Chapter I

INTRODUCTION

Broccoli (Brassica oleracea var. italica) is an annual crop and grows to maturity in 75-95 days depending on cultivar, season and planting date. It is a popular but highly perishable vegetable and an important contributor of antioxidant vitamins in the human diet. The storage life of broccoli is 3 to 4 weeks in air at 0°C (Makhlouf and others 1989a) and 2 to 3 days in air at 20°C (Wang 1977b). The terminal floral cluster and supporting stalk (the part of broccoli plant commercially marketed) have high respiration and transpiration rates. Ice is usually used to reduce the temperature of broccoli heads to retard respiration and increase relative humidity. As the post harvest life of broccoli is short, it should be marketed within 1-14 days after harvest (Brennan and Shewfelt 1988) to appeal to consumers.

Post harvest treatments such as packaging that employs polymeric films and automatic misting preserve nutritional and other quality attributes of whole broccoli products (Rij and Ross 1987; Kader and others 1989; Barth and others 1992). The post harvest life of broccoli florets and spears has been extensively studied but crown-cut heads of broccoli may require a different and improved handling and storage applications different from broccoli florets. Crown-cut broccoli has a large single head with main stem attached while spears and florets are broken parts of broccoli head. It is possible that the shelf life deterioration may be different in crown-cut heads of broccoli than in florets and spears.

Rationale/Justification:

Historically in South West Virginia, Carroll/Patrick/Floyd counties have long been known for quality crucifer (cabbage) production (Tobler 1984). But there has been a significant decrease in acreage under cabbage due to low prices and replacement of cabbage with more profitable crops. Fall broccoli has the potential to be a high value crop if the shrink wrap packaging technology can be disseminated to the experienced growers of this region. Without icing requirement, broccoli marketing would be easy and opens
up significant potential for Virginia producers who can afford relatively inexpensive film wrapping machines (as compared to ice equipment) in conjunction with onsite cooling. Immediate onsite cooling is often not possible and delays may be involved before the harvested produce is cooled. Thus we sought to examine the effects of post harvest cooling delay on post harvest characteristics of broccoli. Overall, this study aims to characterize post harvest shelf life of broccoli using shrink wrap packaging in comparison to a standard ice packaging. The following are the specific objectives of this study.

**Specific Objectives:**

1) To evaluate the performance of shrink wrap polymeric film for water loss, internal atmosphere, color, texture and ascorbic acid compared to ice packaging.

2) To determine the effects of post harvest cooling delay between shrink wrapped single head broccoli to iced single head broccoli with respect to weight loss, color, texture and ascorbic acid content under typical market storage conditions.

3) To compare the shelf life and performance of broccoli cultivars, Everest (50 days to maturity) and Gypsy (61 days to maturity).

4) To study the effects of post harvest treatments on retention of phytochemicals (glucosinolates).
Chapter II

REVIEW OF LITERATURE

A) Modified Atmosphere Packaging

The quality of fresh broccoli primarily depends upon maturity at harvest, minimizing injury due to handling and maintaining optimum temperature and humidity. Once these primary requirements have been met, further maintenance of quality can be enhanced by modifying the atmosphere surrounding the product. Historically atmospheres surrounding produce have been altered in controlled atmosphere storage facilities where the levels of gas are continually monitored and adjusted to maintain optimal concentrations (Zagory and others 1988). Modified atmosphere storage implies a lower degree of control of gas concentrations. Advances in design and manufacture of polymeric films with a wide range of gas permeability characteristics have helped maintain modified atmospheres within flexible film packages.

Modified Atmosphere Packaging (MAP) utilizes polymeric films of differential permeabilities to O\textsubscript{2}, CO\textsubscript{2}, C\textsubscript{2}H\textsubscript{4} and water vapor to extend the shelf life of fruits and vegetables (Barth and others 1993). Atmosphere modification within a film develops as a result of the respiration rate of the plant tissue and diffusion characteristics of the film (Kader and others 1989). Modified atmosphere storage generally results in reduced respiration rates as long as O\textsubscript{2} and CO\textsubscript{2} levels are maintained within levels tolerated by broccoli. The effects of reduced O\textsubscript{2} and elevated CO\textsubscript{2} on respiration are additive and greater than the effects of either alone (Kader and others 1989). However exposure of fresh produce above their CO\textsubscript{2} tolerance limit may cause physiological damage and exposure to levels below their O\textsubscript{2} tolerance limit may increase anaerobic respiration and the development of off flavors due to accumulation of ethanol and acetaldehyde (Zagory and others 1988).
1) Factors affecting modified atmospheres

The conditions created and maintained within a package are the net result of the interplay among several factors generated by environment and fresh produce.

a) Resistance to diffusion of $O_2$, $CO_2$, $C_2H_4$

Most fruits and vegetables are tolerant of $O_2$ levels down to 1-5% and $CO_2$ levels upto 5-10% (Kader 1980). Broccoli can tolerate as low as 1% $O_2$ and upto 10% $CO_2$ (Kader and others 1989). Plant enzymes involved in $O_2$ utilization can function in an environment of less than 1% $O_2$ (Burton 1978). The difference between external $O_2$ (or external $CO_2$) concentration and the amount of $O_2$ (or $CO_2$) available within the cell is determined largely by the resistance of the plant organ to gas diffusion. Resistance to gas diffusion varies among different plants, plant cultivars, plant organs and stage of maturity but little affected by temperature (Cameron and Reid 1982). Anatomical differences are responsible for differing diffusion resistance, rather than biochemical differences in tolerance to low $O_2$ and high $CO_2$ modified atmosphere (Burton 1974).

b) Respiration

Primary effect of modified atmosphere is a lower rate of respiration which reduces the rate of substrate depletion, $CO_2$ production, $O_2$ consumption and release of heat. The result is lowered metabolism and longer shelf life. Modified atmosphere conditions can alter respiratory quotient which in turn will affect the atmosphere created by the respiration of the commodity within the package (Kader and others 1987; Tomkins 1965). The rate of respiration is sensitive to changes in $O_2$ concentration below 8% and $CO_2$ about 1% (Zagory and others 1988). However if $O_2$ is reduced or $CO_2$ is elevated beyond levels of tolerance of the commodity, respiration associated with anaerobic respiration or $CO_2$ damage will increase.
c) Optimum temperature

Metabolic processes such as respiration and ripening rate are sensitive to temperature. Biological reactions generally increase two to three fold for every 10°C rise in temperature. Generally fruits and vegetables will last longer at lower temperatures. However, every commodity has a lower temperature limit and below this limit chilling damage can occur, thus increasing respiration rate, hastening senescence and lowering the value of the commodity.

