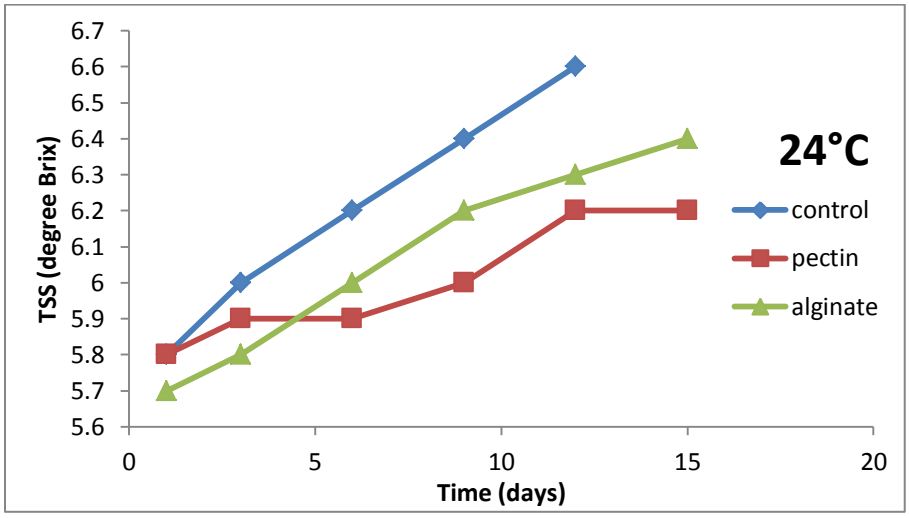


C

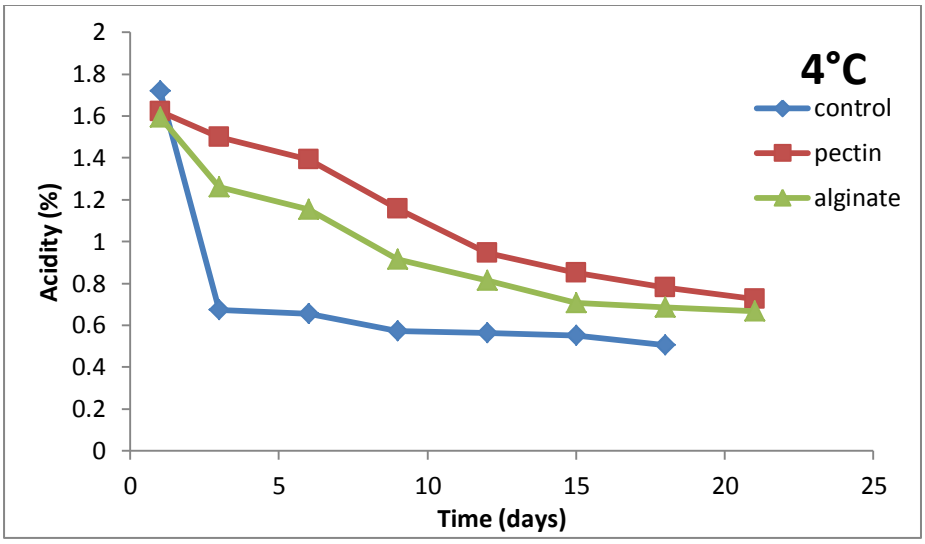
Figure 3.5 Total soluble solids (vs) time for control, pectin and alginate coated samples at 4°C (A), 12°C (B) and 24°C (C)

At 12°C the TSS of the control increased from 5.8 to 6.2 over a period of 15 days, whereas the TSS increased from 5.8 to 6 and 5.7 to 6.1 over a period of 18 days for pectin and algiante coated cherry tomatoes respectively (Figure 3.5B). At 24°C the TSS of the control increased from 5.8 to 6.6 over a period of 12 days, whereas the TSS increased from 5.8 to 6.2 and 5.7 to 6.4 over a period of 15 days for pectin and algiante coated cherry tomatoes respectively (Figure 3.5C). The high value of soluble solids in the control fruits is attributed partly to water loss and drying of cherry tomatoes fruits; on the other hand, the increase in TSS is a direct consequence of the breakdown of complex carbohydrates into water soluble sugars during normal ripening, a consequence which is also observed as pulp softening. Edible coatings delayed ripening as indicated by changes in TSS observed in our experiments, as well as those reported by other researchers (Mitra and Ramaswamy, 2008).

During ripening, starch is degraded rapidly by the combined action of amylases, starch phosphorylase and 1, 6-glucosidase and sucrose synthase, to sugars such as sucrose, glucose, and fructose. At the start of the ripening, sucrose is the predominant sugar in the pulp, and its formation precedes accumulation of glucose and fructose (Kittur *et al.,* 2001). Based on our experiments, the TSS values of pectin-based coated fruits were lower than those of the alginate and control, suggesting that the former synthesized sugars at a slower rate than the latter.

The total soluble solid content, the titratable acidity and the ascorbic acid content of control mango fruit fell greatly after seven days of storage. The chitosan coated sliced fruit had a greater total soluble solid content, titratable acidity and ascorbic acid content, but the total soluble solid contents did not vary significantly among the fruit treated with 0.5%, 1% and 2% chitosan (Chien *et al.*, 2007). Similar trends in total soluble solids were observed when mangoes were coated with pectin (Moalemiyan *et al.*, 2011). Maftoonazad and Ramaswamy, (2008) reported that there was no statistically significant change during storage for soluble solids of peaches when they were coated with methyl cellulose and the sodium alginate based composite coatings.

3.3.6 Titratable Acidity: The samples coated with pectin showed less decrease in titratable acidity and it was greatest in the uncoated samples. The titratable acidity of the coated samples at all temperatures was more in comparison with the control. The coatings reduced ethylene production in coated cherry



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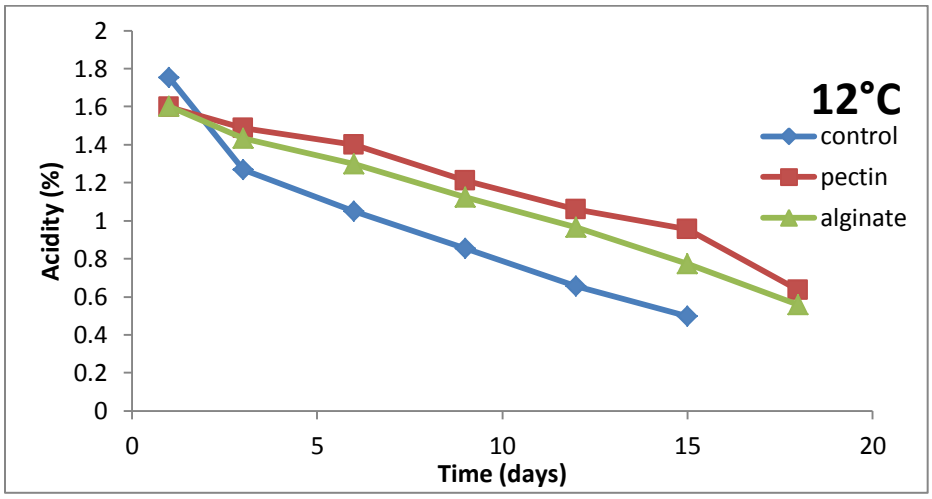

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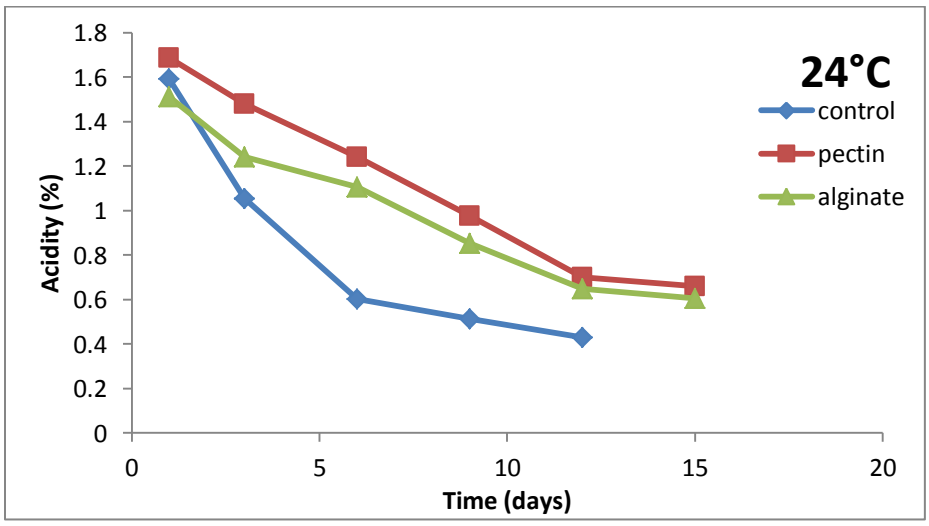


C

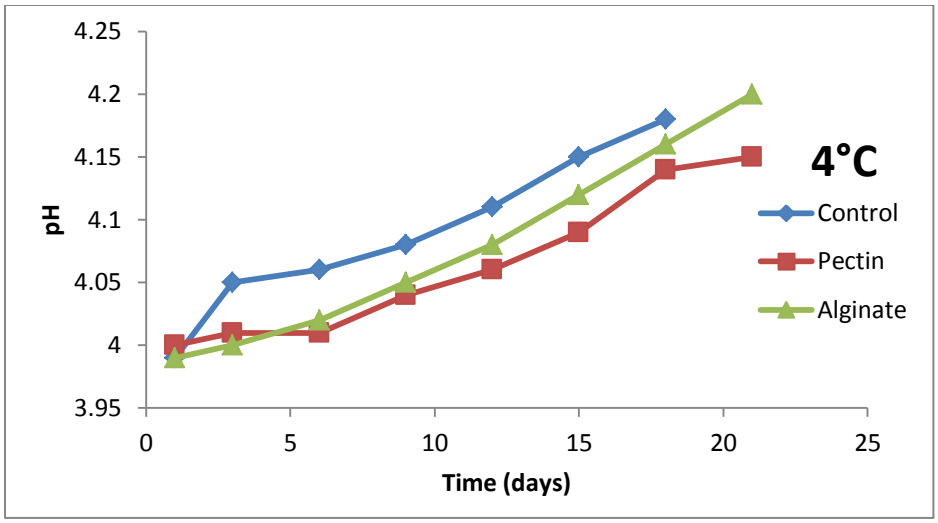
Figure 3.6 Titratable Acidity (vs) time for control, pectin and alginate coated samples at 4°C (A), 12°C (B) and 24°C (C)

tomatoes thereby maintaining acidity in comparision with the control. But acidity of all the samples including pectin and alginate reduced with increase in time at all temperatures. The acidity values decreased and rate of decrease with time was fast at higher temperatures (24°C) and slow at lower temperatures of storage (4 and 12°C).

