AN ABSTRACT OF THE THESIS OF

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Title: Storage Quality of Broccoli Florets and Evaluation of Modified Atmosphere Packaging.

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Daryl G. Richardson

The effect of some factors that can affect quality characteristics of broccoli florets in storage was evaluated. Quality characteristics of broccoli florets with different rates of field nitrogen (N) application (0, 90, 180, and 270 Kg N/ha from urea) were evaluated in modified atmosphere packaging (MAP) (B-900 Cryovac film) storage at 0°C. Increased field N application resulted in more green color and higher chlorophyll concentration but these differences disappeared as chlorophyll linearly decreased by 50% during 28 days of MAP storage. N fertilization up to 180 Kg/ha increased ascorbic acid content at harvest but losses in storage were not affected by nitrogen fertilization treatments. It was noted in several of the packages with B-900 film that off-odors were present after storage. While this was noted late in the evaluation (not quantified) this led to a second experiment.

MAP using three Cryovac polyolefin films (PD-941, PD-961EZ, and PD-900) and perforated polyethylene bags were evaluated for broccoli florets stored at 0°, 5°, and 10°C. Films with differential permeability properties had similar

effects on controlling weight loss but significantly affected in-package CO_2 and O_2 concentrations. Color (Hue angle and Chroma) and chlorophyll retention was better in MAP broccoli florets than perforated bag controls. MAP also delayed loss of ascorbic acid. The beneficial effects of MAP were especially noticeable when the florets were stored at 5° and 10°C in which shelf life was appreciably extended. Broccoli florets in PD-941 film developed no off-odors during 28 days storage even under the highest (10°C) storage temperature.

Seven different broccoli cultivars were evaluated for quality in MAP storage using Cryovac PD-941 film. Weight loss was kept very low (less than 3%) during 21 days at 4°C and even 5 days after transfer to 15°C storage room. Differences in ascorbic acid content, chlorophyll concentration and green coloration were found among cultivars. While these quality characteristics differed greatly between cultivars at harvest, they lost them at different rates in storage. From this study, 'ATX91-307" followed by 'Emerald' appeared to be superior to the others during simulated marketing conditions of 21 days at 4°C plus 5 days at 15°C under near optimal MAP conditions.

Storage Quality of Broccoli Florets and Evaluation of Modified Atmosphere packaging

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Chair of Department of Horticulture

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I understand that my thesis will come part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Amalia Cabezas, Author

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Chapter 1

Review Of Literature

Physiological and Compositional Changes

Broccoli is a compact, rapidly developing floral vegetable that is harvested when the flowering heads are immature and growing rapidly. Harvested broccoli comprises a mass of green floral buds (florets) and thick, fleshy flower branchlets attached to the central plant axis (collectively, the head) (King and Morris, 1994 a, b). When harvested, the floral apices are separated from nutrients, hormones, and energy supplied by the roots and leaves. This separation coupled with a high respiration rate, contributes to the rapid senescence of broccoli florets as is typical of commodities harvested before physical growth has ceased (Huber, 1983).

Harvesting and handling broccoli for market imposes a series of stresses on tissues, including wounding, separation from nutrient source, and dehydration. Harvest stresses are particularly severe on actively growing immature tissues. Those tissues are unable to maintain metabolic homeostasis and senesce rapidly (Huber, 1983).

Yellowing of florets is a relatively late event in the postharvest senescence of broccoli, preceded by major physiological and compositional changes beginning very early after harvest. During the first 6 hours after harvest at 20°C, major losses of sugars (13, 46 and 11%) and organic acids (97, 92 and 80%), occur from floral, middle and basal sections of broccoli branchlets. Organic acid pools were almost totally depleted 24 hours after harvest in floral sections, 48 hours in middle sections, and 72 hours in basal sections (King and Morris, 1994 a, b). The free amino acid content of all sections of branchlets increased during the first 12 hours after harvest at 20°C, with a doubling of the glutamine pool (1.4 mg g^{-1} fresh wt) accounting for most of this increase, while the ammonia content increased only in floral sections from 0.2 mg to 2.5 mg g^{-1} fresh wt after 96 hours (King and Morris, 1994 a, b).

The most obvious characteristic of broccoli postharvest senescence is sepal degreening due to chlorophyll degradation (Wang, 1977; Zhuang, 1994). It has been proposed that ethylene has an important role in this chlorophyll loss (Watada, 1987). Some broccoli cultivars show a climacteric pattern of respiration and ethylene production with increased ethylene and carbon dioxide production associated with tissue degreening (Wang, 1977). Despite the high respiratory activity of broccoli flower heads, ethylene production rates of the entire flower head are relatively low. However, the flower buds are the most susceptible to the adverse effects of ethylene. A few parts per million (ppm) of the gas in the atmosphere can enhance yellowing, bud drop and decay (Aharoni et al., 1985).

Most vegetables are perishable products with active metabolism during the postharvest period. King and Morris (1994a), found changes in respiratory metabolism early in the postharvest life of broccoli. Carbon dioxide produced from heads of container grown broccoli and from heads, branchlets, and florets of field harvested broccoli decreased markedly during the first 12 hours of postharvest storage before stabilizing. The respiratory quotient (amount of CO_2 evolved per O_2 consumed) shifted toward a more oxidative metabolism in parallel with respiratory decline. The changes in respiration, carbon metabolism, and nitrogen metabolism are consistent with changes described for plants in stress situations (King and Morris, 1994 a, b).

Because of the potential for interaction between the florets and the tissues of the rest of the head, the development of senescence may be different in broccoli presented as florets, stalks, or whole heads (King and Morris, 1994a).

Quality Characteristics Important in Fresh Market

Quality, as applied to plant foods, is a complex concept that is usually divided into two categories: general marketability (i.e., the suitability of the product for certain purposes) and nutritional quality (Weichmann, 1986).

Quality has been defined as "the composite of those characteristics that differentiate individual units of a product, and have significance in determining the degree of acceptability by the buyer" (Kramer and Twigg, 1970). Quality is only one of the factors that influence consumer acceptability of fruit and vegetable. However, it is the only factor that is intrinsic to the item and it is the factor most directly affected by handling and storage conditions (Shewfelt, 1993).

External appearance plays the major role in the presentation and selection of fresh produce. Consumers have learned to make their selections on this basis. Internal quality characteristics such as taste and nutritional value are recognized as increasingly important by some buyers but these still play a minor role in marketing (Shewfelt, 1993). Thus, color and general appearance strongly influence the decisions of buyers to purchase particular fruits or vegetables (Weichmann, 1986). Appearance factors include size, shape, gloss, color, and absence of defects (Kramer and Twigg, 1970).

Postharvest longevity (shelf life) of a product is considered to be a very important aspect of quality. Monitoring weight change gives an indication of loss of moisture and turgor and is a direct measure of total loss in marketable weight of produce (Perrin and Gaye, 1986). Weight loss is the major determinant of shelf life of fresh-marketed broccoli (Ryall and Lipton, 1979). Moisture loss in vegetables leads to shriveling, wilting, softening and loss of crispness and turgidity (Kasmire and Cantwell, 1992). Broccoli branchlets and florets lose turgor and become flaccid when water loss is excessive (Brennan and Shewfelt, 1989). Decay commonly occurs on florets, primarily during advanced stages of senescence (Aharoni et al., 1985).

Color

The common feature of leaf, stem or flower type vegetables is color. For these, color is part of the "quality" as it is either an element of attraction or an indication of normal, healthy tissue (Phan, 1987) or freshness.

Color, a primary indicator of maturity or ripeness, is derived from the pigments found in the product. It is the major quality attribute of vegetables and is considered to have the most impact upon consumer selection of produce. For green vegetables, chlorophyll content is associated with greenness (Shewfelt, 1986). Determining chlorophyll concentration provides a sensitive measure of greenness in broccoli (Lebermann et al., 1968b).

Increased CO_2 and decreased O_2 levels in the storage atmosphere reduce the rate of color changes, mainly from green to yellow in vegetables because of reduced breakdown of chlorophyll. This effect has been demonstrated for broccoli (Lieberman and Hardenburg 1954; Lebermann et al., 1968a; Kasmire et al., 1974). Lebermann et al., (1968) showed that an increase in CO_2 is more effective than a reduction in O_2 in slowing color change; they obtained similar results using 3% or 21% O_2 with increased CO_2. Lipton and Harris (1974) recommended 10% CO_2 combined with O_2 at 0.5-1.0%, since higher O_2 concentrations did not influence yellowing as reported by Lebermann et al., (1968). They found that O_2 at less than 1% reduced the breakdown of chlorophyll and that this inhibiting effect persisted even during subsequent aeration. Wang (1979) tested the influence of short-term high-CO_2 treatments (up to 40%) on the preservation of the greenness of broccoli and found that 20% or more CO_2 maintained chlorophyll, even during additional storage time in air. Six days of 20% CO_2 treatment were much more effective than three days, but 40% CO_2 injured the broccoli and thus reduced salability. Chlorophyll of sweet pepper also is retained better in a CO_2-enriched atmosphere than in air. However, this positive effect is lost once the product is exposed to air (Wang. 1977).