The optimum temperature may vary depending upon other conditions. For example, reduced O₂ or elevated CO₂ can overcome the impact of lower temperature injury on the ripening process. Reduced chilling injury has been associated with elevated CO₂ for some commodities (Lyons and Breidenbach 1987). Proper temperature management of fresh produce is perhaps the single most important part of post harvest handling. Cantwell and Suslow (1999) reported that fluctuations during commercial handling of broccoli can produce offensive sulfur containing odors.

d) Optimum relative humidity

Low relative humidity can increase transpirational damage and lead to desiccation, increased respiration and an unmarketable product (Kader 1987). One of the benefits of modified atmosphere packaging in general is the maintenance of adequate relative humidity within the package. There is a danger that relative humidity can get too high causing moisture condensation and conditions favorable for microbial growth, resulting in decay of the commodity. Condensation on the film package surface may adversely affect the gas permeability properties of the film, leading to the evolution of an unfavorable atmosphere. Maintenance of proper temperature throughout the post harvest handling steps is crucial to preventing condensation within the package.
e) Optimum concentrations of $O_2$ and $CO_2$

An optimum atmosphere should minimize respiration rate without danger of metabolic damage to the commodity. Different commodities vary widely in their tolerances to different atmospheres. Since the effects of low $O_2$ and high $CO_2$ on respiration are additive the optimal concentrations of both gases in combination are difficult to predict without actual measurements in a variety of atmospheres (Zagory 1988). The limits of tolerance to low $O_2$ and high $CO_2$ beyond which damage occurs are subject to several variables such as temperature, physical conditions and maturity (Kader and others 1989). Cantwell and Suslow (1999) reported that most modified atmosphere packaging for broccoli is designed to maintain both $O_2$ and $CO_2$ at about 10% to avoid development of undesirable odors.

2) Methods of creating modified atmosphere conditions

Modified atmospheres can be created either passively by the commodity or intentionally.

a) Commodity generated or passive MA

If commodity characteristics are properly matched to film permeability characteristics, an appropriate atmosphere can passively evolve within a sealed package as a result of the consumption $O_2$ and the production of $CO_2$ through respiration (Smith and others 1987). In order to achieve and maintain satisfactory atmosphere within a package, gas permeabilities of the selected film must be such that they allow $O_2$ to enter the package at a rate offset by the consumption of $O_2$ by the commodity. Similarly, $CO_2$ must be vented from the package to offset the production of $CO_2$ by the commodity. Furthermore, this atmosphere must be established rapidly and without the danger of creation of anoxic conditions or injuriously high levels of $CO_2$. 
b) Active modified atmosphere

Atmospheres within MAP can be actively established and adjusted because of the limited ability to regulate a passively established atmosphere. This can be done by pulling a slight vacuum and replacing the package atmosphere with the desired gas mixture. This mixture can be further adjusted through the use of absorbers or adsorbers in the package to scavenge $O_2$, $CO_2$ and $C_2H_4$ (Zagory and Kader 1988).

B) Films for packaging fresh produce

Although many plastic films are available for packaging purposes, relatively few are used to wrap fresh produce and even fewer have gas permeabilities that make them suitable to use for MAP. In a MA package the $O_2$ content would be reduced from an ambient 21% to 2.5% and $CO_2$ will increase from an ambient 0.03% to 16-19% in the package (Zagory and Kader 1988). Because these high levels of $CO_2$ would be injurious to most fruits and vegetables (Smith and others 1987) an ideal film must let more $CO_2$ out than $O_2$ to enter. Several polymers used in film formulation meet this criterion. Polymer film permeability is dependant on many factors including gas-polymer solubility, temperature (Rogers 1985) and micro porosity (Mannapperume and others 1989). Low density polyethylene and poly vinyl chloride are the main films used in packaging fruits and vegetables (Zagory 1988). Therefore, permeability determination of the specific film under the envisaged storage conditions is a requirement for effective use of film. The extension of broccoli shelf life has been demonstrated using perforated polyethylene (Wang 1977b) and vacuum packaging (Wu and Salunkhe 1976).

C) Yellowing in broccoli

Broccoli is a rapidly developing floral vegetable (Wang 1977b) and florets are the most perishable part of broccoli head. Color is an extremely important attribute of fresh
broccoli which is three dimensional, based on responses of three different receptors (red, green and blue) in the human eye (Francis and Clydesdale 1975).

Loss of green color is a major limiting factor in shelf life reduction in the storage of fresh broccoli. ‘Yellowning’ is the term used to describe the adverse condition of broccoli color quality degradation. Yellowning may be caused by over maturity at harvest, high storage temperatures and/or exposure to ethylene. Two types of yellowing occur in broccoli. Bead yellowing is due to senescence, while marginal yellowing develops during growth when florets are not exposed to light. Bead yellowing is a limiting factor in commercial marketability of broccoli.

Floret maturity is likely to have the greatest effect on the rate of broccoli yellowing (Tian and others 1994). The phytohormones, ethylene and cytokinin also play a central role in post harvest yellowing of broccoli. According to Makhlouf and others (1989) ethylene accelerates and cytokinin delays (Rushing 1990) broccoli yellowing.

Ethylene production by reproductive organs (stamens) is greater than that produced by sepals and petals. Ethylene produced from reproductive organs may increase sensitivity of sepals to ethylene (Tian and others 1994). Cytokinins delay yellowing in leaves from many plant species (Adepipe and others 1971; Thimann 1980; Lipton and Mackey 1987; Szewykowski 1992) and may stimulate development of plastids and chloroplasts (Parthier 1989). A number of encoded mRNAs for chloroplast proteins such as small sub unit of ribulose-1,5 bisphosphate carboxylase and light harvest chlorophyll a/b protein are suggested to be regulated at the transcript level by cytokinin (Parthier 1989). Cytokinins have a critical role in regulating chlorophyll biosynthesis and/or degradation (Tetley and Thimann 1974; Shewfelt and others 1983; Szewykowski 1992).

Modified atmosphere storage has been shown to be beneficial in preventing or retarding the yellowing of broccoli (Leberman and others 1968). Low O₂ and high CO₂ concentrations prevent the formation of ethylene, essential to yellowing process (Leberman 1964). Broccoli stored under elevated CO₂ concentrations has a brighter green
color when cooked than broccoli stored in air. Oxygen at 1% level or less inhibited yellowing of broccoli heads during storage at 5°C or 7.5°C (Lipton and Harris 1974). Yellowsing was minimal at 2.5°C regardless of O₂ concentration.

Packaging of broccoli using polymeric films had been shown to retard deterioration and enhanced maintenance of nutrients and color. Shewfelt and others (1983) investigated effects of packaging and icing on color retention of N₆ benzyl adenine treated broccoli. Best color retention was found in packaged samples. Rij and Ross (1987) observed increased green color and turgidity retention as well as reduced water loss in packaged broccoli spears. Forney and others (1989) reported that broccoli packaged in film (which resulted in accumulation of about 10% CO₂ within packages) retained turgidity and moisture significantly better than non packaged controls. Wang (1979) reported that loss of chlorophyll and ascorbic acid and onset of ethylene production were delayed in broccoli spears under high CO₂ (20-40%).