At 4°C the titratable acidity of the pectin coated cherry tomatoes reduced from 1.62% to 0.72% on day 21, Similarly the acidity of the alginate samples reduced from 1.59% to 0.669% on day 21, where as the control samples acidity reduced from 1.72% on day 1 to 0.506% on day 18 (Figure 3.6A). At 12°C the titratable acidity of the pectin and alginate coated cherry tomatoes reduced from 1.59% to 0.63% and 1.60% to 0.56% respectively from day 1 to 18, the control samples acidity reduced from 1.75% on day 1 to 0.50% on day 15 (Figure 3.6B). Similar trends were noticed at 24°C (Figure 3.6C) and it could be seen that the rate of decrease of acidity was fast at 24°C than 4 and 12°C even though the acidity of the coated samples being higher than control.

Dong *et al.* (2004), and Li and Yu (2000) reported slower rate of decreasing acidity for litchi, and peach respectively. Organic acids are substrates for many enzyme catalysed reactions during aerobic respiration in plant cells and a reduction in the acidity may be expected as a result of such activity during the ripening process, thus making the fruits taste relatively sweeter. A decline in acidity demonstrates advancement of maturity of fruit/ripening thus coated fruits contribute to delay of ripening. These results agree with those reported by El Gaouth *et al.* (1991) who analysed the effects of chitosan on strawberries. However, higher titratable acidity could also be the result of formation of carboxylic acid by dark fixation of CO_2 (Pesis and Ben-Arie, 1986).

3.3.7 Changes in pH: Since coated fruits showed less variation in the titratable acidity, the associated variation in their pH was also relatively lower. At any given time, control cherry tomaotes had higher pH than coated fruits, confirming previous results (Medlicott *et al.*, 1987). The rate of increase of pH in control samples was also higher than in coated fruits. Diaz-sobac *et al.* (1996) also reported higher increase in pH of the control samples compared to pH of the mangoes coated with malto dextrin and methyl cellulose. The pectin coating was more effective than alginate coating in retention of pH.



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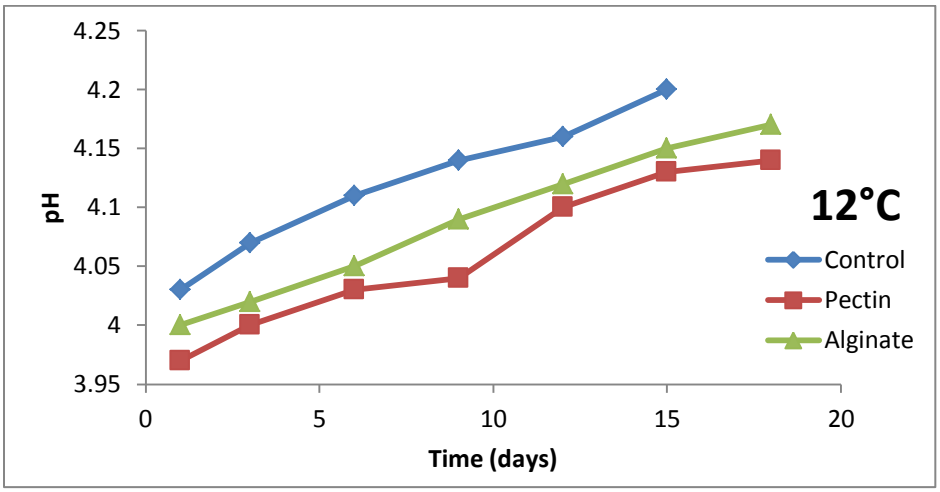

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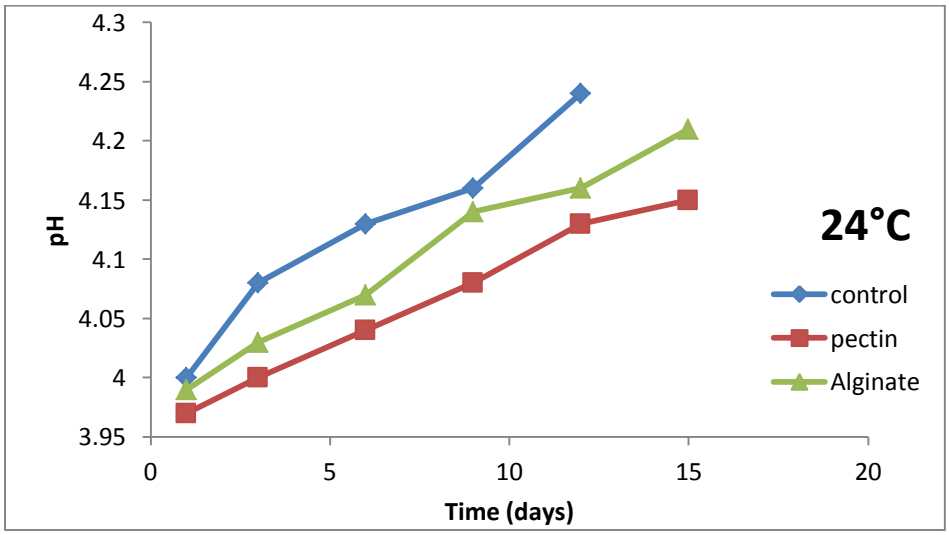


C

Figure 3.7 pH (vs) time for control, pectin and alginate coated samples at 4°C (A), 12°C (B) and 24°C (C)

The pH of the pectin and alginate coated samples was higher than the control cherry tomatoes at all the storage temperatures. The edible composite coatings had protective effect of reducing the ripening rate of the fruit there by maintaining the acidity of the fruit than the control. The pH values of all the samples irrespective of the control eventually increased with time at all the temperatures. The rate of increase was fast at 24°C where as the rate of increase was slow at 4 and 12°C.

At 4°C the pH of the pectin coated samples increased slowly from 4.00 on day 1 to 4.15 on day 21. The pH of the alginate samples increased from 3.99 on day 1 to to 4.20 on day 21, where as the pH of the control increased steadily from 3.99 on day 1 to 4.18 on day 18 (Figure 3.7A). At 12°C the pH increased slowly from 3.97 to 4.14 and 4.00 to 4.17 from day 1 to day 18 for pectin and alginate samples respectively, where as the pH of the control increased steadily from 4.03 on day 1 to 4.2 on day 15 (Figure 3.7B).

Similar trends were noticed at 24°C (Figure 3.7C) for coated and control samples but the rate of increase was more than the refrigerated storage temperatures of 4°C and 12°C. Titratable acidity and pH remained unchanged until day 9 whereas TA values increased for control when minimally processed mangoes were coated with chitosan (0.25% w/v) and stored at 6°C for a period of 9 days (Djioua *et al.*, 2010).

3.3.8 Color (L* and a* values): Color evaluation of the cherry tomatoes was affected by coating and storage time. The color parameters like L* and a* values were evaluated as the other parameters b, hue angle and total color difference did not show any convincing trends. The lightness values of the pectin and alginate coated cherry tomaotoes were higher than the control and changed at much lower rate.

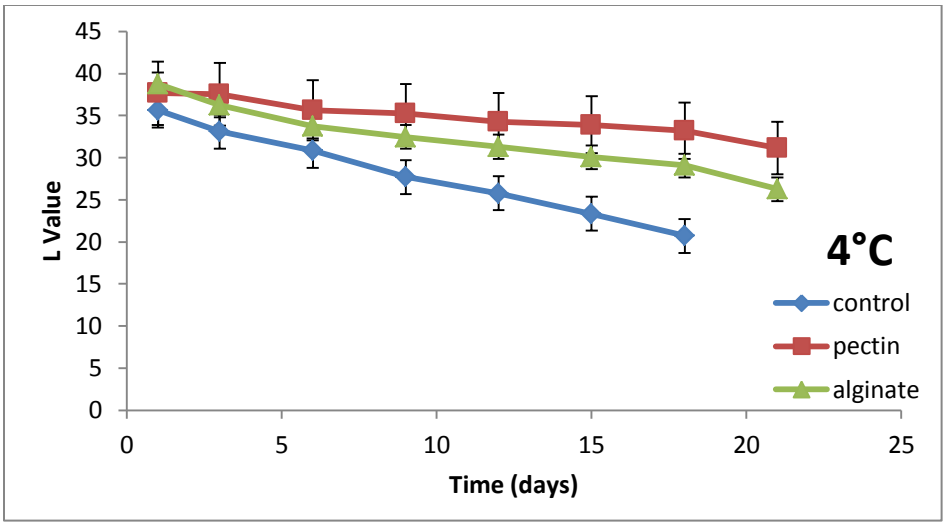
The cherry tomatoes were light yellow shade to orange in color. As the fruit ripens the color of the fruit changes towards red. Hence the lightness value (L*) reduces and redness values (a*) increases as the fruit continues its physiological activities. The pectin and alginate coatings protected the fruit as the barrier for the fruit thereby reducing its repsiration and transpiration rates and ethylene production. Hence the coated fruits ripened at much slower rate when compared to the control, which attributed to the high L* values of the coated fruits than control. The coated fruits ripened at slower rate because of which they have high L* values and low a

values. The L* value decreased with storage time at all temperatures for all the samples, since it is a measure of the color in the light dark axis, this falling value indicates that the fresh fruit was turning less bright.