Packaging of broccoli under elevated CO_2 and reduced O_2 using polymeric films (MAP), have been shown to preserve color during storage (Elkashif et al., 1983; Barth et al., 1992, 1993). Some authors (Zhuang et al., 1994) have reported that MAP storage increased (about 30%) chlorophyll content in broccoli florets within 6 days of storage at 5°C.

Aromas and Flavor

Usually, extremely low O_2 or very high CO_2 levels result in off-flavors because of anaerobic conditions. Broccoli stored for 9 days at 2.5°C in 1% O_2 developed slight off-odors, while storage in 0.5% O_2 resulted in offensive off-odors that were still noticeable after subsequent aeration for 2 days at 5°C (Kasmire et al., 1974). Lipton and Harris (1974) did not report any off-odors of broccoli stored in 1% O_2 for 6 or 13 days at 2.5 or 5.0°C. However, off-odors developed in all samples stored in 0.1% O_2 and in most samples stored in 0.25% O_2. Those off-odors formed at 0.25% O_2 tended to disappear upon aeration. This has also been reported for several types of fruits (Richardson and Kosittrakun, 1995). Broccoli stored in 1% O_2 plus 10% CO_2 (at 7.5°C) developed an unpleasant odor that differed from that induced by low O_2 alone and was accompanied by an off-flavor, but both disappeared during aeration at 10°C and had no influence on culinary quality of cooked broccoli (Lipton and Harris 1974). When 1% O_2 was coupled with 20% CO_2, off-odors were very objectionable (Kasmire et al., 1974).

Texture

Texture is also an important quality attribute of horticultural crops. Texture, like appearance, is evaluated in the context of specific consumer expectations. Crispness and crunchiness are expected of fresh apples, carrots, lettuce and celery, but softness or tenderness is desired in asparagus. Maintaining turgidity is the first and foremost factor for successful preservation of the quality of harvested produce (Weichmann, 1986).

The texture of plant products is a consequence of cell-wall structure and internal pressure within the cell (turgor). Products that maintain structure and turgor during handling and storage remain crisp and may contain an abundance of undegraded cell-wall biopolymers (Shewfelt, 1986). Many factors have been shown to affect the texture of vegetables such as temperature, relative humidity and atmospheric composition. In broccoli, alteration of texture is not related with a specific structure, such as cell wall polysaccharides. Instead, water relations or moisture content in the tissue as a whole contribute to texture in broccoli (Lipton, 1975).

The influence of controlled atmosphere (CA) storage on the texture of vegetables varies with the commodity. No textural changes were reported in raw broccoli stored in 0-20% CO_2 with 2, 5, or 20% O_2 for 14 days at 1° or 7°C. After cooking, however, stalks stored in high CO_2 were softer (Lebermann et al., 1968a). Atmospheres containing 10% or more CO_2 prevented toughening of broccoli and the tissue became more tender than it was at the time of harvest. This tenderization seems to be a direct result of CO_2-mediated decreases in pH of the tissue (Lipton, 1975).

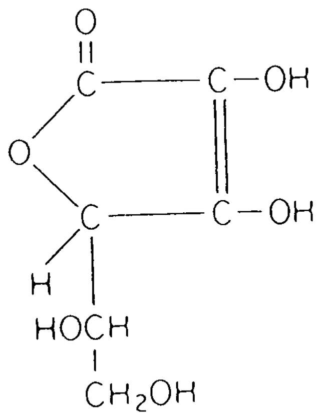
Nutritional Quality

Fruits and vegetables are rich sources of vitamins, particularly A and C. Ascorbic acid, a labile vitamin, is more readily lost through handling and processing than most other constituents of foods. It is subject to leaching from cutting and cooking in water and is highly susceptible to chemical degradation (Fennema, 1977). Retention of vitamin C is often analyzed when evaluating postharvest storage effects on nutritional quality of fruits and vegetables (Klein and Perry, 1982; Vanderslice et al., 1990).

Broccoli is known to be rich in vitamin C which is an important component of its nutritional quality. USDA researchers (1982, 1984) reported that 100 g of broccoli contains 93 mg of ascorbic acid and 1,542 IU of vitamin A. Because of its instability, ascorbic acid may serve as an excellent indicator of broccoli quality (Ezell and Wilcox, 1959).

Overview of Ascorbic Acid

L-ascorbic acid is a highly soluble compound that is both acidic and has strong reducing properties. These qualities are attributed to its enediol structure, which is conjugated with the carbonyl group in a lactone ring. The natural form of the vitamin is the L-isomer (Tannenbaum et al., 1985).



L - ascorbic acid

Ascorbic acid is a γ-lactone of a hexonic acid with an enediol structure at carbons 2 and 3. It is unstable and can be readily oxidized to dehydroascorbic acid. The metabolic pathway (Baig et al., 1970; Isherwood et al., 1954) for synthesis involves the following:

UDP-D-glucose + 2 NAD^+ + H_2O $\longrightarrow$ UDP-D-glucuronic acid + 2 NADH + $2H^+$

D-glucuronic acid $\xrightarrow{\text{reduction}}$ L-gluconic acid $\longrightarrow$

$\xrightarrow{\text{lactonizes}}$ L-gulonolactone $\xrightarrow{\text{oxidized}}$ L - ascorbic acid

Vitamin C appears to be synthesized in leaves and then transported to meristematic regions in the root and shoot tip. Ascorbic acid functions in the respiratory metabolism of plants in oxidation reduction systems (Isherwood and Mapson, 1962).

External factors known to affect the biosynthesis of ascorbic acid are: light intensity , latitude, salts, nitrogen supply, temperature and possibly molybdenum levels (Ezell and Wilcox, 1959; Ottoson, 1979; and Poole et al., 1944). Internal factors affecting biosynthesis appear to be continued production of hexose sugars, the presence of oxygen, and the maintenance of reduced phosphopyridine nucleotides by active reducing systems in the tissue (Isherwood and Mapson, 1962).

Ascorbic acid is highly sensitive to various modes of degradation. Factors that can influence the nature of the degradative mechanism include temperature, salt and sugar concentration, pH, enzymes, metal catalysts, initial concentration of ascorbic acid, and the ratio of ascorbic acid to dehydroascorbic acid. Since ascorbic acid is soluble in water, it is readily lost via leaching from cut or bruised surfaces of foods (Tannenbaum et al., 1985) and from cooking in water.

A major limitation to maintaining ascorbic acid content of vegetables can be in the handling and storage of the crop where losses have ranged up to 76% (Fennema, 1977). Ascorbic acid stability is influenced by precooling, the rate of water loss, and by temperature during storage (Salunke et al., 1973; Zepplin and Elvehjem, 1944). High temperatures decrease ascorbic acid concentrations while low temperatures maintain ascorbic acid levels. In research conducted by

Poole et al.(1944), cabbage lost only 11% ascorbic acid in two weeks of refrigerated storage. More than 40% of the ascorbic acid content of asparagus, 50% of broccoli and 40% of spinach was lost in 24 hours at 21°C (Harris and Von Loesecke, 1972).

Storage

Fresh fruits and vegetables have a limited shelf-life. They are living, respiring tissues that are also beginning the processes of senescing and dying (Shewfelt, 1986). Broccoli is a highly perishable product whose visual and organoleptic qualities greatly depend on the storage conditions. The respiration rate of freshly harvested broccoli is very high, comparable to that of asparagus, spinach, or sweet corn. Thus, like these crops, broccoli must be cooled immediately after harvest in order to delay deterioration and be kept at low temperature for a maximum shelf life (Hardenburg et al., 1986). Rapid cooling to 0° to 2°C is essential, with commensurately high relative humidity (RH) (95%) to prevent shrinkage and loss of turgidity (Nonnecke, 1989).

Storability of broccoli flower heads is very short, especially at temperatures above 5°C. Respiration rates increase sharply and the inflorescence turns yellow due to opening of the flower buds and degradation of chlorophyll in the green tissues. This may be followed by abscission of the florets and axillary leaves (Aharoni et al., 1985). A temperature of 0°C, relative humidity of 95-100% is recommended for storage of broccoli (Lorenz and Maynard, 1988). Refrigeration at 0°C prolongs the storage life of broccoli up to 3 weeks whereas broccoli only keeps satisfactorily one day at ambient (20°C) temperature (Smith, 1940; Wang, 1977).