**D) Effects of post harvest cooling delay on shelf life**

Broccoli has a short post harvest shelf life and is marketed within 1 to 14 days after harvest. The terminal floral cluster and supporting stalk (part of broccoli plant commercially harvested) have high respiration and transpiration rates which continue during market distribution. Ice is used in the field to reduce the temperature, which retards respiration and elevates relative humidity which in turn delays wilting. Successful storage of broccoli for 2 to 4 weeks under ideal conditions (0°C, 95% relative humidity) has been reported (Ryall and Lipton 1974; Shewfelt and others 1984). Small changes in techniques at one stage of handling (cooling after harvest) may have pronounced effects on quality at a later stage (Hackert and others 1987).

**E) Ascorbic acid in broccoli**

Broccoli is one of the most popular and highly perishable vegetable and important contributors of antioxidant vitamins including β carotene and ascorbic acid and other
nutritive components of the human diet. Broccoli ranks 5th among fresh fruit vegetables ranked for vitamin C content (Salunkhe and others 1976). In addition to the value of the vitamin C to human nutrition, ascorbic acid is biochemically important to the plants as a reducing agent in the synthesis of ethylene (Liberman and Mapson 1964).

Ascorbic acid content of fresh produce changes during harvest, handling, storage and transportation (Seelig 1979). Ascorbic acid is labile and its retention is often followed when evaluating post harvest storage effects on nutritional quality of vegetables (Klein and Perry 1982; Vander Slice and others 1990).

Modified atmosphere storage has been shown to result in greater ascorbic acid retention and shelf life extension in contrast to air stored vegetables (Kader 1986; Rij and Ross 1987). Wang (1979) reported that loss of ascorbic acid and chlorophyll were delayed in broccoli spears under high CO₂ (20 - 40%). Hudson and others (1985) reported that floral buds of broccoli head contain 120 ± 26 mg of ascorbic acid per 100 gm of broccoli. Barth and others (1993) estimated a 15% drop in amino acid content from initial value in first 48 hours in packaged broccoli.

F) Glucosinolates in broccoli

Glucosinolates are an important group of β-D-thioglucosides that are widely distributed throughout the Cruciferae, a family that includes a number of vegetables important in human nutrition (Hanley and others 1983; Heaney and Fenwick 1985). Broccoli has been shown to be rich in glucosinolates possessing (methyl-sulfinyl) alkyl side chains (Fenwick and others 1983; Carlson and others 1987). Glucosinolates may be hydrolyzed to form bioactive isothiocyanates, nitriles and/or thiocyanates in plant materials during processing by the action of the endogenous enzyme thioglucoside glucohydrolase commonly called as myrosinase (Cole 1976). These hydrolysis products contribute to characteristic flavors and odors of Brassica vegetables (Wallsgrove and others 1999) and produce a wide range of physiological changes in higher animals and humans (Fenwick
and others 1983). Environmental conditions and agronomic practices markedly affect glucosinolate level and amount and type of breakdown products formed (Heaney and Fenwick 1980). The nature and level of glucosinolates vary in different plant species with seed containing up to 5% and leaf tissue as little as 0.1% of fresh weight (Sang and others 1984). Rangkadorlik and others (2002) reported that at 20°C there was 55% loss of glucoraphanin concentration in broccoli stored in open boxes during first three days.

Hansen and others (1995) studied glucosinolate content of broccoli stored for 7 days at 10°C under air and controlled atmosphere conditions (0.5% O₂, 0.5% O₂ + 20% CO₂ and 20% CO₂) and concluded that controlled atmosphere treatment and storage time had no significant effect on the relative content of methylsulfinylalkyl glucosinolates (glucoiberin and glucoraphanin) and 3-indolyl methyl glucosinolates (glucobrassicin, neoglucobrassicin and 4-methoxy glucobrassicin). In contrast, Howard and others (1997) concluded that sulforaphane, a breakdown product from glucoraphanin decreased after storage of broccoli was held in perforated polyethylene perforated bags at 4°C for 21 days after harvest. Rodrigues and Rosa (1999) suggested refrigeration at 4°C and freezing were the best preservation processes for maintaining high content of glucosinolates in broccoli.


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A. ABSTRACT

The effects of packaging treatments, post harvest cooling delay and storage duration on color, texture, ascorbic acid content, weight loss and glucosinolate retention in crown-cut heads of broccoli were studied. Oxygen and CO\textsubscript{2} levels inside shrink wrap packages were also monitored. Shrink wrap packaging had a significant positive effect on hue angle (\(p\leq0.05\)). Packaging and post harvest cooling delay had no effect on hue difference (\(\Delta H\)) and total color difference (\(\Delta E\)). While post harvest cooling delay had no effect on texture, crown-cut heads of broccoli stored in shrink wrap packaging retained firmness significantly better than ice packaged heads of broccoli (\(p\leq0.05\)). Ascorbic acid was retained better in broccoli held in shrink wrap packages and post harvest cooling delay had a significant negative influence on ascorbic acid content (\(p\leq0.05\)). Packaging and post harvest cooling delay had a significant positive effect on weight loss (\(p\leq0.05\)). Broccoli stored in shrink wrap film lost about 3.7\% of original weight while ice packaging resulted in about 17.4\% weight loss (\(p\leq0.05\)). No consistent trends were observed in the levels of O\textsubscript{2} and CO\textsubscript{2} inside shrink wrap packages. An important glucosinolate, glucoraphanin was retained significantly better in shrink wrapped heads (\(p\leq0.05\)). Between two cultivars, shelf life of cv.Gypsy was better than cv.Everest with respect to color, ascorbic acid retention and weight loss. But cv.Everest retained texture (firmness) better after 35 days of storage. Overall results indicate that shrink wrap packaging and shorter post harvest cooling delays protect quality of broccoli.

Key Words: Broccoli, Shelf life, Shrink wrap packaging, Ascorbic acid, Glucosinolates, Texture, Color
B. INTRODUCTION

Broccoli (*Brassica oleracea var.italica*) is an annual crop and grows to maturity in 75-95 days depending on cultivar, season and planting date. It is one of the most popular and highly perishable vegetables and an important contributor of antioxidant vitamins in the human diet. The storage life of broccoli is 3 to 4 weeks in air at 0°C (Makhlouf and others 1989a) and 2 to 3 days in air at 20°C (Wang 1977b). Post harvest treatments such as packaging that employs polymeric films and automatic misting preserve nutritional and other quality attributes of whole broccoli products (Rij and Ross 1987; Kader and others 1989; Barth and others 1992). Modified Atmosphere Packaging (MAP) utilizes polymeric films of differential permeabilities to O$_2$, CO$_2$, C$_2$H$_4$ and water vapor to extend shelf life of fruits and vegetables (Barth and others 1993). Modified atmosphere conditions can alter respiratory quotient which in turn will affect the atmosphere created by the respiration of the commodity within the package (Kader and others 1989). The rate of respiration is sensitive to changes in O$_2$ concentration below 8% and CO$_2$ about 1% (Zagory and others 1988). Broccoli can tolerate upto 10% CO$_2$ and as low as 1% O$_2$ (Kader and others 1989). Cantwell and Suslow (1999) reported that most modified atmosphere packaging for broccoli is designed to maintain both O$_2$ and CO$_2$ at about 10% to avoid development of undesirable odors.