3.3.8.1 L Value: At 4°C the L* value of the pectin coated cherry tomaotes maintained 37.6 till day 3, decreased to 35.2 on day 9 maintained 33.2 till day 18 and reduced to 31.1 on day 21 at 4°C, where as the L* value of the alginate coated cherry tomatoes reduced from 38.7 on day 1 to 33.7 on day 6 , steadily reduced to 30.1 on day 15 and finally to value of 26.6 on day 21. The L* value of the control decreased steadily from 35.6 on day 1 to 20.7 on day 18 (Figure 3.8A). The rate of decrease of L* value in control was more and faster than the pectin and alginate coated cherry tomaotes. Similar trends were noted in the decrease in L* value of coated and control samples at other temperatures of 12°C (Figure 3.8B) and 24°C (Figure 3.8C) but the rate of decrease in L* value was fast with increase in temperature from 4°C to 24°C. Decreasing in L* and b* values during the storage were noticed for mangoes coated with chitosan and it was observed that hot water dip allowed to maintain color during 3 days (Djioua *et al.*, 2009, 2010). A decrease in L* value, which is evident by the loss of brightness, is an indicator of browning in fresh-cut fruits (Gonzalez Aguilar *et al.*, 2008).

3.3.8.2 a* value: The a* value of the pectin coated cherry tomaotes at 4°C increased slowly from 15.7 on day 1 to 22.76 on day 21. The a* value of the alginate coated cherry tomaotes increased slowly from 17.46 on day 1 to 23.70 on day 21. Whereas in control the value increased from 21.53 on day 1 to 24.67 on day 18 (Figure 3.9A).

Similar trends were noticed in increase in a* value at 12°C (Figure 3.9B) and 24°C (Figure 3.9C). But it was noticed that the rate of increase in a* value was faster in all the samples including control with increase in tempeature from 4°C to 24°C.The rate of increase of a value in control was more and faster than the pectin and alginate coated cherry tomaotes at all temperatures of storage. The pectin edible coating is more effective in decelerating the ripening rate of the fruit, which can be noticed from the slow increase in a* value (redness) when compared to alginate and control samples. No proper trends were noted in the b value of the cherry tomatoes over the period of time for all the samples at all storage temperatures of 4, 12 and 24°C.



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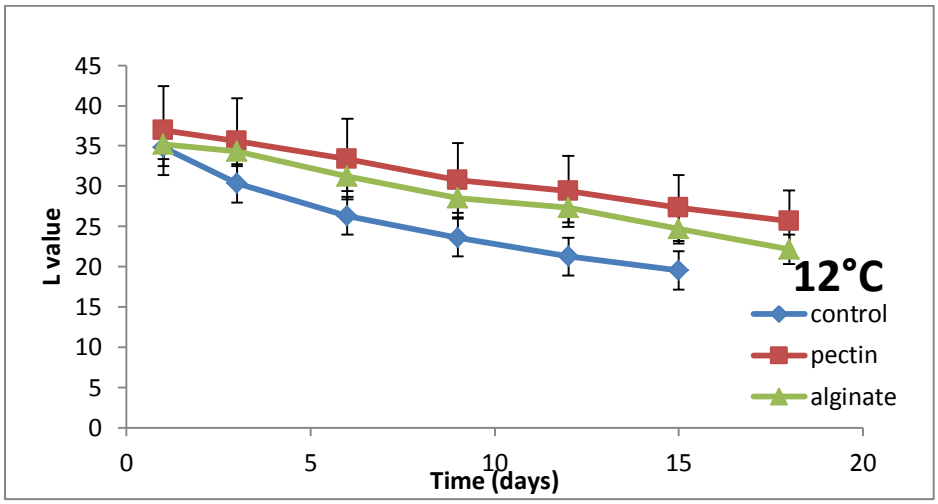

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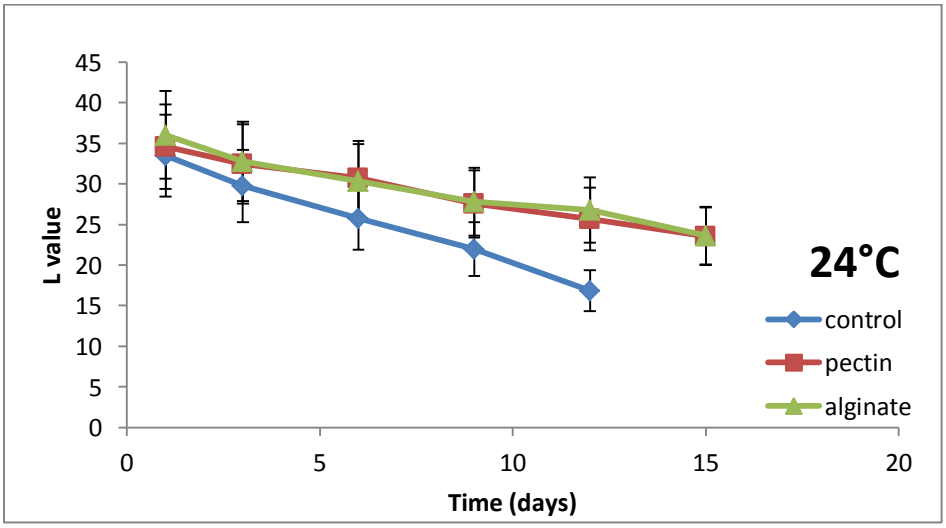

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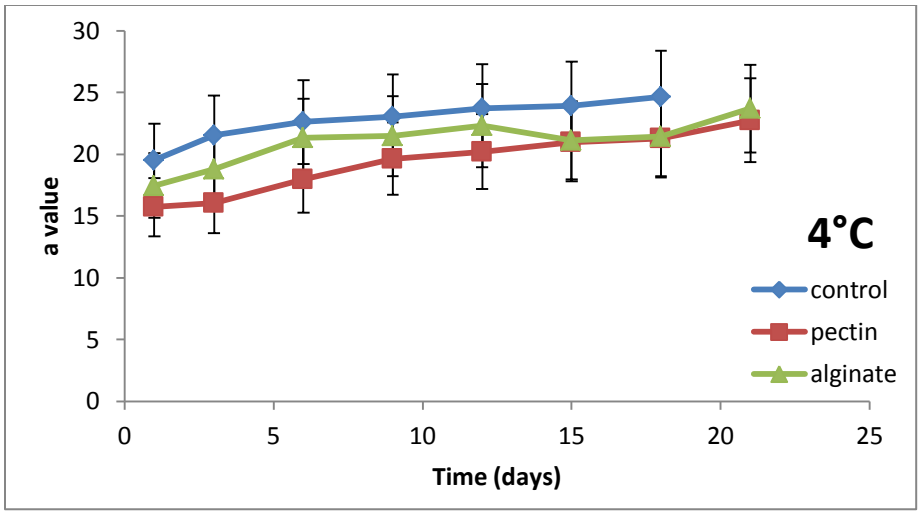
Figure 3.8 L* value (vs) time for control, pectin and alginate coated samples at 4°C (A), 12°C (B) and 24°C (C)



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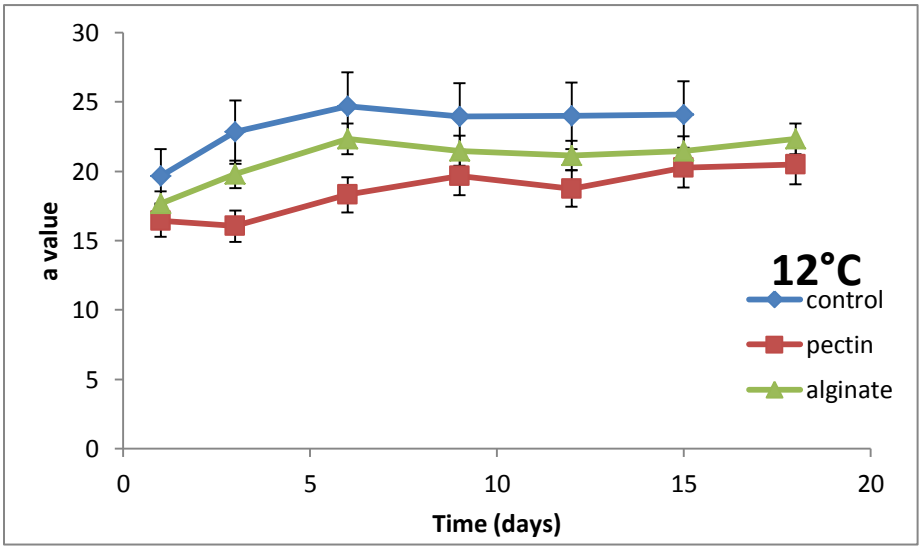

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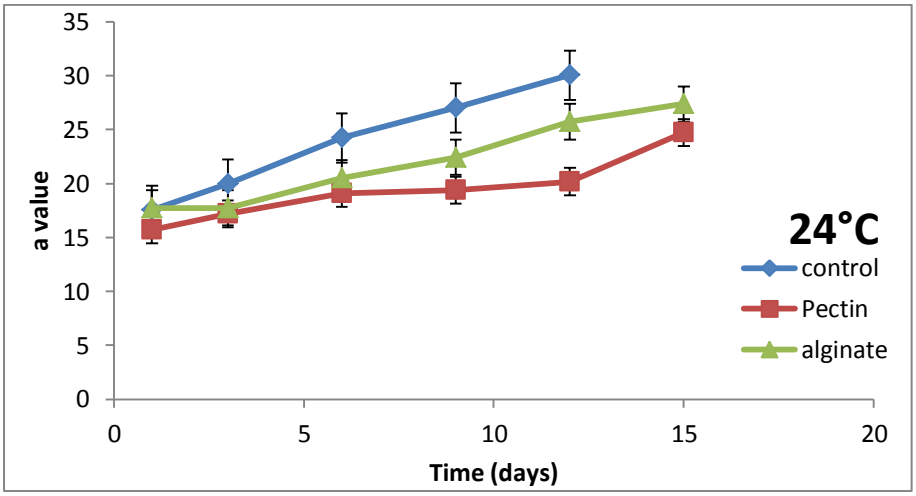