Controlled and Modified Atmosphere

Fruits and vegetables continue to actively metabolize during their postharvest existence. Techniques used to extend shelf life of such commodities include: 1) lowering the temperature to slow respiration, senescence, and growth of pathogens, 2) maintaining an optimal relative humidity to slow water loss without accelerating decay, 3) adding chemical preservatives to slow physiological processes and prevent microbial losses, and 4) modification of O_2, CO_2, and C_2H_4 concentrations in the atmosphere surrounding the commodity to levels different from those in air . This is referred to as controlled atmosphere (CA) or modified atmosphere (MA). CA implies a greater degree of precision than MA in maintaining specific levels of O_2, CO_2, and other gases (Exama et al., 1993; Kader et al., 1989; Shewfelt, 1986).

Reduced O_2 and increased CO_2 environments generally reduce respiration and ethylene production rates, retard softening, and slow physiological and compositional changes associated with ripening and senescence of fruits and vegetables as long as O_2 and CO_2 levels are maintained within levels tolerated by the commodity (Kader et al., 1989). The use of modified or controlled atmosphere should be considered as a supplement to, but not as a substitute for proper temperature and relative humidity management (Kader, 1992).

The senescence-retarding effect of high CO_2 and low O_2 in the atmosphere of broccoli flower heads has long been recognized (Kasmire et al., 1974; Lipton and Harris, 1974; Smith, 1940; Wang, 1979). The presence of high concentrations of CO_2 has been shown to have an antagonistic action to that of ethylene (Phan, 1987). Oxygen concentrations under 1% are needed to retard broccoli flower head senescence, whereas a wide range of CO_2 concentrations between 5-20% were effective. Increasing CO_2 concentrations were more effective than decreasing O_2 concentrations for slowing senescence (Phan, 1987). Presently, controlled and/or modified atmospheres in traditional CA - buildings have limited commercial use for broccoli because construction of CA buildings are not cost effective for the relatively short storage duration and because of the possibility of offensive odors and off-flavors which are known to occur under insufficient aeration (Kasmire et al., 1974; Lebermann et al., 1968a; Lipton and Harris, 1974; Smith, 1940; Wang, 1979).

Controlled atmosphere storage of vegetables has resulted in greater ascorbic acid retention and shelf-life extension in contrast to air-stored vegetables (Kader, 1986). Storage of broccoli in elevated CO_2 atmospheres (e.g. 5-20%) has resulted in suppression of respiration and C_2H_4 production rates, and consequently slowing of senescence (Forney et al., 1989; Lebermann et al., 1968a; Wang, 1979; Lieberman and Hardenburg, 1954).

One of the major beneficial effects of controlled atmospheres on green vegetables is green color retention, probably due to competitive inhibition of ethylene action by carbon dioxide and reduced ethylene production at reduced oxygen partial pressure (Isenberg, 1979) which may regulate chlorophyllase activity. Results obtained by Aharoni et al. (1985) indicated that the inhibitory effect of CO_2 on chlorophyll degradation can't be ascribed primarily to suppressive effects on respiration. They concluded that the senescence-retarding effect of CO_2 was related to its ability to block ethylene action. Lebermann et al. (1968a) observed better retention of color and chlorophyll after 16 days of storage at 7°C, especially with increased CO_2, but also with less O_2 than in air. Broccoli stored in an atmosphere of 2% O_2 with no added CO_2 retained an average of 53% of its original chlorophyll content, while samples stored in an atmosphere of 21% O_2 with 20% CO_2 retained an average of 88%. In a similar study, Lipton and Harris (1974) found comparable results under 1% or less O_2, but the broccoli developed undesirable odors at 0.25% O_2 or less. These authors recommended an atmosphere containing 0.5 to 1% O_2 and 10% CO_2 for short-term storage at temperatures $\geq$ 0°C. Off-odors developed in CO_2-enriched (10% or more) atmospheres, and they increased as temperature was lowered and storage prolonged (Kasmire et al., 1974).

Modified Atmosphere Packaging

Historically, atmospheres surrounding produce have been altered in controlled atmosphere storage facilities where the levels of gases are continually monitored and adjusted to maintain the optimal concentrations. This high degree of atmospheric regulation associated with CA is capital intensive and expensive to operate. Hence it is more appropriate for commodities that are amenable to long-term storage such as apple, cabbage, kiwifruit, and pear (Kader et al., 1989). Modified atmosphere packaging (MAP) of fresh fruits and vegetables refers to the still evolving technique of matching the respiration of the product with the O_2 and CO_2 permeability (or breathability) of packages in order to modify the O_2 and CO_2 concentrations of the atmosphere to desired levels within the package (Beaudry and Lakakul, 1995). Modified atmosphere storage implies a lower degree of control of gas concentrations. Typically, initial atmospheric conditions are established for a temporary period of time, and the interaction of the commodities physiology and the physical environment maintain those conditions within broad limits (Zagory and Kader, 1988).

Advances in the design and manufacture of polymeric films with a wide range of gas permeability characteristics have motivated interest in creating modified atmospheres via flexible film packages which result in decreased O_2 and/or increased CO_2 concentrations inside the package. A modified atmosphere is created naturally in a sealed package, as a direct result of counterbalancing of the O_2 uptake and CO_2 production by the produce with diffusion gases across the membrane (Kader et al., 1989). Eventually, at a given temperature, the two gases approach steady-state when the rate of gas permeation through the package film equals the rate of respiration. The magnitude of the CO_2 increase and O_2 decrease inside the package is largely dependent on gas permeabilities of the film. In order to obtain maximum benefit, such steady-state gas concentrations should correspond to storage optima of the packaged commodity (Exama et al., 1993). Such modified atmosphere packaging could be applied to shipping containers, retail packages containing several intact or sliced commodity units, or retail packages for individual units of the commodity (Kader et al., 1989), and represents considerable costs savings compared to controlled atmosphere facilities.

One important goal of MAP is to generate an atmosphere sufficiently low in O_2 or high in CO_2 to influence the metabolism of the product being packaged or the activity of decay-causing organisms resident on that product such that storability and/or shelf life is extended (Beaudry and Lakakul, 1995). MAP can produce all the positive and negative effects of any modified atmosphere. The positive effects of film packaging, independent of the creation of modified O_2 and CO_2 atmosphere can include : (1) maintenance of high relative humidity and reduction of water loss; (2) possible carriers of fungicides or ethylene absorbers (Kader at al., 1988); (3) protection from surface abrasions by avoiding contact between the commodity and the material of the shipping container; (4) possible protection from the deleterious effects of light for commodities such as potatoes and Belgian endive; (5) provision of a barrier to the spread of decay from one unit to another; (6) improved sanitation by reducing contamination of the commodity during handling; and (7) facilitation for brand identification (Zagory and Kader, 1988). Some negative effects include excessive humidity which favors mold growth, and the possibility of off-flavors if CO_2 or O_2 are changed excessively.

If atmosphere modification for packaged fruits or vegetables is a goal, knowledge of the effect of packaging on the atmosphere obtained in the package and the effect of the obtained atmosphere on the quality and physiology of the enclosed product is essential. The effects of reduced O_2 and elevated CO_2 on respiration and fruit ripening are additive and can be greater than the effects of either alone (Kader et al., 1989). However, exposure of fresh produce to levels above their CO_2 tolerance limit may cause physiological damage and off-flavors, and exposure to levels below their O_2 tolerance limit may increase anaerobic respiration and the development of off flavors due to accumulation of ethanol, acetaldehyde and other metabolites (Richardson and Kosittrakun, 1995; Beaudry and Lakakul, 1995; Zagory and Kader, 1988).

Benefits of appropriate MAP include reduced rates of nutritional and quality losses. Generally, lower oxygen concentrations during storage result in lower ascorbic acid losses in vegetables (Weichmann, 1986). Ascorbic acid, chlorophyll and moisture retention were greater in broccoli spears packaged in a semipermeable film compared to non-packaged, when they were stored 4 days at 20°C (Barth, et al 1993). Packaging and storage temperature of 10°C generally maintained the various quality attributes of vegetables (green beans, spinach and bell peppers) better than unpackaged conditions and/or a higher storage temperature of 20°C. The degree of benefit differed with vegetables and quality attributes (Watada et al., 1987). MAP used during simulated prolonged transit (3 days at 15°C + 2 days at 20°C or 14 days at 0.5°C + 2 days at 2°0C) allowed the broccoli heads to be kept in high quality, whereas the PVC-film wrapped heads were unsaleable (Aharoni et al., 1985).