Broccoli is a rapidly developing floral vegetable (Wang 1977b) and florets are the most perishable part of broccoli head. Color is an extremely important attribute of fresh broccoli which is three dimensional, based on responses of three different receptors (red, green and blue) in the human eye (Francis and Clydesdale 1975). Loss of green color is a major limiting factor in shelf life reduction in the storage of fresh broccoli. ‘Yellowing’ is the term used to describe the adverse condition of broccoli color quality degradation. Yellowing may be caused by over maturity at harvest, high storage temperatures and/or exposure to ethylene. Two types of yellowing occur in broccoli. Bead yellowing is due to senescence, while marginal yellowing develops during growth when florets are not exposed to light. Bead yellowing is a limiting factor in commercial marketability of broccoli. Modified atmosphere storage has been shown to be beneficial in preventing or
retarding the yellowing of broccoli (Leberman and others 1968). Oxygen, at 1% level or less inhibited yellowing of broccoli heads during storage at 5°C or 7.5°C (Lipton and Harris 1974). Rij and Ross (1987) observed increased green color and turgidity retention and reduced water loss in packaged broccoli spears.

Broccoli is an important contributor of antioxidant vitamins including β carotene and ascorbic acid and other nutritive components of the human diet. Broccoli ranks 5th among fresh fruit vegetables ranked for vitamin C content (Salunkhe and others 1976). In addition to the value of the vitamin C to human nutrition, ascorbic acid is biochemically important to the plants as a reducing agent in the synthesis of ethylene (Liberman and Mapson 1964). Ascorbic acid is labile and its retention is often followed when evaluating post harvest storage effects on nutritional quality of vegetables (Klein and Perry 1982; Vander Slice and others 1990).

Glucosinolates are an important group of β-D-thioglucosides that are widely distributed throughout the Cruciferae, a family that includes broccoli and a number of vegetables important in human nutrition (Hanley and others 1983; Heaney and Fenwick 1985). Broccoli has been shown to be rich in glucosinolates possessing (methyl-sulfinyl) alkyl side chains (Fenwick and others 1983; Carlson and others 1987). Glucosinolates may be hydrolyzed to form bioactive isothiocyanates, nitriles and/or thiocyanates in plant materials during processing by the action of the endogenous enzyme thioglucoside glucohydrolase commonly called as myrosinase (Cole 1976). These hydrolysis products contribute to characteristic flavors and odors of cruciferous vegetables (Wallsgrove and others 1999) and produce a wide range of physiological changes in higher animals and humans (Fenwick and others 1983).

Hansen and others (1995) studied glucosinolate content of broccoli stored for 7 days at 10°C under air and controlled atmosphere conditions (0.5% O₂, 0.5% O₂ + 20% CO₂ and 20% CO₂) and concluded that controlled atmosphere treatment and storage time had no significant effect on the relative content of methylsulfinylalkyl glucosinolates (glucoiberin and glucoraphanin) and 3-indolyl methyl glucosinolates (glucobrassicin,
neoglucobrassicin and 4-methoxy glucobrassicin). In contrast Howard and others (1997) concluded that sulforaphane, a breakdown product from glucoraphanin decreased after storage of broccoli was held in perforated polyethylene perforated bags at 4°C for 21 days after harvest. Rodrigues and Rosa (1999) suggested refrigeration at 4°C and freezing were the best preservation processes for maintaining high content of glucosinolates in broccoli

The post harvest life of broccoli florets and spears has been extensively studied but crown heads of broccoli may require a different and improved handling and storage applications different from broccoli florets. It is possible that the deterioration may be different in crown-cut heads of broccoli than in florets and spears. In the present study we investigated the effects of packaging treatments and post harvest cooling delay on the shelf life characteristics of two cultivars (cv.Everest and cv.Gypsy) of broccoli.

C. MATERIALS AND METHODS

1) Plant material, packaging and storage

Everest (50 days to maturity) and Gypsy (61 days to maturity) commercial cultivars of broccoli were grown at Whitethorn farm (Blacksburg, VA) during fall 2003, using standard field practices (O’Dell 1993). Seed material for cv.Everest was procured from Syngenta Seeds Inc. (Golden Valley, MN) for cv.Gypsy was provided by Sakata Seed America Inc. (Morgan Hill, CA). Broccoli cv.Everest was planted on July 20, 2003 and harvested on September 12, 2003 while cv.Gypsy was planted on August 16, 2003 and harvested on October 18, 2003. At maturity (Cantwell and Suslow 1999) whole heads (120 heads for each cultivar) of broccoli were hand harvested during morning coolness (harvest temperature for cv.Everest was 13°C and for cv.Gypsy 17°C), stripped of any remaining leaves and transported to Food Science and Technology laboratory, Virginia Polytechnic Institute and State University located 9 miles (approx) from growing location (Blacksburg, VA). Three levels of post harvest cooling delay i.e. one hour, three hours and six hours and two packaging methods i.e. shrink wrap packaging and ice packaging
were considered. The heads of broccoli were randomly distributed between two packaging treatments. One third of the broccoli heads from both packaging treatments were air cooled at one hour after harvest, 1/3 were cooled at 3 hours after harvest and the remaining 1/3 were cooled at 6 hours after harvest. Broccoli heads were held at 20°C until they were subjected to respective treatments. Each treatment for cv. Everest was replicated 4 times while cv. Gypsy was replicated 3 times.

Packaging material, PD-941 (Cryovac, Duncan, SC) multilayered polyolefin film and an OptiR Shrink Packaging System (Sealed Air, Ayer, MA) was used for shrink wrapping broccoli heads. Samples were stored in a walk-in cooler at a standard temperature of 3±1°C F and relative humidity was 85±3%. Ice was replenished as necessary on a daily basis. Samples of stored broccoli heads were taken out of cooler after 7, 14, 21, 28 and 35 days (after harvest) and subjected to analyses.