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Figure 3.9 a* value (vs) time for control, pectin and alginate coated samples at 4°C (A), 12°C (B) and 24°C (C)

Similar trends in changes in color l* and a* values were observed in mangoes treated with pectin (Moalemiyan *et al.,* 2011), strawberries (Hernandez-Munoz *et al.,* 2008) and on fresh-cut rose apple (Worakeeratikal *et al.,* 2007) with chitosan. The use of antimicrobial edible coatings had a significant ($p \leq 0.05$) effect in the color parameters L*, a*, b* and h* of 'Fuji' apples. The apples were coated with the apple puree alginate films with lemon grass, oregano oil and vanillin. It was reported that the external color of the apples coated with the lemon grass tend to be greenish, but the internal color was seriously affected leading to a darken color from the first days of storage. Lowest changes in color were observed when vanillin was incorporated in the film (Rojas-grau *et al.,* 2007b). The presence of a high percentage of O_2, combined with chitosan coating, seemed to affect color positively when strawberries were treated with a solution of 1% chitosan, packaged in modified atmosphere (MA) with high (80%) and low (5%) percentage of O_2 and then stored at 4, 8, 12 and 15°C (Tamer and Copur, 2009). The use of alginate and gellan edible coating in fresh-cut Fuji apples had a significant effect in the color parameter hue angle. In this study, low h* values were indicative of browning in apple wedges. Alginate and gellan edible coatings containing N-acetyl-cysteine as anti-browning agent maintained apple wedges free from browning during 21 days of storage (Rojas-grau *et al.,* 2008).

3.3.9 Water Solubility and Swelling Ratio: Water Solubility is one of the most important properties studied in food and pharmaceutical applications, as unlike water permeability, it is determined by the chemical structure and defines the material resistance or tolerance to water, therefore indicating its stability in water. As stated by Lee *et al.,* (2004), while films can be used as coating materials to inhibit for instance exudation in frozen foods, the higher the water solubility the poorer the stability of such films. In the case of high moisture fresh-cut fruits, resistance of coatings to dissolve in water is relevant. Swelling ratio (SR) defines the amount of water absorbed by films and it reflects an important property of carbohydrate and protein films, as these biopolymers initially swell when suspended in water, with resulting changes in their structure. SR has been employed as a measure of the extent of cross-linking, as used in protein films based on collagen (Lee *et al.,* 2004). Thus, examination of swelling ratio is a requisite for the efficient application of biopolymer films. Low SR values indicate a high tolerance to water. Water solubility and Swelling Ratio for the pectin composite film was found to be 2.3 and 0.47 where as the water solubility and swelling ratio for the pectin composite film was found to be 2.6

and 0.55. from the above results it can be understood that the lower the swelling ratio, the greater the tolerance to water, hence reducing the weight loss of fruit.

3.3.10 Statistical Analysis: Statistical Analysis by two-way ANOVA with coatings and temperature as variables on the quality parameters gave the following results

- Temperature had significant effect on a* value of the cherry tomatoes ($p \leq 0.05$) at all temperatures but the coatings had no significant effect ($p \geq 0.05$).
- Coatings and temperature had significant effect on response L* value, total soluble solids, respiration rate, weight loss and firmness of the cherry tomatoes ($p \leq 0.05$).
- Coatings had significant effect ($p \leq 0.05$) but temperature had no significant effect ($p \geq 0.05$) on the pH, titratable acidity of the samples.
- Both temperature and coating had no significant effect on the polyphenols of the cherry tomatoes ($p > 0.05$).

Table 3.1 Analysis of variance: effect of coatings and temperature on responses

Response	**Variables**	**Sum of squares**	**Mean Square**	**F**	**p**
Weight loss	Temp	82.05	41.02	174.25	0.000
	Coatings	6.89	3.44	14.64	0.014
Respiration rate	Temp	17142.8	8571.42	21.49	0.007
	Coatings	12466	6233.01	15.63	0.013
Firmness	Temp	4.66	2.33	36.13	0.003
	Coatings	0.88	0.44	6.84	0.048
Titratable acidity	Temp	0.009	0.004	4.63	0.091
	Coatings	0.058	0.029	28.82	0.004
Total soluble solids	Temp	0.18	0.09	27.00	0.005
	Coatings	0.10	0.053	16.00	0.012
pH	Temp	0.001	0.0007	2.27	0.219
	Coatings	0.005	0.002	9.08	0.033
Total polyphenols	Temp	638.20	319.099	2.62	0.188
	Coatings	524.66	262.329	2.15	0.232
L* value	Temp	36.36	18.18	6.52	0.055
	Coatings	92.346	46.1732	16.56	0.012
a* value	Temp	52.94	26.47	9.27	0.031
	Coatings	22.12	11.06	3.87	0.116

3.4 Summary and Conclusions: Application of pectin and alginate coatings to cherry tomatoes was shown to be beneficial in retarding the ripening process. The coatings acted as a physical barrier for the gas exchange between the fruit and the environment. It lowered both the rate of substrate catabolism and the ability to generate the energy required to drive the biochemical reactions associated with fruit ripening. Coatings favorably influenced several physiological properties of the fruits during storage. Coating slowed down the rate of respiration, reduced the color changes in both skin and flesh, reduced the softening of the tissue and increased the shelf-life.

Application of the pectin-based coating on cherry tomatoes was more effective than alginate in reducing the associated physiological changes and extending the storage life than alginate however both had similar shelf life. The coatings limited the transpiration and consequently diminished weight reduction by water evaporation, also reduced respiration rate and prevented or delayed responses to ethylene. For post-harvest fruit handling these are considered to be key factors. It favorably affected several physiological and chemical properties of the fruit during storage. The coating also imparted sheen and enhanced the visual appeal of the fruits, reduced decay, reduced the color changes in both skin and flesh, and lowered the softening of the tissue. It delayed ripening as indicated by changes in TSS, pH, and titratable acidity. However, coating can sometimes result in anaerobic respiration and off flavor development. To prevent this and to get the best results, the formulations need to be optimized to strike a balance between gas and water vapor transmission which would depend on the fruit and/or vegetable cultivar (especially the respiration rate). Furthermore study is recommended on the properties of the film like tensile strength, improving the mechanical properties of the film by adding other functional plasticizers etc., and also incorporation of anti-microbial substances like essential oils in the coatings.

CONNECTING STATEMENT

TO CHAPTER 4

In Chapter 4, the use of an hormetic abiotic stress with hot water to enhance the post-harvest quality of the cranberries in terms of the total polyphenols, color and texture is described. The idea of subjecting the fruits to stress was inspired from previous studies on stress induction of polyphenols in fruits. Fruits like cranberries which are a significant source of polyphenols but are not actually very much consumed (as whole fruits) were chosen for this study. The central hypothesis was that willingly subjecting to fruits to little doses of hormetic stress (hormesis) would trigger the defense mechanism of the fruits, thereby stimulating the synthesis of polyphenols. These polyphenols would be of high value because of their antioxidant and anti-carcinogenic properties.

The cranberries were subjected to hormesis by hot water dipping at 40, 50 and 60°C for 1, 5 and 10 min respectively. The stress subjected fruits were given a time of 24 h to overcome the stress and the changes in color, texture and polyphenols were evaluated. As expected the fruits which underwent hormesis showed more total polyphenols than the control, and improved the firmness and color of the cranberries, which is highlighted in this chapter.

Part of the results of this study has been prepared for publication: **Narayanapurapu, P.T.R.** and Ramaswamy, HS., 2012. Effect of an abiotic stress on the post-harvest quality of cranberries (to be submitted).

Experimental work and data analysis were carried out by the candidate under the supervision of Dr. HS Ramaswamy.

CHAPTER 4

EFFECT OF ABIOTIC STRESS ON THE POST HARVEST QUALITY OF THE CRANBERRIES

Abstract

Cranberry fruit is rich in phenolic phytochemicals such as phenolic acids, flavonoids and ellagic acid. Consumption of cranberry has historically linked to lower incidences of urinary tract infections and has now been shown to have a capacity to inhibit peptic ulcer-associated issues. Subjecting respiring produce to temporary stresses has been seen as a way to accumulate the hypersensitivity stress metabolites in the produce. The changes in the color, firmness and the total polyphenols in the cranberry (*Vaccinum macrocarpon*) subjected to abiotic stress in the form of hot water treatment at different temperatures for different period of times were investigated. Three temperatures, 40, 50 and 60°C, for a period of 1, 5 and 10 min were selected for treatment after preliminary experiments to refine the temperature and time combinations. Fresh cranberries were subjected to the abiotic stress for the selected combination of temperature and time, removed from the hot water, cooled, drained of excess water, and transferred to polyethylene tetra phthalate preformed containers and stored at 4°C for a period of 24 h allowing the fruits to overcome the sustained stress. After 24 h the total polyphenols, color and firmness changes were evaluated in comparison with the control. The treatment of hot water at 60°C for 1 min resulted in an increase of the total polyphenols to 258 mg in comparison to the control having 120 mg and showed more color difference because of the accumulation of more anthocyanins. The treatment of 40°C for 1 min improved the firmness of the fruit.

4.1 Introduction

Cranberry fruits are grown mainly for processing purposes, but in the U.S and Canada, strong markets for fresh fruit exist for the Thanksgiving and Christmas holiday seasons. In addition to the conventional aspects of cranberry consumption, health benefits of the fruit have become increasingly important. Fruits of Vaccinium sp. including cranberries are particularly rich in flavonoids and other phenolic compounds (Wang and Stretch, 2001). These compounds have health-promoting benefits as antioxidants and anti-carcinogens (Prior and Cao, 2000; Ames *et al.,* 1993; Hakkinen *et al.,* 1999). In addition to direct units, the phytochemicals are usually expressed as Trolox equivalents per gram or gallic acid equivalent for antioxidant and

polyphenolic activity. Wang and Stretch (2001) found that the concentrations of antioxidants in 10 cranberry cultivars ranged from 8.6 to 14.1 units of Trolox equivalents per gram, anthocyanins from 0.20 to 0.66 mg of cyanidin-3-galactoside per gram and total phenolics, from 1.20 to 1.76 mg of gallic acid equivalents per gram of fresh fruits.