Methods Of Creating Modified Atmospheres

Atmosphere modification necessitates a film or package through which gas exchange is restricted. Modified atmosphere can be established either passively by the commodity or by initially charging the atmosphere with O_2 and/or CO_2 concentrations expected to be near equilibrium.

Commodity-generated or Passive Modification

Modified atmospheres are generated through the natural process of respiration of the enclosed product, which reduces O_2 and increases CO_2 under restricted gas exchange through the film barrier. Because the process of respiration is relatively slow, atmospheric modification by these methods can be relatively slow as well, taking several days at low temperatures and much of the benefit of the MA may be lost (Beaudry and Lakakul, 1995; Kader, 1992).

Passive modification depends on both the characteristics of the commodity and the packaging film. If the 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 of O_2 and the production of CO_2 through respiration (Smith et al., 1987). In order to achieve and maintain a satisfactory atmosphere within the package, the gas permeabilities of the selected film must be such that they allow O_2 to enter the package at a rate balanced by the consumption of O_2 by the commodity. Similarly, CO_2 must be vented from the package to balance the production of CO_2 by the commodity. Furthermore, this atmosphere must be established rapidly and without danger of creation of anoxic conditions or injuriously high levels of CO_2 (Zagory and Kader, 1988). The conditions created and maintained within a package are the net result of the interaction among several factors, both commodity generated and environmental (Zagory and Kader, 1988):

Respiration and Diffusion Characteristics of the Commodity

Gas exchange between a plant and its environment includes the following steps: (1) diffusion in the gas phase through the dermal system, (2) diffusion in the gas phase through the intercellular system, (3) exchange of gases between the intercellular atmosphere and the cellular solution (cell sap) or vice versa, which is a function of the distribution and effectiveness of the intercellular spaces and respiratory activity, and (4) diffusion in solution within the cell from centers of CO_2 production to centers of O_2 consumption (Kader, 1987).

As a result of respiratory metabolism, CO_2 and C_2H_4 are produced in the mitochondria and cytoplasm, and this local increase in concentration diffuses outside toward the cell-wall surface adjacent to the intercellular space. These gases then move into the intercellular space below the dermal system. From there, CO_2 and C_2H_4 diffuse through the openings in the surface of the commodity to the ambient atmosphere (Burton, 1982). Oxygen diffusion follows a reverse pattern of that for CO_2 and C_2H_4. Oxygen diffuses inside from the ambient air into the centers of consumption inside the cells. In senescent tissues the intercellular spaces may become filled with cellular solution, which impedes O_2 movement and results in anaerobic conditions within the tissue (Kader et al., 1989).

Gas diffusion rate within a fruit or vegetable depends on the properties of the gas molecule, the magnitude of the gradient, and the physical properties of the intervening barriers (thickness, surface area, density, and molecular structure). CO_2 moves more readily than O_2. Diffusion of C_2H_4 and CO_2 are similar. Three different routes are available for the exchange of gases between horticultural commodities and their surrounding atmosphere: lenticels and stomata, the cuticle, and the pedicel opening or floral end. In leaves, gas diffusion is regulated by control of stomatal aperture, but most bulky organs have no functional stomata or other active controls of gas exchange (Banks, 1984). Fick's First Law of Diffusion states that the movement or flux of a gas in or out of a plant tissue depends on the concentration drop across the barrier involved, the surface area of the barrier, and the resistance of the barrier to diffusion (Salisbury and Ross, 1992).

In turn, the respiration rate of a commodity inside a polymeric film package will depend on the kind of commodity, maturity stage, physical condition, concentrations of O_2, CO_2 and C_2H_4 within the package, commodity quantity in the package, temperature, and possibly light (Kader et al., 1989).

Film Characteristics and Permeability

Gas diffusion across a film is determined by the film structure, film permeability to specific gases, thickness, area, concentration gradient across the film, temperature and differences in pressure across the film. Relative humidity may affect diffusion characteristics of some films (Kader et al., 1989).

Other variables that can affect gas permeation into and out of polymeric film packages are free volume inside the package, effectiveness of seal or closure of the package, air velocity around the package (Kader et al., 1989), and presence of water droplets condensed on the film surface.

Equilibrium Gas Concentrations

There are somewhat different strategies of regulating gas exchange to achieve desired gas concentrations. In one strategy, all the O_2 and CO_2 that move through the package do so through the film itself. After a short period of adjustment, steady-state conditions will be established inside an intact polymeric film package once the appropriate relationship among produce and package variables is achieved. Oxygen inside the package is consumed by the produce as it respires and an approximately equal amount of CO_2 is produced. The second strategy involves the use of perforations (either small holes or patches containing microperforations) for the major route of gas movement (Beaudry and Lakakul, 1995). For both routes of gas exchange, the reduction in O_2 concentration and increase in CO_2 concentration create gradients that, according to Fick's Law, cause O_2 to enter and CO_2 to exit the package. Initially, however, the gradient is small and the flux across the package is not sufficient to replace the O_2 that was consumed or to diffuse out all the CO_2 that was generated. Thus, inside the package, the O_2 content decreases, the CO_2 content increases and new equilibrium concentrations of the gases surrounding the product are established. Steady state (constant) O_2 levels can be achieved in the package when the O_2 uptake by the product is equal to that permeating into the package (Jurin and Karel, 1963). Similarly, steady-state CO_2 levels in the package are achieved when CO_2 production by the product equals CO_2 escape from the package (Geeson et al., 1985; Smith et al., 1987; Beaudry and Lakakul, 1995).

External Factors

Any change in temperature will affect the rate of respiration and the equilibrium conditions within the package unless the rate of diffusion of gases through the film can be changed by temperature to exactly the same extent as respiration. A decrease in the internal concentration of O_2 and an increase in the internal concentration of CO_2 within bulky plant organs in response to an increase of temperature has been demonstrated for various commodities including oranges (Eaks et al., 1960) and pears (Maxie et al., 1974). The permeability of films has been reported to rise from two to five times with every 10°C increase in temperature. This implies that a film that is appropriate for MAP at a low temperature may result in a harmful atmosphere at higher temperatures (Zagory and Kader, 1988).

Films that are available for use in MAP commonly have very low permeability to water vapor. The high relative humidity generated within the packages can cause condensation and favor the development of fungi and bacteria (Kader et al. 1989). Condensation on the surface of a commodity or on the film may also affect gas diffusion across the barriers and solubility of gases. Carbon dioxide is more soluble in water than O_2 or C_2H_4 (Kader et al., 1989).

Active or Initially Established Modified Atmosphere

Because of the limited ability to regulate passively, particularly high CO_2 established atmospheres, it is advantageous that atmospheres within MAP will be actively established and adjusted. This can be done by pulling a slight vacuum and replacing the atmosphere of the package with the desired gas mixture just prior to sealing. Here, the main advantage is that the atmosphere can be modified immediately while packaging (Kader et al., 1989).

The concentration of gases can be further adjusted by the use of absorbers or adsorbers in the package to scavenge O_2, CO_2, or C_2H_4. Carbon dioxide absorbers can prevent the build up of CO_2 to injurious levels, a situation that can occur for some commodities during passive modification of the package atmosphere (Zagory and Kader, 1988).

Films Suitable for MAP

In 1989, Kader et al. listed the following desirable characteristics of plastic films for MAP of fresh produce: 1) required permeabilities for the different gases 2) good transparency and gloss 3) light weight 4) high tear strength and elongation, resistance to puncture 5) low temperature heat-sealability 6) nontoxic and no transfer of odors or flavors 7) nonreactant with produce 8) good thermal and ozone resistance 9) good weatherability 10) commercial suitability 11) ease of handling 12) ease of printing for labeling purposes 13) biodegradable or recyclable 14) thermally responsive to the same extent as the commodity.

Selection of a film that will result in a favorable MA should be based on the expected respiration rate of the commodity at the transit and storage temperature to be used and on the known optimum O_2 and CO_2 concentrations for the commodity (Kader, 1986). Many plastic films are available for packaging purposes (Table 1.1), but relatively few have been used to wrap fresh produce. Even fewer have gas permeabilities that make them suitable to use for MAP. Because oxygen content in a MAP is typically being reduced from an ambient 21 percent O_2 down to two to five percent within the package, there is a danger that CO_2 will increase from ambient 0.003 % to 16 to 19 % in the package. This is because normally there is a one-to-one correspondence between O_2 consumed and CO_2 produced. Because these high levels of CO_2 would be injurious to most fruits and vegetables, an ideal film must let more CO_2 exit than O_2 enter. The CO_2 permeability should be somewhere in the range of three to five times greater than the oxygen permeability, depending upon the desired atmosphere. Several polymers used in film formulation meet this criterion. Of these, low density polyethylene and polyvinyl chloride are the main films employed in packaging fruits and vegetables (Kader, 1992).