2) Chemical and physical analyses of broccoli

a) Weight loss

Initial weight of broccoli heads was measured on a balance, Model I-20W (Ohaus Corporation, NJ) after subjecting them to respective post harvest cooling delay treatments. Final weight of the shrink wrapped broccoli heads was measured after removing the film and for the heads of broccoli stored in ice, final weight was taken after shaking off any remaining ice and water. Weight loss was measured as percentage of the initial weight.

b) Color analysis

Color of all samples was measured non destructively using CR-300 colorimeter (Minolta. Ramsey, NJ). The instrument was calibrated using a standard white tile (Y= 92.13; x = .3415; y = .3200). Reflectance of each replicate was measured at two equally spaced sites around circumference (varying sizes) of head and a third measurement was taken at
the center of head. Derived functions for color difference (ΔE) and hue difference (ΔH) were calculated using the formulae

Hue angle = tan⁻¹ (b* / a*)

ΔE = [(ΔL)² + (Δa)² + (Δb)²] ½

ΔH = [(ΔE)² -- (ΔL)² -- (ΔC)²] ½ (Gnanasekharan and others 1992; Shewfelt and others 1984)

L* a* b* values were used to quantitatively determine the color changes in broccoli.
Where ΔL, Δa, Δb and ΔC are differences in the color values of each sample from standard tile (Anon 1979). L* value is related to the darkness of the sample, chroma (C) is a function of color saturation and hue angle (tan⁻¹ (b*/a*)) is related to the greenness / yellowness of broccoli head. A decrease in hue angle corresponds to the color changing from green to yellow. ΔE (saturation index) is a measure of total color difference and ΔH (hue difference) indicates the color shift from green to yellow.

c) Texture analysis

Texture (firmness) was measured by determining the energy needed to shear the vegetable (Berrang and others 1989). Shear values of broccoli stalks were determined using Instron Universal Testing machine Model 1011 (Instron Corp. Canton, MA) with a Warner-Bratzler attachment. A 20 kg shear cell was used with a cross head speed of 10 cm/min. For each measurement a uniform stalk of 6 cm length (varying diameter) was placed perpendicular to the path of the Warner Bratzler blade and deformation measurements were made. Texture is reported as joules/mm² or energy needed to cut the vegetable.

d) Analysis of ascorbic acid

Ascorbic acid was measured (mg/100 gm broccoli) by titrimetric assay (AOAC 1995). Ground broccoli (100 gm) was extracted with 100 ml of 2% metaphosphoric acid for 3
min using a blender and filtered through Whatman #42 filter paper. Aliquots (3.5 ml each) of the filtrate were titrated with 2,6- dichloroindophenol and ascorbic acid retention (mg/gm) was calculated.

e) Head space analysis

Oxygen and CO₂ measurements of the head space inside packages were conducted using Hewlett Packard Gas Chromatograph, Model 5890 (Hewlett Packard. Avondale, PA) with thermal conductivity detector. Separation was completed on a 15 M x 0.53 mm column (0.5µ; Nukol®) (Sigma-Aldrich. St.Louis, MO). Duplicate 0.5 ml gas samples were taken by syringe from the package for each measurement. The temperature program began at 35°C for 10 min, and then the temperature was raised to 225°C in 15 min. Oxygen and carbon dioxide identification and quantification were based on retention time and peak area results for air gas standard.

f) Separation and identification of glucosinolates by HPLC

i) Sample preparation

Individual florets with ~3 cm stalk were cut from heads of broccoli. Samples of broccoli (50 gm) and 62.5 ml water (Howard and others 1997) were blended (Waring blender). The slurry was frozen (-18°C) and lyophilized. A sample mill (Thomas Scientific. Swedesboro. NJ) was used to grind the lyophilized samples.

ii) Extraction of glucosinolates and HPLC analysis

Glucosinolates were analyzed according to Wathelet and others (1991) and AOCS official method (1995) with slight modifications. Approximately 200±0.1 mg of prepared sample was taken in a 10 ml tube and heated for 10 min at 75°C in 2.5 ml of 70% aqueous methanol. After centrifugation (5 min, 5x10³ g) a second extraction was achieved using 2.5 ml of 70% aqueous methanol. One ml of centrifuged extract of each
sample was pipetted on the top of ion-exchange column containing 0.5ml DEAE Sephadex A 25 (Sigma-Aldrich. St.Louis, MO). Desulfation was carried out by the addition of 75 µl of purified (AOAC official method, 1995) sulfatase (Type H₁ from Helix Pomatia) (Sigma-Aldrich, St.Louis.MO). Desulfated glucosinolates were eluted with (2 ml. 1.0 ml x 2 times) HPLC grade water (Sigma-Aldrich. St.Louis, MO) and analyzed in a Agilent HPLC system (Agilent Technologies. Waldbronn, Germany) on a C₁₈ column (250 X 4.6 mm i.d; 5 µ) (Phenomenex. Torrance, CA) at 229 nm detection. HPLC grade water and 20% acetonitrile (v/v) were used as mobile phases at a flow rate of 1.5 ml/min. BCR-190R (Commission of European Community, Bureau of Reference, Brussels, Belgium) was used as reference material.

iii) LC/MS analysis

LC/MS was also used to identify glucosinolates. Desulfated glucosinolates were analyzed (Griffiths and others 2000) on an Agilent HP1100 HPLC system on C₁₈ column (250x4.6 mm i.d: 5µ) (Phenomenex. Torrance, CA) at 226-228 nm. HPLC grade water +1%FA (A), CH₃CN +1%FA (B) were used as mobile phases at a flow rate of 0.2ml/min. Mass spectra were obtained using Micromass Quattro II triple quadrupole mass spectrophotometer (Micromass, UK). Full scan spectra were obtained with a range of 200-600 m/z with a scan time of 0.4s flow injection.

g) Statistical analyses

The experiment was 2x2x3x5 factorial design. Early fall cv.Everest was replicated four times while late fall cv.Gypsy was replicated three times. SAS (SAS institute. Cary, NC) program was used for statistical analyses including means, standard error and LSD analysis for mean comparisons. Mean separation was determined by Tucky’s test. The interaction between the main effects viz. cultivars, packaging treatments, post harvest cooling treatments and storage treatments was evaluated. Statistically significant differences (p≤0.05) between treatments were determined using analysis of variance (ANOVA).
D. Results and Discussion

i) Weight loss

Results in Table 1 indicate that shrink wrap film markedly reduced the weight loss of broccoli heads. Significant differences in weight loss were found between heads of broccoli held in film wrap and broccoli samples stored in ice. Shrink wrap film helps to maintain high relative humidity inside the package so less weight losses were observed.

Table 1 -Effects of packaging, post harvest cooling delay and storage duration on percent weight loss in crown-cut broccoli

<table>
<thead>
<tr>
<th>Number of observations</th>
<th>Weight loss (%) (mean ± S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cultivar Treatment</strong></td>
<td></td>
</tr>
<tr>
<td>Broccoli cv.Everest</td>
<td>120</td>
</tr>
<tr>
<td>Broccoli cv.Gypsy</td>
<td>90</td>
</tr>
<tr>
<td><strong>Package Treatment</strong></td>
<td></td>
</tr>
<tr>
<td>Shrink wrap air cooled</td>
<td>105</td>
</tr>
<tr>
<td>Ice cooled</td>
<td>105</td>
</tr>
<tr>
<td><strong>Post harvest Cooling Treatment</strong></td>
<td></td>
</tr>
<tr>
<td>One hour cooling delay</td>
<td>70</td>
</tr>
<tr>
<td>Three hour cooling delay</td>
<td>70</td>
</tr>
<tr>
<td>Six hour cooling delay</td>
<td>70</td>
</tr>
<tr>
<td><strong>Storage Treatment at 4°C</strong></td>
<td></td>
</tr>
<tr>
<td>7 days storage</td>
<td>42</td>
</tr>
<tr>
<td>14 days storage</td>
<td>42</td>
</tr>
<tr>
<td>21 days storage</td>
<td>42</td>
</tr>
<tr>
<td>28 days storage</td>
<td>42</td>
</tr>
</tbody>
</table>

** p ≤ 0.05
Cooling delay also had significant negative influence on weight loss. Heads of broccoli that were cooled within one hour after harvest had smaller weight losses (9.5%). But as the post harvest cooling delay progressed, losses in weight were increased (36.2%). As expected, storage duration also had significant influence on weight loss.