The growing environment strongly affects fruit quality. Factors such as soil fertility, pH, water availability, fruit pollination, pruning, plant age, vigor, and the presence of abiotic and biotic stresses can effect fruit growth, composition, and quality. Cranberry fruit maturity is based primarily on color. Fruits develop color as a response to environmental temperatures and light. Therefore fruit in the upper canopy of the plant that receive the most light develop better color and ripen first, while fruit buried deep in the canopy are slow to develop color and some fruit may remain white. Ceponis and Stretch (1983) found that the "*Early Black*" fruits which were highly colored had less physiological breakdown than the less colored fruit within a harvest.

Cranberries are harvested by wet or dry methods. The most common method, wet-harvesting, involves scooping or raking of the fruit in flooded bogs with a motorized water reel. Dry harvesting involves hand picking, hand scooping, and machine scooping (Eck, 1990; Bailey, 2000). Proper temperature management is the most critical factor in the postharvest handling of fruit. The recommended storage temperature for fresh cranberries in various handbooks ranges from 2 to 7°C based on chilling sensitivity of fruit (Levine *et al.*, 1941; Wright *et al.*, 1937). In addition to the effect of temperature on breakdown and decay, temperature also affects fruit color. At 2°C and above color tends to darken. Therefore, color can be improved in early harvested fruit, which tend to be pale in color by storing at 7 to 10°C for several weeks (Levine *et al.*, 1941). Precooling cranberries can be performed using cold air (forced-air) or water (hydrocooling). If significant field heat is present at the time of harvest, fruit may benefit from its rapid removal (Kaufman *et al.*, 1958).

Cultivar and fruit maturity can also affect storage quality (Stretch and Ceponis, 1986). Stark *et al.* (1969) found that cranberries stored at 22°C for 3 weeks in atmospheres of 5 or 10 kPa CO_2 with 3 kPa O_2 had the same levels of rot as air stored fruit, while fruit held in 100 kPa N_2 became dull and water soaked in appearance and had a fermented odor. Ozgen *et al.*, (2002) studied the relationship between postharvest life of cranberry (*Vaccinium macrocarpon Ait. cv. Stevens)* fruit and reported that red fruit have longer postharvest life, possibly because of lower respiration

rates. Treatments with N_2 gas have been shown to improve cranberry storage life by reducing fungal decay (Lockhart *et al.,* 1971). Wang and Stretch (2001) found that the antioxidant activities of cranberries increased during storage with maximum increases occurring at 15°C compared with 0, 5, 10, and 20°C.

Craker (1971) reported that exposure of harvested fruit to 0.1 to 10 µL/L ethylene increased color formation in poorly colored fruit and it was greatly enhanced when fruit were exposed to light. Heat treatments using hot water reduced decay by killing pathogens or altering the physiology of the product (Fan *et al.,* 2008; Anderson and Smith 1971). Anthocyanin content was increased 10% by 2 and 4 kJ m^{-2} UV-C light treatments in '*Bluecrop*', but was reduced by the 4 kJ m^{-2} UV-C light treatments in '*Collins*' and UV-C treatments had no effect on weight loss, firmness, titratable acidity, or soluble solids content of the fruit (Forney, 2008). Cranberries (*Vaccinium macrocarpon Aiton*) were treated with high voltage electric fields (HVEF) of 2, 5 or 8kVcm^{-1} in strength for 30, 60 or 120 min in a parallel plate electrode system, and stored at ambient conditions (23°C and 65% RH) for three weeks to study the effect of treatments on their respiration rate, physiological loss of mass, color, total soluble solids (TSS) and skin puncture strength (Palanimuthu *et al.,* 2009).

Peonidin-3-glucoside (41.9%) and cyanidin-3-glucoside (38.3%) were the main anthocyanins isolated from fruits of *Vaccinium oxycoccus* L. (small cranberry) (Andersen, 1989). The major anthocyanins in cranberry are galactosides and arabinosides of cyanidin and peonidin (Fuleki and Francis, 1968). Vaccinium fruits are among the most plentiful food sources of anthocyanin. Content varied widely among cranberry cultivars, averaging 25–65 mg/100 g of ripe fruit at harvest (Wang and Stretch, 2001), with reports of anthocyanin content as high as 100 mg/100 g fresh fruit weight (Vvedenskaya and Vorsa, 2004).

4.2 Abiotic stresses: Generally a living commodity (plant, fruit) can be subjected to two types of stresses called as biotic and abiotic stresses. The stresses which are caused by the living organisms are called as the biotic stresses. Examples of biotic stresses are pathogenic microorganisms, insects, weeds etc. The stresses which are caused by nonliving things to plants are categorised as abiotic stresses. For example high temperature, ultra violet radiation, pulsed electric field, drought conditions, extreme weather conditions etc. Plants produce or stimulate synthesis of various stresses. Certain stresses can induce polyphenols production in plants.

Research has been conducted to find out the relation between abiotic stresses and the accumulation of polyphenols and increase in antioxidant activity of the plant material. There are many evidences of abiotic stresses reaction caused by wound, light, temperature and ultra violet radiation which have been found to increase the accumulation of polyphenols and improve the overall antioxidant activity (Cisneros-Zevallos, 2003)

4.2.1 Effect of abiotic stresses on various fruits and vegetables: It has been observed that various abiotic stresses can be used to induce polyphenol accumulation in purple flesh colored potato (Ryes and Cisneros Zevallos, 2003). Ethylene, methyl jasmonate, temperature and light were found to increase the polyphenolic content and anti-oxidant activity significantly to about 60%. It was found that wounding increased the polyphenols content to about 60% and antioxidant activity to about 85%. Lewis *et al*. (1999) reported that lowering the storage temperature increased the total flavonoid content in potatoes. Kalt *et al*. (1999) investigated the effect of storage temperature of 0, 10, 20 and 30°C for 8 days on anthocyanin and antioxidant activity of fresh berries. All fruits showed increased amount of anthocyanin and antioxidant activity for temperatures above and below the 20°C control temperature. Kalt *et al.* (1999) showed that anthocyanin increased in strawberry about 1.7 folds at 0°C while the increase was higher at higher temperature. Phenylpropanoid compounds are responsible for disease resistance in many fruits during post-harvest storage. Appropriate dosage of UV-C irradiation treatment duration can enhance the production of stress induced phenyl-propanoids within the treated product (Ben-Yehoshua *et al.,* 1998). Cantos *et al.,* (2003) showed that UV-C irradiated grapes are a good raw material for the production of stilbene enriched red wines as compared to the grapes without UV-C radiation. The wine made from UVC radiated grapes had nearly 1.5 to 2 times higher piceatannol and resveratrol than wine made from the non UV-C irradiated grapes. Strawberries treated with UVC dosage of 0.25kJ/m^2 maintained good quality with higher anthocyanin content while increasing the storage period by 3-4 days as compared to the control fruits (Baka *et al.,* 1999). Similarly Arakawa (1993) proved that the UV radiation can increase the anthocyanin pigment in the cherries while maintaining the quality for the fruit.

Enhancing the health benefit properties of fresh produce will add value and create new opportunities for growers and processors by reaching these health-oriented markets. To achieve this goal, there is a need to provide technologies that can ensure the delivery of high quality

products with high levels of the desired nutraceuticals (Cisneros-Zevallos, 2003). The main objective of this research is to produce a post-harvest produce with high anti-oxidant content. This work investigates the effect of hot water hormesis on secondary metabolites especially total anti-oxidants like polyphenols etc., The hypothesis brought forward is that the treatment of post-harvest produce with high pressure to enhance their antioxidant content by stimulating the fruits' stress response mechanisms.

To our knowledge, there was no published data about the use of hot water hormetic treatment for increasing the polyphenol content of berries especially cranberries. Consequently, the objective of this study was to evaluate the effect of hot water hormetic treatment on polyphenolic activity, color and firmness of the cranberry fruits.

4.3 Materials and methods

4.3.1 Selection of temperature and time: Cranberries were first heat treated at 80°C for 1 min, which resulted in softening of the fruit, with cell rupture and leaching of the juices of the fruit. The fruit was almost mushy in texture and difficult to hold its own shape. So heat treatment at 80°C was dropped from the experiment. The same test was repeated at 70°C for 1 min, which also resulted in similar results making the fruit mushy in texture, with cell rupture with loss in shape and color. Hence treatment at 70°C was also discontinued. Heat treatments at 40°C, 50°C, and 60°C showed no significant effect on the fruit shape and firmness; hence these were selected for this study.

The treatment time was chosen in such a way that the fruit was still able to carry out its physiological process like respiration and transpiration even after the stress; so that it could respond to and repair the stress it is subjected to. The fruits could withstand a maximum treatment time of 10 min without major changes in its firmness and color. Hence the time limit was set at 10 min. The selected time levels were 1, 5 and 10 min each at 40°C, 50°C and 60°C.

4.3.2 Treatment: The fresh cranberries were obtained from the market and cleaned with fresh water to remove the extraneous matters. From the whole lot of cranberries, the fruits which were of similar shape, size and maturity level were selected and weighed approximately 100g for each treatment. The weighed fruits were placed in a netted Nylon mesh bag and placed in the water bath maintained at selected temperatures of 40, 50 or 60°C and held for 1, 5 and 10 min. After

the selected treatment, the cranberries were removed from the water bath; water cooled and were wiped with a dry cloth to remove the excess moisture. The fruits were packed in polyethylene tetrapthalate containers and stored in a refrigerator at 4°C for 24h allowing the fruit to respond to the induced abiotic hormesis. After 24 h the fruits were removed from the refrigerator and evaluated for quality parameters of color, firmness and total polyphenols in comparison with the control cranberries.