Table 1.1. Permeabilities of available polymers used for film formulation (Kader et al., 1989).

Film type	Permeabilities (cc/m^2/ mil/day at 1 atm) CO_2	O_2	CO_2:O_2 Ratio	WVTR*
Polyethylene: low density	7,700 - 77,000	3,900 - 13,000	2.0 - 5.9	
Polyvinyl chloride	4,263 - 8,138	620 - 2,248	3.6 - 6.9	
Polypropylene	7,700 - 21,000	1,300 - 6,400	3.3 - 5.9	
Polystyrene	10,000 - 26,000	2,600 - 7,700	3.4 - 3.8	
Saran™	52 - 150	8 - 26	5.8 - 6.5	
Cellulose acetate	13,330 - 15,500	1,814 - 2,325	6.7 - 7.3	1,613 -1,395
Polyvinylidine chloride	59	15.5.	3.8	3.1
Rubber Hydrochloride	4,464 - 209,250	589 - 50,375	4.2 - 7.6	7.8 - 10.9
Nylon	31	15.5	2.0	126
Polycarbonate	23,250 - 26,350	13,950 - 14,725	1.7 - 1.8	10.9 - 17.1
Ethylcellulose	77,500	31,000	2.5	310
Methylcellulose	6,200	1,240	5.0	3,100
Polyvinyl alcohol	near 0	near 0	-	1,240
Polyester	180 - 390	52 - 130	3.0 - 3.5	-
Polyvinyl fluoride	171	50	3.4	-
Polychlorotriflour-oethylene	124	11.8	10.5	0.3
Cellulose triacetate	13,640	2,325	5.9	74 - 93
Vinyl chlorideacetate	853	233	3.7	62

*Water vapor transmission rate.

Chapter 2

Effect of Nitrogen Fertilization on Storage Quality of Broccoli Florets

Abstract

The storage quality of broccoli florets (*Brassica oleracea* L group Italica cv 'Excelsior') supplied with nitrogen (N) at different levels during development was examined. Field nitrogen treatments consisted of 0, 90, 180, and 270 Kg N/ha from urea. Broccoli florets (250 grams) of all treatments, were packaged in Cryovac B-900 polyolefin film and stored at 0°C. Chemical and quality analyses were performed on the day of harvest and after 8, 12 and 28 days of storage. Green color intensity (L*, a*, b* values) in response to different rates of nitrogen fertilizer was measured with a Minolta Chroma Meter. Increased field N application resulted in more green color and higher chlorophyll concentration. With no N application the chlorophyll concentration was 84 μg/g fresh wt and hue angle of 138.0 compared with 127 μg/g fresh wt and hue angle of 151.0 for the 270 Kg N/ha treatment after 12 days storage. At harvest, N fertilizer rates up to 180 Kg/ha increased the ascorbic acid content. Ascorbic acid losses were not affected by nitrogen fertilization rates. However, the effect of storage time on ascorbic acid loss was significant. Weight loss was kept low in all samples due

to the film packaging of the florets. However, there was a tendency for broccoli florets with the highest N fertilization rate to lose more weight in storage.

Introduction

Broccoli is a popular market and home garden crop, widely used as a fresh and processed vegetable. The popularity of broccoli consumption places greater demands upon growers to supply high quality produce.

Broccoli production is highly dependent on soil fertility. Broccoli typically shows large yield responses to nitrogen fertilizer (Arjona and Greig, 1984; Cutcliffe at al., 1968). However, fertilization with nitrogen and other mineral elements may affect some quality and nutritional characteristics of fruits and vegetables. In experiments with container-grown vegetables, high levels of potassium (K) nutrition generally increased the content of vitamin C, whereas higher N levels decreased vitamin C (Stanislaw et al., 1987). Increased nitrogen fertilization was also shown to decrease vitamin C content in tomato fruits (Stanislaw et al., 1987).

Plant pigments, especially chlorophyll, are very important appearance factors in the quality of vegetables. Nitrogen (N) encourages vegetative growth and is an integral component of the chlorophyll molecule, as well as enzymes, proteins and many other compounds. The importance of nitrogen levels in the synthesis of chlorophyll in plants has been shown. While more N usually means more chlorophyll, excessive nitrogen soil applications may also reduce chlorophyll synthesis (Eheart, 1966). In some cases, Eheart (1966) noted decreases in chlorophyll content of field-grown broccoli heads with the application of excessive amounts of nitrogen.

Several studies on N fertilization for maximization of broccoli yield has been published (Dufault, 1988; Arjona, 1984; Cutliffe, 1971). However, there is limited information on the impact of nitrogen fertilization on various aspects of quality. The purpose of this study was to determine the effect of nitrogen fertilization rates on quality characteristics of MAP broccoli florets in storage.

Materials and Methods

Broccoli (cv Excelsior) heads were harvested from field plots at the Oregon State University Vegetable Research Farm, Corvallis in which treatments of 0, 90, 180 and 270 Kg N/ha were arranged in a randomized complete block design with 4 replications. The nitrogen was applied as urea (46-0-0) to all treatments (except for the 0 Kg/ha treatment) seven days after planting. The fertilizer was side dressed between the pairs of broccoli rows. Thirty five days after the first application, the remaining nitrogen for the N-rate treatments was applied in a similar manner. Broccoli was harvested at the commercial maturity stage and after cutting and removal of leaves, heads were immediately brought to the laboratory and kept overnight at 0°C. What we will call "florets" through this study, are actually the florets plus a portion of the stem, the total length of which was about 2.5 cm. Botanically, the term floret refers to "an individual flower of a definite cluster". Since cut broccoli can be used in various forms such as complete heads or spears, we wanted to deal mainly with the most physiologically active tissues (florets). We therefore tried to minimize the stem tissue. The next morning, the heads were divided with a sharp knife into individual pieces of florets of about 20 g each, and surface-sterilized by dipping for 2 minutes in 100 ppm sodium hypochlorite (NaOCl), then drained on paper towels. Florets were packaged into 25 X 48 cm B-900 Cryovac polyolefin bags, sealed with a V-300 Fuji Impulse vacuum sealer and immediately transferred to a 0°C storage room. Gas transmission rates given by the manufacturer for the polyolefin film were 0_2-3-6 cc/m^2/24 hr at 40°F and water vapor transmission-0.5-0.6 g/100 in^2/24 hr at 100°F. Broccoli florets were used at 250 g per individual replicate (1 bag) and each treatment had four replicates for each of the four evaluation times. All bag samples were stored at 0°C for analyses after 8, 12 and 28 days.

A randomized block experimental design was used with four blocks corresponding to the actual arrangement in the field.

Determinations

Quality of broccoli florets was evaluated on the day after harvest and after 8, 12, and 28 days of storage. For each of the four replications per treatment (4 nitrogen fertilizer rate), the following quality attributes were monitored: weight loss, ascorbic acid level, chlorophyll concentration and color change.

Each experimental unit (1 bag) was weighed initially, and again at each time interval. Weight loss was calculated from the difference between initial and final weight at each time interval and expressed as a percentage of the initial fresh weight.

Chemical extractions and analyses were performed to evaluate the loss of green coloration (chlorophyll) and ascorbic acid (Vitamin C). These analyses were performed on tissue samples containing mostly floral portions with relatively little stalk tissue. Samples were obtained by thorough mixing and randomized removal of the buds of florets in each bag.

Chlorophyll Concentration

Total chlorophyll determinations were made using a spectrophotometric assay (Anderson and Boardman 1964) as adapted by Barth et al., (1992) with a Baush & Lomb model 2000 UV-visible spectrophotometer. Broccoli tissue (9 g) was extracted in 54 ml acetone: 3 ml 0.1N NH_4OH solution by homogenization using a Virtis homogenizer for 1 min. under cold conditions and then stored briefly in the dark prior to filtration. Homogenate was filtered through Whatman #2 paper. Aliquots of samples were transferred to 1.0 cm quartz cuvets and absorbance read at 700, 663, 645 and 626 nm. Total chlorophyll was calculated and expressed as µg of total chlorophyll per gram fresh weight (µg/g FW).

Ascorbic Acid Analysis

Reduced ascorbic acid (mg/g fresh weight) was determined by the titrimetric assay described by Pelletier (1985) as reported by Barth et al. (1992). Floral tissue was cut up and a 5 gram sample placed into 63 ml 3% metaphosphoric-acetic acid extracting solution. A Virtis homogenizer was used to grind samples for 2 minutes. Macerated samples were filtered through Whatman # 2 filter paper into 50 ml volumetric flasks and made up to volume by adding 3% metaphosphoric-acetic acid. Five ml aliquots were transferred into a small beaker with a magnetic stirring bar. The samples were titrated with 2,6-dichloroindophenol indicator solution until the pink color remained for at least one minute.