**Table 2 - Effects of treatment interactions between crown-cut broccoli varieties and packaging on percent weight loss**

<table>
<thead>
<tr>
<th>Treatment interactions</th>
<th>Weight loss (%) (mean ± S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cv.Everest x shrink wrap air cooled</td>
<td>4.6** ± 0.40</td>
</tr>
<tr>
<td>cv.Everest x ice cooled</td>
<td>19.9** ± 1.0</td>
</tr>
<tr>
<td>cv.Gypsy x shrink wrap air cooled</td>
<td>3.2** ± 0.30</td>
</tr>
<tr>
<td>cv.Gypsy x ice cooled</td>
<td>14.7** ± 1.2</td>
</tr>
</tbody>
</table>

**p≤0.05

The interaction between variety and packaging (Table 2) had a significant effect on percent weight loss (p≤0.05). For both cultivars weight loss was significantly lower in heads of broccoli stored in film wrap when compared to broccoli stored in ice packaging. However, cv.Gypsy lost less weight in comparison with cv.Everest for both packaging treatments.

**ii) Color analysis**

Instrumental color readings (Table 3) provided a meaningful insight into quality deterioration during post harvest handling and storage. Color changes in both cultivars of broccoli under study (cv.Everest and cv.Gypsy) were significantly (p≤0.05) affected by packaging, post harvest cooling delay and storage duration.
Table 3 - Color difference values of crown-cut broccoli as affected by packaging, post harvest cooling delay and storage duration

<table>
<thead>
<tr>
<th>Overall mean ± S.E. color difference values</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of observations</td>
<td>tan⁻¹(b/a)</td>
<td>ΔE</td>
<td>ΔH</td>
</tr>
<tr>
<td><strong>Cultivar Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broccoli cv.Everest</td>
<td>120</td>
<td>-60.9** ± 0.42</td>
<td>54.2** ± 0.19</td>
</tr>
<tr>
<td>Broccoli cv.Gypsy</td>
<td>90</td>
<td>-56.0** ± 0.49</td>
<td>56.7** ± 0.22</td>
</tr>
<tr>
<td><strong>Package Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shrink wrap air cooled</td>
<td>105</td>
<td>-53.6** ± 0.45</td>
<td>55.3 ± 0.20</td>
</tr>
<tr>
<td>Ice cooled</td>
<td>105</td>
<td>-63.3** ± 0.45</td>
<td>55.3 ± 0.30</td>
</tr>
<tr>
<td><strong>Post harvest Cooling Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One hour cooling delay</td>
<td>70</td>
<td>-57.2** ± 0.56</td>
<td>54.1 ± 0.55</td>
</tr>
<tr>
<td>Three hour cooling delay</td>
<td>70</td>
<td>-58.8**a ± 0.56</td>
<td>54.5 ± 0.57</td>
</tr>
<tr>
<td>Six hour cooling delay</td>
<td>70</td>
<td>-59.3**a ± 0.56</td>
<td>54.2 ± 0.43</td>
</tr>
<tr>
<td><strong>Storage Treatment at 4°C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days storage</td>
<td>42</td>
<td>-49.0** ± 0.72</td>
<td>55.6**c ± 0.33</td>
</tr>
<tr>
<td>14 days storage</td>
<td>42</td>
<td>-55.1**b ± 0.72</td>
<td>55.8**c ± 0.33</td>
</tr>
<tr>
<td>21 days storage</td>
<td>42</td>
<td>-58.5**b ± 0.72</td>
<td>55.7**c ± 0.33</td>
</tr>
<tr>
<td>28 days storage</td>
<td>42</td>
<td>-62.8** ± 0.72</td>
<td>56.3**c ± 0.33</td>
</tr>
<tr>
<td>35 days storage</td>
<td>42</td>
<td>-66.7** ± 0.72</td>
<td>53.7**c ± 0.33</td>
</tr>
</tbody>
</table>

** p≤ 0.05

Means within the column with the same superscript are not significantly different from each other.

L* a* b* - CIE lab hunter values

Hue angle = tan⁻¹((b*/a*)

Total color difference ΔE = [(ΔL)² + (Δa)² + (Δb)²]¹/²

Hue difference Δ H= [(ΔE)² - (ΔL)² - (ΔC)²]¹/²
Heads of broccoli stored in shrink wrapped film showed less decline in hue angle and ΔH values compared with broccoli heads stored in ice indicating better retention of green color. Total color difference (ΔE) was not affected by packaging. Post harvest cooling delay had a negative effect on hue angle but ΔE and ΔH were not affected. Post harvest cooling delay resulted in an expected pattern of decreased hue angle (less greenness). Broccoli heads cooled at one hour after harvest retained green color better than broccoli cooled 3 hours and 6 hours after harvest. No significant differences in hue angle were found between 3 hour and 6 hour cooling delays after harvest. Storage duration had a significant effect on hue angle, ΔE and ΔH. As the storage duration increased from 7 days to 35 days, there was continuous decline in hue angle, ΔE and ΔH indicating color change from green to yellow. These results were in confirmation of other reports (Leberman and others 1968; Makhlouf and others 1989). Broccoli cv.Gypsy harvested in September, 2003 was greener at 35 days in storage than cultivar cv.Everest which was harvested in August, 2003. Our experiment results indicate that color retention in broccoli is a consequence of two factors viz. shrink wrapping and delay before cooling.

iii) Texture analysis

Results of texture analysis are shown in Table 4. Broccoli heads held in film wrap were significantly (p≤0.05) easier to shear (4.0 X 10⁻⁴ j/mm²) throughout storage than were broccoli heads stored in ice (4.5X 10⁻⁴ j/mm²). It is possible that the increase in shear resistance was slower in film wrapped broccoli heads because of lower O₂ and higher CO₂. According to Lipton and Harris (1974) CO₂ not only retards toughening of broccoli tissue but even induced softening of tissue when combined with low O₂. Post harvest cooling delay and storage period did not affect the texture (firmness) of broccoli significantly. Broccoli cv.Everest harvested early in fall was significantly easier to shear (3.2 X 10⁻⁴ j/mm²) than broccoli cv.Gypsy which was harvested in late fall (5.3 X 10⁻⁴ j/mm²).
Table 4 - Effects of packaging, post harvest cooling delay and storage duration on Warner-Bratzler texture values of crown-cut broccoli