4.3.3 Color: The color was assessed using a tristimulus Minolta Chroma Meter (Minolta Corp, Ramsey, NJ, USA) to determine color parameters L value (lightness or brightness), a* value (redness or greenness) and b* value (yellowness or blueness) of cranberry samples. The colorimeter was warmed up for 20 min and calibrated with a white standard. Measurements were taken for four samples and the average of L*, a* and b* values were obtained. Color of fruit was determined on two different locations on the surface of the fruit. The instrument was calibrated with a white standard tile: L=95.87, a = 0.86 and b=2.47. The total color difference in the cranberries after 24h was measured using the following relationship (Maftoonazad *et al.,* 2008).

$$\Delta E = [(\Delta L)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{0.5} \text{ ……..(Equation 4.1)}$$

4.3.4 Firmness: The firmness of the cranberries was estimated by a puncture test using a TX2 TA Texture Analyzer (TA-XT plus, Texture Technologies Corp, USA). The samples were compressed to 30% deformation in a single compression decompression mode at a steady speed of 50 mm min^{-1} using a 2 mm diameter puncture probe. Maximum force (N) needed to penetrate the fruit was used as an index of firmness. Sample of ten fruits were subjected to puncture and average of the firmness was represented (Gunes *et al.,* 2002).

4.3.5 Sample preparation for chemical analyses: The sample preparation was based on the method of Lim *et al.* (2007) with slight modification. Test samples (peels and pulps) (20 g) were crushed to a paste-like state consistency for approximately 2 min (with intermittent stops to minimize heating) using a blender. Approximately 5g blended sample was added to 20ml of 50% ethanol in a 100-ml volumetric flask. The mixture was shaken manually for 10 min, followed by centrifugation at 1500G. The supernatant liquid was filtered using Whatmann #1 filter paper and used for total polyphenols estimation.

4.3.6 Total polyphenols*:* Total phenolic content was determined according to the method of Lim *et al.* (2007). Samples (0.3 ml) were measured into test tubes followed by the addition of 1.0 ml of Folin-Ciocalteu's reagent (diluted 10 times with water) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with Para film and allowed to stand for 30 min in the dark. Absorbance was measured at 765 nm against a reagent blank. If the sample absorbance value exceeded 1, the sample was appropriately diluted to give reading less than 1 and then multiplied with the dilution factor. A standard calibration curve was prepared using gallic acid. Total phenolic contents were expressed in gallic acid equivalents in mg per 100 g fresh fruit.

4.3.7 Statistical Analysis*:* The statistical analysis was performed using MINITAB 16. The Analysis of variance was performed using two-way ANOVA at 95% level of confidence and 5% level of significance. The effect of time and temperature on the total polyphenols, color and firmness was evaluated two-way ANOVA.

4.4 Results and Discussion

4.4.1 Texture: The texture of the cranberries expressed as firmness after different treatments for 1, 5 and 10 min at 40, 50 and 60°C were evaluated for their significance with respect to process variables (temperature and time). The two-way ANOVA indicated that variables, time and temperature have significant effects on the response firmness of the cranberries ($p<0.05$) (Table 4.1). Temperature was found to be more significant than treatment time.

Table 4.1 Analysis of variance for firmness

Effect of time and temperature on firmness				
Source	**Sum of Squares**	**Mean Square**	**F**	**Sig.**
Temperature	6.84	3.42	30.95	0.004
Time	2.62	1.31	11.85	0.021

Results showed a mixed trend for the texture of cranberries after 24 h of storage following the after hormetic treatment (Figure 4.1). At a given treatment temperature, the firmness showed a decreasing trend with an increase in time. In addition, with increase in the treatment temperature,

the firmness of the cranberries decreased further for all treatments. Some conditions however, contributed to enhancement of texture.

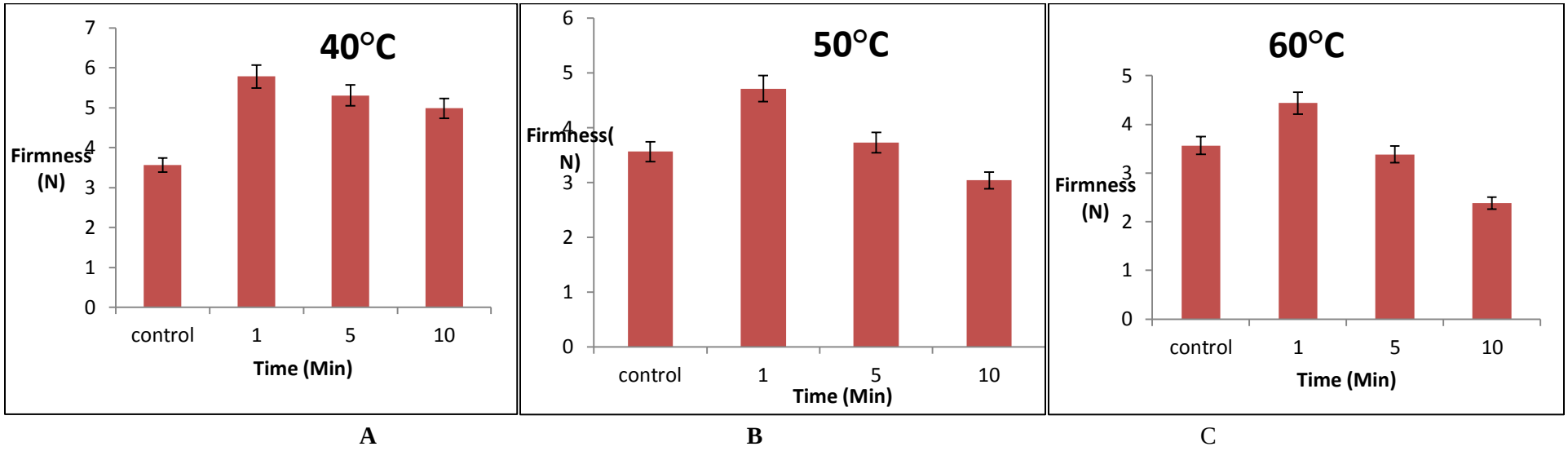
At 40°C, the exposure time of 1, 5 and 10 min increased the firmness of the fruit to 5.78N, 5.48N and 5.18N, respectively (Figure 4.1 A). The treatment of 40°C for all the times improved the firmness of the cranberry. At 50°C, the exposure time of 1, 5 min increased the firmness of the fruit to 4.15 N, 3.72N respectively, but the treatment for 10 min reduced the firmness of the fruit to 3.04N (Figure 4.1B). The temperature of 50°C for 10 min resulted in the softening of tissue which resulted in a decrease of firmness of the fruit. Similarly at 60°C, the exposure time of 1 min increased the firmness of the fruit to 4.15 N, but the treatment for 5 and 10 min reduced the firmness of the fruit to 3.38N and 2.38N respectively (Figure 4.1C).

Comparing the firmness of the treated samples with that of the control (3.65N), the firmness of the cranberries was improved by 1.62, 1.53, 1.45 times at 40°C for 1, 5 and 10 min respectively. Similarly the firmness of the cranberries was improved by 1.32, 1.04 times at 50°C for 1, 5 min whereas the firmness reduced by 0.85 times for 10 min. The temperature of 50°C for 10 min resulted in the softening of tissue which resulted in a decrease of firmness of the fruit. At 60°C the firmness was improved by 1.16 times at 1 min and reduced by 0.92 and 0.66 times for treatment time of 5 and 10 min.

Comparing the firmness for 40, 50 and 60°C at same times, the following observations were made.

1. Among the samples treated for 1 min, the samples treated at 40°C had higher firmness of 5.78N than 4.15N at 60 and 50°C, respectively.
2. Among the samples treated for 5 min, the samples treated at 40°C had greater firmness of 5.48N than 3.72N and 3.38N at 50 and 60°C respectively.
3. Similarly among the samples treated for 10 min, the samples treated at 40°C had higher firmness of 5.18N than 3.04N and 2.38N for 50 and 60°C respectively.

Comparison of firmness for all the nine treatment combinations (Figure 4.1) reveals that the treatments at 40°C for 1, 5 and 10 min improved the firmness of the fruit followed by 50°C and 60°C for 1 min respectively.



A **B** C

Figure 4.1 Changes in firmness in comparison with control at 1, 5 and 10 min at 40°C (A), 50°C (B) and 60°C (C)

The firmness of the cranberries treated at 40°C for 1 min had shown highest increase in firmness to 5.78N.

4.4.2 Color: The color parameters like L*, a* and b* values were collected from the cranberries after 24h of storage at 4°C. Figure 4.2 shows changes in the L*, a* and b* values of the samples at all the treatments. It was difficult to elucidate meaningful trends for these values as influenced by the process variables, the total color difference was computed from the L*, a* and b* parameters for further analyses.

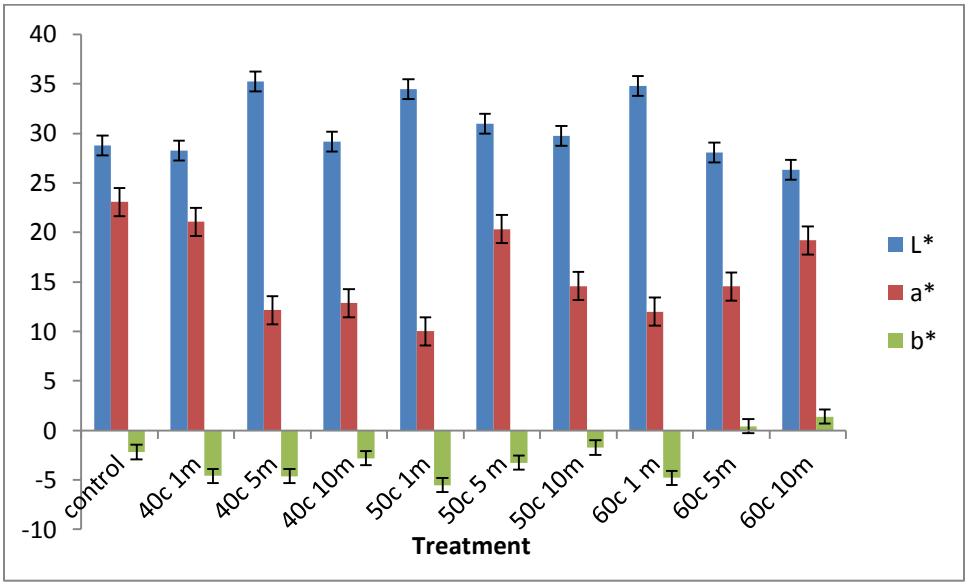


Figure 4.2 L*, a* and b* values of the samples in comparison with control

The two-way ANOVA (table 4.2) reported that time has a significant effect on the response of total color difference of the cranberries ($p<0.05$) whereas the temperature had no significant effect on total color difference of the cranberries ($p> 0.05$).