Color Assessment

Colorimetric measurements of broccoli florets were recorded at each time interval using a Minolta Chroma Meter CR - 300 tristimulus colorimeter in CIE L* a* b* (Commission Internationale D'Eclairage) Color Space coordinates by placing the 8-mm-diameter measuring area against the floret surface. The instrument was calibrated to a white standard under illuminant condition C (6774K). The L*a*b* color space was used based on its recommended adequacy for theoretically quantifying color changes in green vegetables (Shewfelt et al., 1988). The L* variable lightness index ranges the scale from 0 for black to 100 for white. The a* scale measures the degree of red (+a*) or green (-a*) color and the b* scale measures yellow (+b*) or blue (-b*) color. Color is reported as chroma values ($(a^{*2} + b^{*2})^{1/2}$) and hue angle values ($\tan^{-1} b^*/a^*$). L is related to darkness of the sample, chroma is a function of color saturation, and hue angle tonality is related to the greenness/yellowness for this specific vegetable. A decrease in hue angle corresponds to the color changing from green to yellow.

Color of each replicate was measured at the center of each floret on six individual florets selected at random (n =24 for each treatment).

Results and Discussion

Weight Loss

The influence of N fertilizer rates on weight loss of broccoli florets during storage is indicated in Figure 2.1 and appendix Table A2.1. The florets from 0 Kg/ha of nitrogen lost about 0.6% of their initial fresh weight after 8 days and 2.6% after 28 days. The percentage of water loss increased in samples from 90 Kg N/ha and further increased in samples from 270 Kg N/ha at all time intervals. However, among the treatments with N fertilizer, broccoli florets with 180 Kg N/ha showed the least percentage of water loss (Figure 2.1). No significant differences were noticed between 90 and 270 Kg/ha of nitrogen treatments at all time intervals.

The loss of weight was 40% less in samples from 180 Kg N/ha than 270 Kg N/ha, and 35% less than samples from 90 Kg N/ha after 28 days storage (Figure 2.1).

The results show that as storage time increases the weight loss of broccoli florets increases. Also, broccoli florets treated with 90, 180, and 270 kg N/ha exhibited significantly higher weight loss than those with 0 kg N/ha (controls). Ben-Yehoshua (1987) considered 4% weight loss the limit for consumer acceptance.

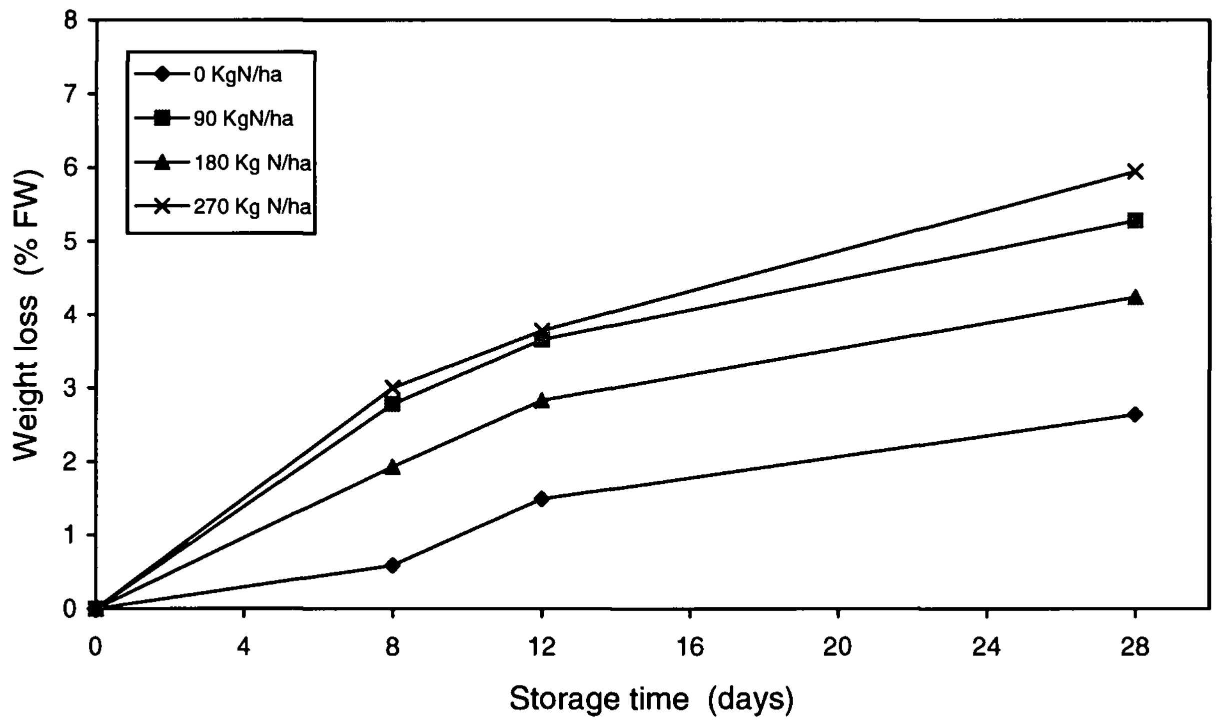


Figure 2. 1. Weight loss of MAP (Cryovac B-900 film) broccoli florets stored at 0°C as affected by urea nitrogen fertilizer rates during development.

Ascorbic Acid

Application of nitrogen fertilizer up to 180 kg/ha generally increased the ascorbic acid content of broccoli florets at harvest (Table 2.1). The initial ascorbic acid concentration of the 0, 90, 180 and 270 Kg/ha of N treated broccoli florets was 0.91, 1.09, 1.37, and 1.28 mg/g, respectively. Results from this study differ with previous reports concerning ascorbic acid content and N application. Eheart (1966) reported some decrease in the content of vitamin C of broccoli heads with increasing N application rates to 112 Kg N/ha. However, this study showed some increase in ascorbic acid with increasing N fertilization rates. Burrel et al. (1940) reported increased ascorbic acid content in cabbage with increasing N application while Sorensen (1984) observed opposite results.

Moreover, N fertilization had no significant effect on vitamin C losses of broccoli florets over a 28 day storage period (Table 2.1). The highest broccoli ascorbic acid content was observed in the application rate of 180 kg N/ha after 8, 12 and 28 days of storage and the lowest content in the samples from 0 kg N/ha. These results parallel those of Toivonen et al. (1994) which indicate that N fertilization rates did not significantly affect the vitamin C losses of broccoli heads over a 20-day storage period at 1°C. Also Freyman et al. (1991) found that N fertilization did not significantly affect the chemical quality or storage of cabbage.

Broccoli samples in 0°C storage for 8 days which were treated with 180 Kg/ha of N fertilizer, showed the highest ascorbic acid value (0.90.mg/g FW) and samples from 270 Kg N/ha, the lowest value (0.49 mg/g FW). Furthermore, broccoli ascorbic acid values from the 270 Kg/ha N fertilizer plots tend to exhibit more variation over time than values from the other three application rates. This greater variation might explain the difference exhibited at 8 days of storage.

Table 2.1. Effects of nitrogen fertilization rates and 0°C storage time on ascorbic acid concentration of broccoli florets sealed in B-900 Cryovac film.

Storage time (days)	Nitrogen fertilization rates (Kg /ha)	ascorbic acid concentration (mg/g fresh wt)
0	0	0.91 b*
	90	1.09 a
	180	1.37 a
	270	1.28 a
8	0	0.68 b
	90	0.84 ab
	180	0.90 a
	270	0.49 c
12	0	0.37 b
	90	0.52 a
	180	0.56 a
	270	0.51 a
28	0	0.21 b
	90	0.27 ab
	180	0.36 a
	270	0.11 c

*Means within a column in each storage time followed by the same letter are not different, FPLSD, $\alpha = .05$ (each value is the mean of 4 observations)

Generally ascorbic acid trends from the N treatments were similarly affected by storage time.

The effect of storage time on ascorbic acid levels was significant. After an initial sharp decline between days 8 and 12, the ascorbic acid content decreased less rapidly to the end of the experiment at 28 days. Ascorbic acid levels were very low (about 25% of initial values) after 28 days and was significantly different than after 8 or 12 days (Figure 2.2). The overall decrease in ascorbic acid which occurred over time was similar to that found by Fennema (1977). The decrease in ascorbic acid by the end of the storage period was more or less expected. The concentration of CO_2 in the packages after 96 hours reached about 18%. The continued loss of ascorbic acid was apparently not benefited by high CO_2, or low O_2 concentration. The problem with controlled, or modified atmosphere storage of broccoli is the susceptibility of the vegetable to CO_2 injury (Kasmire et al., 1974; Wang, 1979). Broccoli has tolerance limits of at least 1% O_2 and no more than 15% CO_2, beyond which physiological damage can occur (Brecht, 1980).