<table>
<thead>
<tr>
<th>Cultivar Treatment</th>
<th>Number of observations</th>
<th>Stress (j/mm$^2$) ($\times 10^{-4}$) (mean ± S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli cv.Everest</td>
<td>120</td>
<td>3.2** ± 0.09</td>
</tr>
<tr>
<td>Broccoli cv.Gypsy</td>
<td>90</td>
<td>5.3** ± 0.11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Package Treatment</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrink wrap air cooled</td>
<td>105</td>
<td>4.0** ± 0.10</td>
</tr>
<tr>
<td>Ice cooled</td>
<td>105</td>
<td>4.5** ± 0.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post harvest Cooling Treatment</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>One hour cooling delay</td>
<td>70</td>
<td>3.1 ± 0.22</td>
</tr>
<tr>
<td>Three hour cooling delay</td>
<td>70</td>
<td>3.5 ± 0.15</td>
</tr>
<tr>
<td>Six hour cooling delay</td>
<td>70</td>
<td>3.3 ± 0.25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Storage Treatment at 4°C</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days storage</td>
<td>42</td>
<td>3.4 ± 0.16</td>
</tr>
<tr>
<td>14 days storage</td>
<td>42</td>
<td>3.1 ± 0.16</td>
</tr>
<tr>
<td>21 days storage</td>
<td>42</td>
<td>3.1 ± 0.18</td>
</tr>
<tr>
<td>28 days storage</td>
<td>42</td>
<td>3.4 ± 0.20</td>
</tr>
<tr>
<td>35 days storage</td>
<td>42</td>
<td>3.4 ± 0.16</td>
</tr>
</tbody>
</table>

** $p \leq 0.05$
The interaction between variety and packaging (Table 5) was found to affect the stress needed to shear the vegetable significantly (p≤0.05). For both the cultivars, the heads of broccoli stored in film wrap were easier to shear than the broccoli stored in ice. However, the magnitude of increase in stress needed to shear the vegetable was higher for ice packaged broccoli of cv.Gypsy than that of cv.Everest. The interaction between variety

**Table 5 - Effects of treatment interactions between crown-cut broccoli varieties and packaging on Warner-Bratzler texture values**

<table>
<thead>
<tr>
<th>Treatment interactions</th>
<th>Stress (J/mm² x 10⁴) (mean ± S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cv.Everest x shrinkwrap air cooled</td>
<td>3.1** ± 0.10</td>
</tr>
<tr>
<td>cv.Everest x ice cooled</td>
<td>3.3** ± 0.10</td>
</tr>
<tr>
<td>cv.Gypsy x shrinkwrap air cooled</td>
<td>4.9** ± 0.20</td>
</tr>
<tr>
<td>cv.Gypsy x ice cooled</td>
<td>5.9** ± 0.17</td>
</tr>
</tbody>
</table>

**p≤0.05

**Table 6 - Effects of treatment interactions between crown-cut broccoli varieties and post harvest cooling delay on Warner-Bratzler texture values**

<table>
<thead>
<tr>
<th>Treatment interactions</th>
<th>Stress (J/mm² x 10⁴) (mean ± S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cv.Everest x 1 hour post harvest cooling delay</td>
<td>3.0 ± 0.11</td>
</tr>
<tr>
<td>cv.Everest x 3 hour post harvest cooling delay</td>
<td>3.6** ± 0.14</td>
</tr>
<tr>
<td>cv.Everest x 6 hour post harvest cooling delay</td>
<td>3.1** ± 0.14</td>
</tr>
<tr>
<td>cv.Gypsy x 1 hour post harvest cooling delay</td>
<td>5.4** ± 0.26</td>
</tr>
<tr>
<td>cv.Gypsy x 3 hour post harvest cooling delay</td>
<td>5.2** ± 0.26</td>
</tr>
<tr>
<td>cv.Gypsy x 6 hour post harvest cooling delay</td>
<td>5.6** ± 0.26</td>
</tr>
</tbody>
</table>

**p≤0.05
and post harvest cooling delay (Table 6) was also found significant (p≤0.05) but no trends were observed. For both the cultivars, the heads of broccoli subjected to <1 hour post harvest cooling delay were easier to shear than the broccoli heads that were subjected to longer post harvest cooling delays (3 hours and 6 hours).

iv) Ascorbic acid analysis

The effects of packaging, post harvest cooling delay and storage duration on ascorbic acid retention in crown-cut broccoli are presented in Table 7. The ascorbic acid content

Table 7 -Effects of packaging, post harvest cooling delay and storage duration on ascorbic acid retention in crown-cut broccoli

<table>
<thead>
<tr>
<th></th>
<th>Number of observations</th>
<th>Ascorbic acid (mg/gm of broccoli) (mean ± S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cultivar Treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broccoli cv.Everest</td>
<td>120</td>
<td>0.39** ± 0.001</td>
</tr>
<tr>
<td>Broccoli cv.Gypsy</td>
<td>90</td>
<td>0.40** ± 0.001</td>
</tr>
<tr>
<td><strong>Package Treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shrink wrap air cooled</td>
<td>105</td>
<td>0.41** ± 0.001</td>
</tr>
<tr>
<td>Ice cooled</td>
<td>105</td>
<td>0.38** ± 0.001</td>
</tr>
<tr>
<td><strong>Post harvest Cooling Treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One hour cooling delay</td>
<td>70</td>
<td>0.40** ± 0.001</td>
</tr>
<tr>
<td>Three hour cooling delay</td>
<td>70</td>
<td>0.39** ± 0.001</td>
</tr>
<tr>
<td>Six hour cooling delay</td>
<td>70</td>
<td>0.36** ± 0.001</td>
</tr>
<tr>
<td><strong>Storage Treatment at 4°C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days storage</td>
<td>42</td>
<td>0.44** ± 0.001</td>
</tr>
<tr>
<td>14 days storage</td>
<td>42</td>
<td>0.43** ± 0.001</td>
</tr>
<tr>
<td>21 days storage</td>
<td>42</td>
<td>0.39** ± 0.001</td>
</tr>
<tr>
<td>28 days storage</td>
<td>42</td>
<td>0.36** ± 0.001</td>
</tr>
<tr>
<td>35 days storage</td>
<td>42</td>
<td>0.34** ± 0.001</td>
</tr>
</tbody>
</table>

** p≤ 0.05
was determined in 100 gm dry matter samples. Ascorbic acid retention in shrink wrapped samples was significantly higher \((p \leq 0.05)\) than samples stored in ice. A steady decline in ascorbic acid content was observed in samples packaged in ice throughout storage duration. Post harvest cooling delay also significantly affected ascorbic acid retention. As the post harvest cooling delay increased from one hour to six hours, steady loss in ascorbic acid was noticed. The loss of ascorbic acid may be caused by accelerated oxidative, enzymatic and non enzymatic activities associated with ascorbic acid degradation (Hudson and others 1985). Losses were minimal throughout rest of storage period.

v) Head space analysis

Atmospheric modification inside shrink wrap packages i.e. \(O_2\) and \(CO_2\) levels over 5 week period was observed (Table 8). Although differences were observed in the \(O_2\) and \(CO_2\) levels for different treatments, no consistent trends were noticed. The \(O_2\) and \(CO_2\) levels inside the packages were increased to 21.7\% and 0.09\% respectively, 7 days in storage. The \(O_2\) concentration decreased for the rest of storage period and no trend was observed in \(CO_2\) concentration.