Table 4.2 Analysis of variance for total color difference

Effect of time and temperature on total color difference				
Source	**Sum of Squares**	**Mean Square**	**F**	**Sig.**
Temperature	7.56	3.78	1.78	0.291
Time	83.33	41.66	18.79	0.009

Among the treatments at 40°C for 1, 5 and 10 min, the total color difference of the samples showed a decreasing trend (Figure 4.3A). The total color difference was highest at value of 3.09 for the cranberries subjected to heat treatment at 40°C for 1 min, followed by 2.44 and 2.11 for 5 and 10 min respectively. Similar trends are observed at 50°C for 1, 5 and 10 min (Figure 4.3B). The total color difference was highest at value of 7.14 for the cranberries treated for 1 min, followed by 6.66 and 4.72 for five and ten min respectively. Among the treatment at 60°C for 1, 5 and 10 min was 12.8, 9.04 and 8.10 respectively (Figure 4.3C).

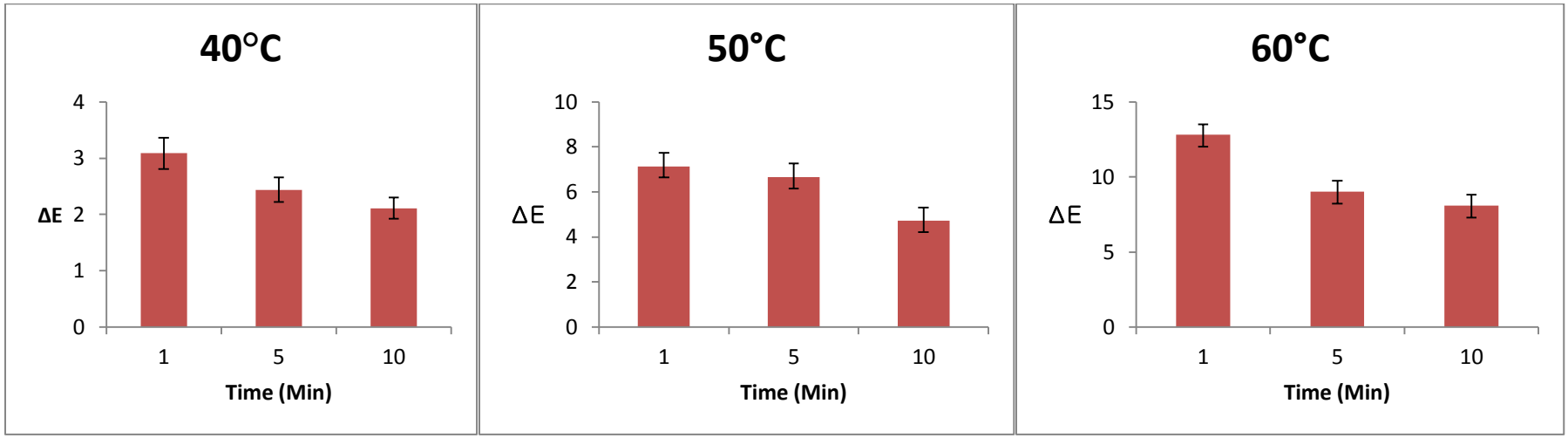
The total color difference of the samples showed a decreasing trend, with increase in time for all the temperatures. From the above observations, the total color difference for cranberries subjected to 40°C for 1 min was highest, followed by 50°C for 1 min and 60°C for 1 min.

Comparing the total color difference for 40, 50 and 60°C at same times, the following observations are made.

1. Among the samples treated for 1 min, the samples treated at 60°C had more total color difference of 12.85 than 7.14 and 3.09 at 50 and 40°C respectively.
2. Among the samples treated for 5 min, the samples treated at 60°C had shown more total color difference of 9.04 than 6.66 and 2.44 at 50 and 60°C respectively.
3. Similarly among the samples treated for 10 min, the samples treated at 60°C had more color difference of 8.10 than 4.72 and 2.11 for 50 and 40°C respectively.

This demonstrates that the samples subjected to less severe thermal stresses induced a greater color difference than the other treatments. This could be due to the accumulation of polyphenols in these samples as will be discussed later. In addition to the effect of temperature on breakdown and decay, temperature also affects fruit color. At 2°C and above, color tends to darken. Therefore, color can be improved in early harvested fruit, which tend to be pale in color by storing at 7 to 10°C for several weeks (Levine *et al.,* 1941).

In a drying experiment, dried samples with hot air had higher total color difference than dried samples with MW assisted hot air due to longer drying time in hot air method and expansion of texture (Andres *et al.,* 2004; Askari *et al.,* 2009). The “a” value decreased in dried samples with hot air. This decrease can be attributed to the heat degradation of coloured compounds (anthocyaninis) that occurs during dehydration. With heat treatment, reddish anthocyanin are



(A) (B) (C)

Figure 4.3 Total color difference (ΔE) at 1, 5 and 10 min at 40°C (A), 50°C (B) and 60°C (C)

converted into a colorless carbinol base, such that the bluish-brown "co-pigments" dominate the color of cranberries, and total color difference was increased (Fazaeli *et al.,* 2011).

The effects of light and abscisic acid on the postharvest ripening of late-harvested white cranberries (*Vaccinium macrocarpon Ait*.) were determined. After drying, fruit were held in a single layer at 20°C with or without 300 μmol $m^{-2}s^{-1}$ light from a combination of fluorescent and incandescent bulbs. Storing cranberries in light resulted in visibly more red fruit with a 4-fold higher concentration of anthocyanins than fruit held in the dark (Forney and Walt, 2009).

4.4.3 Total Polyphenols: The total polyphenols expressed as mg of gallic acid equivalents per 100 g of fruit sample showed an increasing trend in the cranberries subjected to heat stress with increase in temperature. The two way ANOVA reported that both variables had significant effects on the response of total polyphenols in the cranberries with $p<0.05$ (Table 4.3).

The total polyphenols of the cranberries was evaluated after one day of storage at 4°C temperature, giving the cranberries enough time to overcome the stress they were subjected to. It was noticed that the total polyphenols of the cranberries increased after one day of storage at 4°C when compared to control which had total polyphenols expressed as 120.15 mg of gallic acid/ 100 g of fresh cranberries. For all the 9 treatments, the cranberries showed an increase in total polyphenols over the control.

Table 4.3 Analysis of variance for total polyphenols

Effect of time and temperature on total polyphenols

Source	Sum of Squares	Mean Square	F	Sig.
Temperature	15991.6	7995.78	147.31	0.000
Time	2088.5	1044.24	19.24	0.009

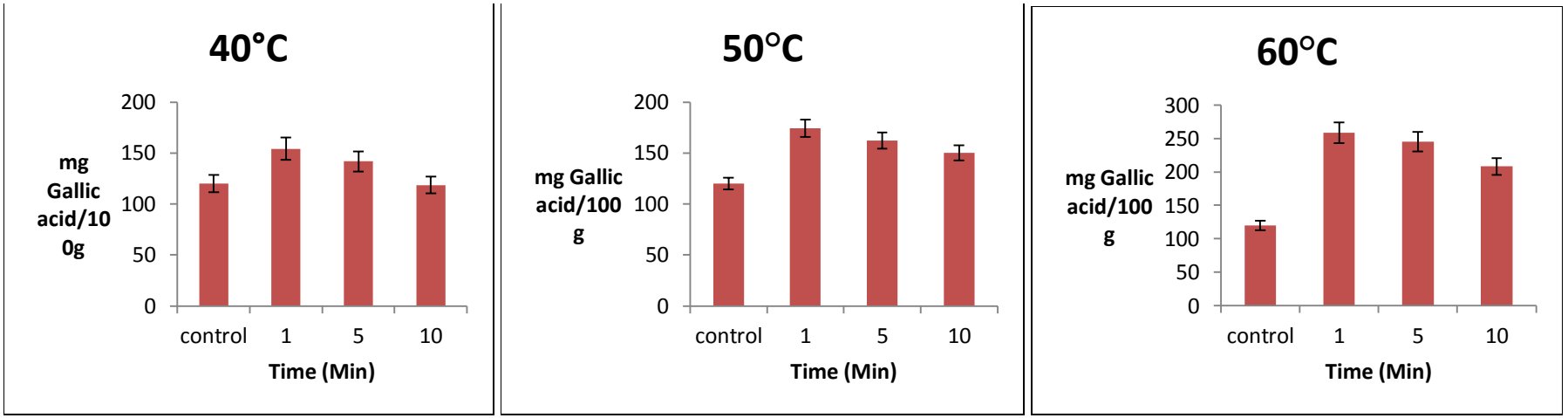
Among the treatments at 40°C for 1, 5 and 10 min, the total polyphenol was increased to a value of 154.3 mg and 141.8 mg of gallic acid/100g for the cranberries subjected for 1 and 5 min respectively, whereas total polyphenol was decreased to 118.7 mg of gallic acid per 100g of fruit sample for 10 min (Figure 4.4A). Thus the total polyphenols increased to 1.28 and 1.18 times for 1 and 5 min respectively, whereas they reduced by 0.98 times at 10 min.