Chlorophyll Concentration

Analyses at harvest, indicated that the chlorophyll concentration increased as the N fertilization level increased (Appendix Table A2.2). This pattern generally held during storage. Significantly more chlorophyll was retained after 8 days of storage in samples treated with 270 Kg /ha of nitrogen fertilizer compared to the other treatments (Appendix Table A2.2). Dufault (1988) reported floret chlorophyll content of greenhouse grown broccoli to increase linearly with increasing nitrogen rate to 5.6 g N/pot. However, Eheart (1966)

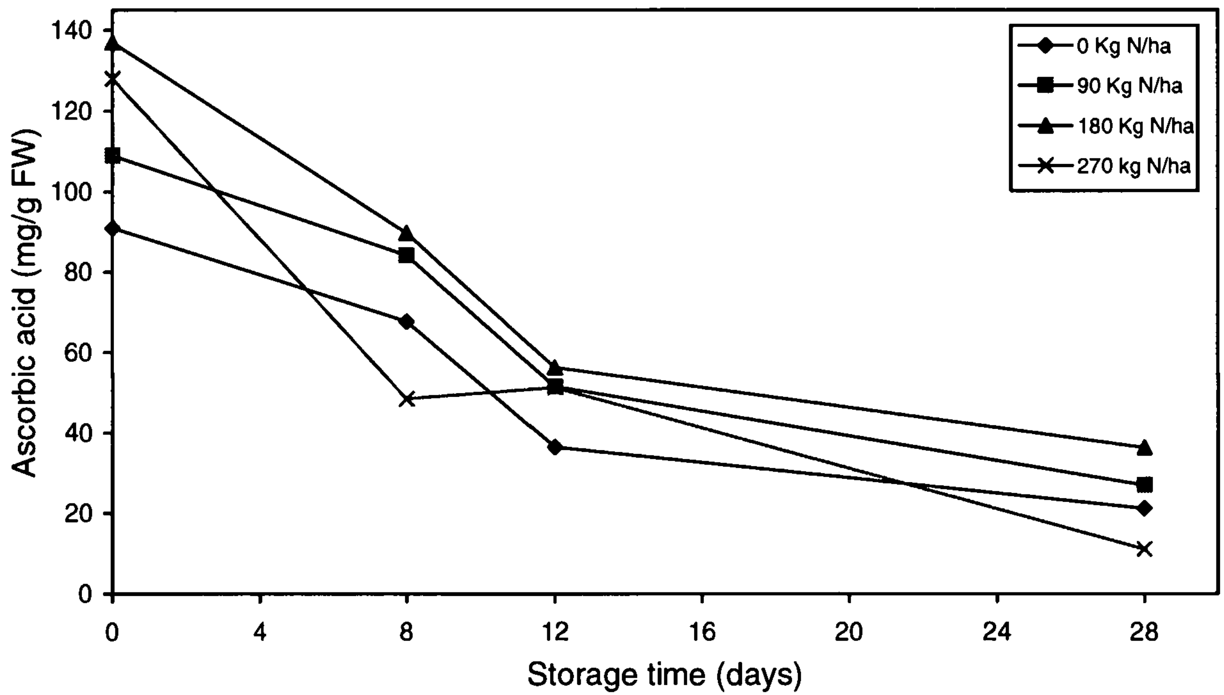


Figure 2.2. Effects of sampling time in storage on ascorbic acid content of broccoli florets at 0°C, selaed in Cryovac B-900 film.

showed that there was some decrease in the content of chlorophyll with increasing N application to 112 kg/ha.

Chlorophyll was best retained in samples with 270 Kg/ha of nitrogen fertilizer at all time intervals analyzed (Figure 2.3, Appendix Table A2.2). This is consistent with the results of color measurements (Table 2.2). The lowest content of chlorophyll concentration was observed after 8 days (102.1 μg/g FW) and 12 days (84.3 μg/g FW) storage in the broccoli treatments without added nitrogen. The large chlorophyll loss observed in all treatments after 28 days of storage may also have been affected by the high CO_2 level inside the package (CO_2 injury) and less the effects of the nitrogen fertilization treatments. Florets showing CO_2 injury symptoms were noticed at the last (28 days) evaluation period. Lebermann et al. (1968b) reported that the pH of broccoli stalks increased progressively with increased concentrations of CO_2 in the storage atmosphere. The pH increased from 6.7 in atmospheres containing 10% CO_2 to 7.0 in atmospheres containing 20% CO_2. Sweeney et al. (1961) found that pH changes between 6 and 7 were crucial in chlorophyll retention in green vegetables. These authors reported chlorophyll retention of 77.2% in spinach with a pH of 6.7 and 44.9% in broccoli of pH 6.4.

While levels of 90 and 180 Kg/ha of nitrogen fertilization showed some increased total chlorophyll by 13 and 10.7% respectively by 12 days (relative to levels at 8 days) these were not statistically different. Other reports have shown that some mature green tissues are capable of chlorophyll biosynthesis in storage and the capacity to synthesize chlorophyll is probably a form of chlorophyll restoration (Bazzaz and Rebeiz, 1978). Further, packaging and environmental conditions influence the retention of chlorophyll in stored vegetables (Perrin and Gaye, 1986 and Watada et al., 1987). Makhlouf et al.

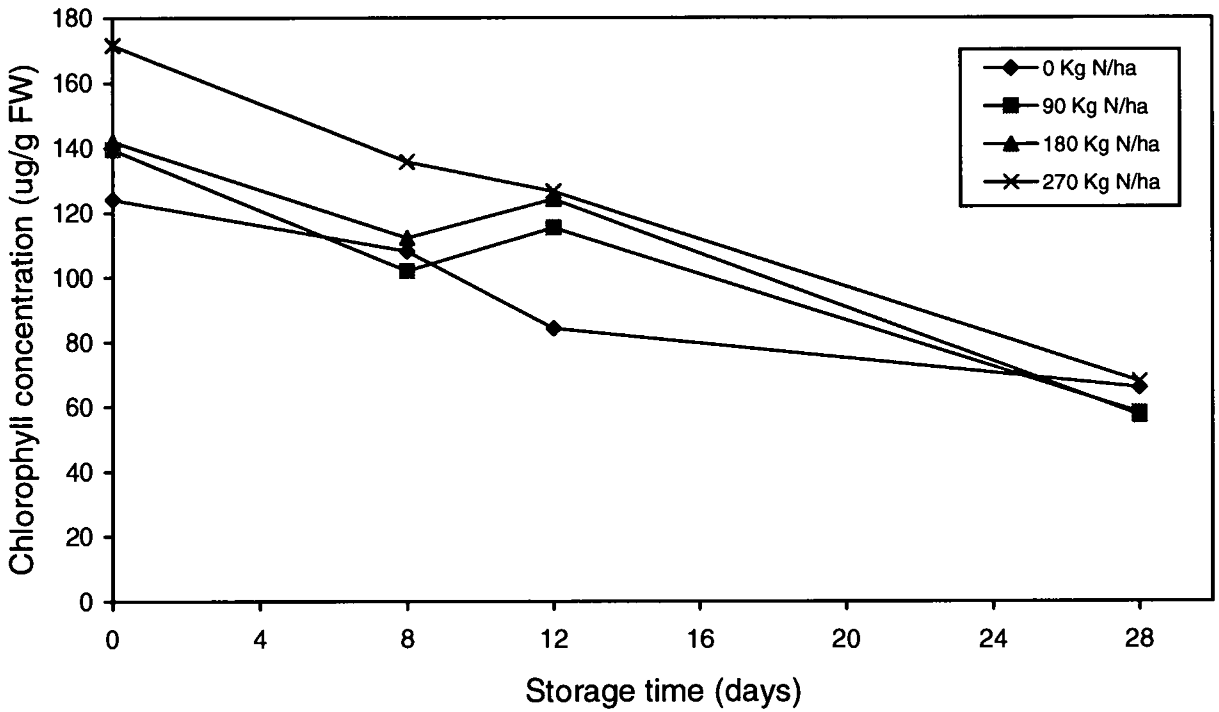


Figure 2.3. Chlorophyll concentration of 0°C stored MAP (Cryovac B-900 film) broccoli florets as affected by Urea N-fertilization rates during development.

(1989) showed that chlorophyll content was maintained in broccoli stored in high CO_2.