Interestingly our results were in contrast to those of Barth and Zhuang (1996). They used polyolefin film PD-941 without micro perforations for MAP of broccoli and noticed that \(O_2\) and \(CO_2\) equilibrated to 11.2\% and 7.5\% respectively within 48 hours. Usually atmosphere modification inside package depends on respiration rate of broccoli (commodity) and gas permeation properties of the packaging film to determine the package atmosphere (Kader and others 1989; Labuza and Breeve, 1989; Varraino Marston 1989). In our experiment the micro perforated packaging does not permit atmospheric over modification which occurs with MAP (Toivonen and others 1997). It was possibly due to micro perforations combined with high \(O_2\) (1065 cc/100 sq. in/24 hours) and \(CO_2\) transmission rates of the film. In spite of non modification of atmosphere within package, we observed that shelf life of broccoli can be prolonged by using micro
Table 8 - Effects of packaging, post harvest cooling delay and storage duration on levels of $O_2$ and $CO_2$ inside shrink wrap packages of crown-cut broccoli

<table>
<thead>
<tr>
<th>Cultivar Treatment</th>
<th>Number of observations</th>
<th>$O_2$ (%) (mean ± S.E)</th>
<th>$CO_2$ (%) (mean ± S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli cv.Everest</td>
<td>39</td>
<td>20.3 ± 0.01</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>Broccoli cv.Gypsy</td>
<td>39</td>
<td>21.2 ± 0.05</td>
<td>0.04 ± 0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Post harvest Cooling Treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>One hour cooling delay</td>
<td>26</td>
<td>20.6 ± 0.27</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Three hour cooling delay</td>
<td>26</td>
<td>20.7 ± 0.13</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>Six hour cooling delay</td>
<td>26</td>
<td>20.7 ± 0.21</td>
<td>0.07 ± 0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Storage Treatment at 4°C</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days storage</td>
<td>12</td>
<td>21.7 ± 0.12</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>14 days storage</td>
<td>21</td>
<td>19.6 ± 0.14</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>21 days storage</td>
<td>18</td>
<td>20.6 ± 0.16</td>
<td>0.03 ± 0.04</td>
</tr>
<tr>
<td>28 days storage</td>
<td>20</td>
<td>20.7 ± 0.14</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>35 days storage</td>
<td>17</td>
<td>20.7 ± 0.19</td>
<td>0.16 ± 0.06</td>
</tr>
</tbody>
</table>

perforated shrink wrap. This was reflected in the results of color, texture, vitamin C, and weight loss. Post harvest cooling delay had no significant effect ($p \leq 0.05$) on the modification of atmosphere inside shrink wrap film. But our results with respect to different response factors (color, texture, ascorbic acid and weight loss) indicate that rapid cooling i.e. within one hour after harvest helped to maintain quality of broccoli by slowing rate of respiration (Brennan and Shewfelt 1989; Ryall and Lipton 1979).
vi) Glucosinolates

Through our experiment five predominant glucosinolates were identified viz. glucoraphanin, glucobrassicin, progoitrin, gluconapin, gluconapoleiferin based on their relative abundance in reference material (Rapeseed, BCR-190R; Figure 1) and broccoli samples (Figure 2 and Figure 3). Table 9 shows the relative amounts of different glucosinolates in broccoli. Results (not shown) did not provide any insight with respect to the effects of post harvest cooling delay and storage duration on retention of glucosinolates. Shrink wrap packaging had a significant (p≤0.05) positive effect on the retention of glucoraphanin. Other glucosinolates were not influenced by packaging. We could not identify all the glucosinolates because the analysis was performed six months after harvest and it is possible that the minor glucosinolates may have degraded with time.

Table 9 - Effects of packaging on individual glucosinolate concentration in crown-cut broccoli

<table>
<thead>
<tr>
<th>Peak</th>
<th>Glucosinolate</th>
<th>Shrink wrap packaging (mean ± S.E)</th>
<th>Ice packaging (mean ± S.E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucoraphanin</td>
<td>44.3**± 2.6</td>
<td>32.4**± 3.6</td>
</tr>
<tr>
<td>2</td>
<td>Glucobrassicin</td>
<td>20.6**± 0.8</td>
<td>24.5**± 1.2</td>
</tr>
<tr>
<td>3</td>
<td>Progoitrin</td>
<td>10.0**± 0.5</td>
<td>12.0**± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>Gluconapin</td>
<td>16.1**± 0.9</td>
<td>19.9**± 1.3</td>
</tr>
<tr>
<td>5</td>
<td>Gluconapoleiferin</td>
<td>8.7**± 0.9</td>
<td>10.9**± 1.3</td>
</tr>
</tbody>
</table>

*a assumed based on relative abundance of different glucosinolates in rapeseed reference material (BCR-190R) and broccoli

** p≤ 0.05

According to Kushad and others (1999) plant genetics and environment plays an important role in broccoli contents. They found more than 50% variation in glucosinolate
amounts in different cultivars of broccoli. So it is possible that the two cultivars, Everest and Gypsy may be lacking in other glucosinolates.

Fig 1 - HPLC profile of desulfoglucosinolates in rapeseed reference material (BCR-190R) Peaks: (1) Progoitrin; (2) Gluconapin; (3) 4-hydroxy glucobrassicin; (4) Glucobrassiccanapin; (5) Gluconapoleiferin; (6) Epiprogoitrin.

Fig 2 - HPLC profile of desulfoglucosinolates in broccoli heads packaged in shrink wrap film. Peaks: (1) Glucoraphanin; (2) Glucobrassicin; (3) Progoitrin; (4) Gluconapin; (5) Gluconapoleiferin
E) CONCLUSIONS

The effects of packaging treatments, post harvest cooling delay and storage duration on color, texture, ascorbic acid content, weight loss and glucosinolate retention in crown-cut heads of broccoli were studied. O₂ and CO₂ inside shrink wrap packages were also monitored. Our results indicate that heads of broccoli stored in shrink wrap packages had far lower weight losses, lesser decrease in green color and ascorbic acid. The texture analysis showed that firmness was maintained better in broccoli stored with shrink wrap film. An important glucosinolate, glucoraphanin was retained better in shrink wrapped heads of broccoli. As expected decrease in post harvest cooling delay (<1 hour) helped maintain the quality of broccoli heads better. Between the two cultivars, shelf life of cv.Gypsy was better than that of cv.Everest in terms of color, ascorbic acid retention and weight loss. But cv.Everest retained texture (firmness) better after 35 days of storage. Finally, it can be concluded that shrink wrap packaging and shorter post harvest cooling delays protect quality of post harvest broccoli.
F) ACKNOWLEDGEMENTS

The authors would like to thank Virginia Agricultural Council for financial support which facilitated the completion of this work.
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Vita

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