Heat stress at 50°C for 1, 5 and 10 min shows an increase in total polyphenol of the samples with respect to the control. The total polyphenol was increased to values of 174.4 mg, 162.3 mg and 150.2 mg of gallic acid/100g for 1, 5 and 10 min respectively (Figure 4.4B). Even though there was an increase in the total polyphenols, decreasing trend was noticed with increase in time. The total polyphenols increased to 1.45, 1.35 and 1.25 times for 1, 5 and 10 min respectively at 50°C. Similar trends were noticed for total polyphenols for heat stress treatments at 60°C for 1, 5 and 10 min. The increase in total polyphenol of the samples showed a decreasing trend with time (Figure 4.4C). The total polyphenols increased to 258.6 mg, 245mg and 208.2 mg of gallic acid/100g for cranberries subjected to heat treatment at 60°C for 1, 5 and 10 min respectively. The total polyphenols increased to 2.15, 2.03 and 1.73 times for 1, 5 and 10 min respectively at 60°C. Within the same temperature treatments, the increase in polyphenols was more for samples subjected to shorter time.

Comparing the total polyphenols increase for 40, 50 and 60°C at same times, the following observations were drawn.

1. Among the samples treated for 1 min, the samples treated at 60°C had more polyphenols of 258mg than 174.4 mg and 154.3 mg at 50°C and 40°C respectively.
2. Among the samples treated for 5 min, the samples treated at 60°C had shown more total polyphenols of 245 mg than 162.3 mg and 141.8 mg at 50 and 40°C respectively.
3. Similarly for 10 min, the samples treated at 60°C had shown more total polyphenols of 208.2 mg than 150.2 mg and 118.7 mg at 50 and 40°C respectively.

From the above observations, the total polyphenols for cranberries subjected to 60°C for 1 min was highest, followed by 60°C for 5 min and 60°C for 10 min among all the nine treatments. The increase in polyphenols reduced with an increase in treatment time from 1 to 10 min for all the temperatures. Samples subjected to lower temperatures showed less increase in the polyphenols than those subjected for higher temperatures.



(A) (B) (C)

Figure 4.4 Changes in total polyphenols for 1, 5 and 10 min at 40°C (A), 50°C (B) and 60°C(C)

The cranberry cultivars *Cropper, Pilgrim, Stevens, Howes, Wilcox, Ben Lear*, and *#35*, had the lowest anthocyanin contents at the time of harvest. Increasing storage temperatures up to 15°C also significantly increased the anthocyanin contents of the cranberries, and in some cultivars, there was a 3 to 5 fold increase compared to those of freshly harvested berries. For instance, *Ben Lear* had initial anthocyanin content of 25.0 mg/100 g, but at storage temperatures of 0, 5, 10, and 15°C, the anthocyanin contents increased to 46.0, 54.6, 62.0, and 76.6 mg/100g, respectively. Similar to *Ben Lear, the cultivars, Early Black, Crowley, Franklin, #35, Wilcox, Howes, Stevens, Cropper,* and *Pilgrim* had the highest anthocyanin content when stored at 15°C for three weeks. Studies on the relationship between postharvest life of cranberry (*Vaccinium macrocarpon Ait. cv. Stevens)* fruit and ripeness stage at harvest reported that the color changed for each grade and the ethylene production was nearly the same for all the ripeness stages. Studies suggest that red fruit have longer postharvest life, possibly because of lower respiration rates, thicker cuticle and wax accumulation (especially at the calyx end) on these fruit may retard the entry of microorganisms into the fruit during wet harvest and may mitigate mechanical injury by harvesting equipment (Ozgen *et al.*, 2001). The effects of light and abscisic acid (ABA) on the postharvest ripening of late-harvested white cranberries (*Vaccinium macrocarpon Ait*.) were determined. After drying, fruit were held in a single layer at 20°C with or without 300 μmol $m^{-2}s^{-1}$ light from a combination of fluorescent and incandescent bulbs. Fruits had 28% more phenolics and 24% higher antioxidant capacity than fruit held in the dark (Forney and Walt 2009). An increment in total phenolics of around 50% and 40% with a 1.1 $J.cm^{-2}$ pulse for a 10 and 5 s treatment exposure respectively, was observed in elderberries. Even though most of the treatments increased in total polyphenols, some treatments decreased the total polyphenols of 2 to 9% for 0.45 $J.cm^{-2}$ for 30 s indicating that there is a limit to the response of the treatment (Murugesan, 2010).

4.5 Summary and Conclusions: In this study the potential of increasing the antioxidant capacity of cranberry with hot water treatment was clearly demonstrated. Hot water treatment as an abiotic stress enhanced the antioxidant activity, color and firmness properties of cranberry fruits by increasing their total polyphenolic content. The polyphenolic content of the cranberries increased to 258mg/100 g of sample for hot water dipping at 60°C for 1 min which in turn showed more total color difference, whereas for the temperature of 40°C for 1 min, it improved the firmness of the fruit. Overall observation shows that with the increase in temperature, the

polyphenolic content increased and firmness values could be reduced. This illustrates the potential use of hot water hormetic treatment to enhance the nutritional content of fruits and vegetables. However the exact mechanism through which the enhancement of the color, firmness and polyphenol occurred is not clear, but it is well supported by the previous research done in similar areas and further study is necessary to describe the mechanism. Since this method of treatment does not consume any significant amount of energy, it can be exploited to enhance the nutritive values of cranberry and other fruits.

CHAPTER 5

General Conclusions and Recommendations for Future Research

This research was focused on first evaluating the suitability of previously established pectin based formulation for cut melons (Narayanapurapu PTR and Ramaswamy HS, 2011) not included in the thesis). This was not very successful although the cut fruits could be kept well for up to a week under refrigerated conditions. The coating was not properly attaching to the cut surface of the fruit. Research on cut fruits were not continued due to lack of time, rather the focus was changed to explore the effect of previously tested pectin-based and alginate based emulsion formulations for coating of cherry tomatoes for their quality enhancement and shelf life extension. To achieve these objectives, pectin and sodium alginate emulsions were formulated according to Maftoonazad (2006) and Maftoonazad *et al.,* (2007), (2008) and applied on whole cherry tomatoes. Their associated quality changes were then evaluated under refrigerated and/or ambient storage conditions. Along with also to explore the effect of induced stress on accumulation of phenolics in cranberries. The major findings are detailed below:

Coating of cherry tomatoes: The pectin and alginate based coating on cherry tomatoes was effective in reducing the physiological activities and associated quality changes thereby contributing to shelf life extension. The coatings effectively reduced the produce transpiration and consequently lowered the weight loss by water evaporation. The produce respiration rate was significantly reduced and the climacteric peak was both suppressed and delayed for both the coatings. These were considered key factors for extending the post-harvest shelf life of cherry tomatoes. They also slowed down the associated physiological and chemical degradation processes properties of the fruit during storage. The composite coatings imparted sheen to the fruits, reduced decay, retarded the color changes of both skin and flesh, and lowered the softening rate of the tissue. The coatings helped to delay the ripening as indicated by changes in TSS, pH, and titratable acidity. The cherry tomatoes with pectin based coating had a slightly better quality than those coated with alginates. Pectin coated samples had the added gloss over the surface adding value whereas the alginate coatings formed a whitish translucent layer hiding the real color of the fruit which might affect the consumer acceptability.

Overall, based on the results obtained in this research, coating of cherry tomatoes with an emulsion using beeswax as hydrophobic phase, high methoxy pectin and alginate as hydrophilic polymer, sorbitol as plasticizer, and monoglycerides as emulsifying agent can be considered effective in extending their shelf-life by at least 25%. This simple and inexpensive coating procedure is effective in slowing down the metabolism of these commodities to give prolonged storage life.

Enhancement of cranberry polyphenols: Improving the health benefit properties of fresh produce like fruits and vegetables will add value and create new opportunities for growers and processors by reaching these health-oriented markets to meet the standards of the dynamic consumers whose focus is shifting towards the health promoting foods. Hormesis is the application of potentially unfavorable agents at low doses to living organisms in order to induce stress responses. Light, wind, ultra violet radiation, pulsed light, pulsed electric field, high pressure are sources of abiotic stress as they are factors external to the living tissues. Abiotic stresses have been shown to induce hypersensitive responses in the host resulting in the synthesis of stress metabolites like terpenes, phenolics, and nitrogen-containing compounds.

This research work investigated the potential use of abiotic heat stress for enhancing the anti-oxidant content of cranberries. This research highlights the potential application for enhancing the polyphenols of cranberries. The abiotic heat stress of 60°C for 1 min improved the total polyphenolic activity of the cranberries two folds, whereas the heat stress at 40°C for 1 min improved the firmness and showed significant color difference ($p<0.05$) of the fruits. The results can be potentially used for enhancing the antioxidant properties of cranberries which can be applied to the fortification of juices.

Future research: In order to improve the feasibility of using pectin and alginate based coating on a commercial scale, additional larger scale post-harvest tests (e.g. sensory tests to determine consumer acceptance, analysis of biological parameters, etc.) are necessary.

The preliminary application of coating research to cut fruits has good scope. The formulations should be modified to make them more hydrophobic and at the same time their adhesive properties should be enhanced so that the coating adheres well to the surface. With these

modifications, the coatings can have a significant effect on extending the shelf life of cut fruits and prevent their weight loss.

Coating research can be extended to include other functional property modifiers by incorporating plasticizers with anti-microbial properties. For example incorporation of essential oils from cinnamon, lemon grass, etc. The films should be evaluated for transmission and mechanical properties to ensure better performance. Finally to get the best results, it is recommended to optimize the formula for each fruit and/or vegetable cultivars as each fruit respires, transpires and ripens differently.

Various methods of abiotic stresses are given to fruits and vegetables in order to explore their potential in enhancing the nutritive value of the produce. Literature shows UV light, controlled atmosphere storage conditions, wounding and temperature were explored to enhance the nutritional content of the fruits. Hence heat stress increased the polyphenolic contents of the cranberries; it could be explored for other potential fruits which are rich in polyphenols like blue berries, black berries and elderberries. Along with heat stress, various novel technologies like high pressure, microwave, and pulsed electric shocks can be used for a short period of time at low levels to check the effect of the abiotic stress on enhancing the nutritional compounds of the post-harvest produce.

In terms of insuring foo security Hence post-harvest losses and food waste are alarming issues in developing and developed countries. This thesis provides a basic insight on reducing post-harvest losses with coatings and enhancing the nutritional compounds in fresh berries with heat stress.

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