Total chlorophyll concentration was better maintained in broccoli florets with the highest level of nitrogen fertilizer treatment, whereas the control broccoli with no added had the lowest content after 8 and 12 days storage (Figure 2.3). Chlorophyll and total N are often correlated. Nitrogen is an integral component of many compounds, including chlorophyll and enzymes, essential for plant growth processes. Although chlorophyll does not have a major N component, it is usually associated with a complex of proteins including those of photosystem I, photosystem II, and the CO_2-fixing machinery. These proteins constitute the bulk of N in the leaf (Khemira et al., 1994).

Color Analysis

Treatments were distinguished clearly on the basis of hue and chroma- aspects of color. As the rate of nitrogen fertilization increased, hue value (a measure of greenness) increased and conversely, chroma (an index somewhat analogous to color saturation or intensity) decreased. Color was retained in the samples with the highest nitrogen fertilization treatment more than in samples from the other two nitrogen fertilization treatments. But, compared with the control (without N fertilization), all nitrogen treatments resulted in better green color retention, particularly in the later periods of the study. Broccoli florets that did not have nitrogen fertilizer had the lowest hue and the highest chroma color measurements at each storage interval (Table 2.2). Color was significantly

Table 2.2. Color changes in broccoli florets sealed in B-900 Cryovac film as influenced by different rates of N fertilization and storage time at 0°C.

Storage time (days)	Nitrogen fertilization rates (Kg /ha)	Hue angle ($\tan^{-1}$ b/a)	Chroma $(a^2+b^2)^{1/2}$
0	0	136.3 b*	22.2 a
	90	155.3 a	16.7 ab
	180	163.2 a	12.0 b
	270	168.9 a	13.8 b
8	0	138.0 c	13.8 a
	90	124.8 b	11.3 a
	180	142.5 ab	11.4 a
	270	150.7 a	10.8 a
12	0	115.3 b	20.9 a
	90	117.7 b	18.5 b
	180	122.7 a	18.8 b
	270	124.8 a	18.3 b
28	0	109.6 c	22.5 a
	90	116.9 b	20.1 b
	180	122.3 a	19.0 b
	270	123.7 a	17.5 c

*Means within a column in each storage time followed by the same letter are not different, FPLSD, α= .05 (each value is the mean of 24 observations) better retained in the samples that were fertilized with 180 or 270 Kg N/ha as judged by the hue values. However, after 28 days storage duration they differed mainly in the chroma value. Dufault (1988) reported head color of greenhouse grown broccoli to intensify from lime/olive to an olive/ emerald with N rate to 5.6 kg/ha and he suggested that higher rates than this may intensify and increase the incidence of emerald color.

Though nitrogen fertilization indeed resulted in samples that were greener than the samples from the 0 Kg N/ha control, as indicated by the initial evaluation and after storage up to 28 days, there was no significant difference between 0 and 90 kg N/ha treatments after 12 days (115.3 and 117.7 hue angle values).

Modified atmosphere has been shown to improve retention of the green color in storage of green vegetables. However, the low color retention in all treatments as judged by instrumental color evaluation was probably due to the detrimental effect of the high CO_2 concentration inside the packages. Since we had no air control for comparison, this is a bit conjectural.

Changes in color during storage were closely related with changes in chlorophyll concentration (Figure 2.3). The increase in chlorophyll concentration in broccoli florets with 90 and 180 Kg N/ha was related to an increased chroma values from 11.3 and 11.4 (8 days after storage) to 18.5 and 18.8 after 12 days.

The chroma value has been previously used successfully to estimate chlorophyll content of green leaves (Singha and Townsend, 1989; Khemira et al, 1993).

Conclusions

1. Weight loss in MAP packaged broccoli florets was generally low (< 6%) and essentially linear during 28 days of 0°C storage.
2. The higher the N-fertilizer during development, generally the greater was weight loss compared to control treatments.
3. Urea-N fertilization rates up to 180 Kg N/ha increased floret ascorbic acid concentration at harvest, but during storage the highest N-rate lost ascorbic acid most rapidly.
4. During 28 days of 0°C storage ascorbic acid decreased by about 65% from initial values. Even though MAP may have had some beneficial effect on conserving ascorbic acid, it did not prevent substantial losses.
5. Increased N-fertilizer rates resulted in progressively greater floret green color (hue angle and chroma) and chlorophyll concentrations, but these differences disappeared as chlorophyll linearly decreased by 50% during 28 days of MAP storage at 0°C.

Chapter 3

Storage Quality of Broccoli Florets: Effects of Temperature and Package Types

Abstract

Modified atmosphere packaging films for preservation of broccoli *(Brassica oleraceae* L Italica group) were evaluated using Cryovac PD-941, PD-961EZ, and PD-900 film and compared to perforated film controls. Storage temperatures were 0°, 5°, and 10°C. Weight loss was greatly reduced in the film packaged florets compared to the vented package control. Films differential permeability properties had only a slight effect on weight loss but it significantly affected in-package CO_2 and O_2 concentrations. Concentrations of these gases inside the package were also influenced by respiration in response to the storage temperature. Internal atmospheres of PD-941 packages tended to equilibrate at about 5% O_2 when stored at 0C but decreased to about 2% for most all sealed packages kept at 5° or 10°C. CO_2 levels in PD-900 packages equilibrated at about 11% at 0°C but increased to about 17% at 10°C. Color (hue angle value of 124 vs 87) and chlorophyll retention (123 µg/g FW vs 42 µg/g FW) was better in the MAP treatments than vented bag controls after 21 days at 5°C. MAP also delayed loss of ascorbic acid. The beneficial effects of MAP were especially noticeable when the florets were stored at 5° or 10°C in which shelf life was

appreciably extended. At 0°C storage temperature no significant differences in ascorbic acid content were found between PD-941 and PD-961EZ film packaged broccoli florets and vented bag control up to 21 days. However, in modified atmosphere packaging with PD-900 film containing more than 11% CO_2, undesirable odor developed. Among the films tested, Cryovac PD-941 was the best for storage maintenance of broccoli florets quality. There was no off-odor buildup in the bag at any temperature in the range of 0° to 10°C.

Introduction

Atmosphere modification, by lowering O_2 and/or increasing CO_2, has been shown to maintain quality and extend the storage life of many fresh commodities. Atmosphere modification using controlled atmosphere (CA) storage facilities are technically complex and expensive. Applications of CA or modified atmospheres (MA) include products that are amenable for long-term storage such as apples, pears, kiwifruits, cabbage (Kader et al., 1989). Packaging techniques can also be used to achieve these beneficial atmospheres. One technique, referred to as modified atmosphere packaging (MAP), depends on the respiration rate of the commodity, the gas permeation properties of the packaging films, and the temperature to determine the composition of the atmosphere (Kader et al., 1989; Labuza and Breene, 1989;).

Minimum safe low temperatures (above freezing or the critical temperature for chilling injury) and high relative humidity control are the most important tools for extending shelf life in most vegetables. A supplement to appropriate temperatures and relative humidity conditions during storage of some vegetables which is currently being actively researched has been modified atmosphere packaging (MAP). This utilizes polymeric films of semipermeable characteristics which result in decreased O_2 and/or increased CO_2 concentrations inside the package. Reduced O_2 and increased CO_2 environments generally reduce respiration and ethylene production rates, retard softening, and slow compositional changes associated with ripening and senescence (Kader et al., 1989). Benefits from appropriate MAP include reduced rates of nutritional and quality losses and increased storage life (Weichmann, 1986). Reduced respiration rates combined with lowered C_2H_4 production and reduced sensitivity to C_2H_4 results in better retention of chlorophyll (green color) and improved texture (less softening and lignification).

Consumption of fresh broccoli has increased rapidly in recent years. Unfortunately, both the growing season in some areas and the shelf life of fresh broccoli are short. Many factors lower the market quality of broccoli after harvest, but one of the most serious is yellowing of the florets. Florets turn yellow very rapidly, especially at high temperatures (Lieberman and Hardenburg, 1954). Retardation of the yellowing, therefore, is important in maintaining the salability of broccoli. It has been shown that yellowing could be delayed by elevated CO_2 atmosphere (Leberman et al., 1968., Lieberman and Hardenburg, 1954; Lipton and Harris, 1974). Although broccoli is considered to be relatively tolerant of high CO_2 (Lipton, 1975; Wang, 1979), prolonged exposure to high CO_2 or low O_2 were found to induce off-odor and off-flavor (Kasmire et al., 1974; Lipton and Harris, 1974).

Optimal storage conditions for broccoli heads have been reported (Makhlouf et al., 1989) but such conditions may not be adequate for broccoli florets. They have a larger cut surface area, and are subject to greater wounding stress. Rushing (1990) observed an increase in respiration and ethylene production in broccoli florets as compared to intact heads during the first 24 hr storage at 16°C.

With the recent development of new films and packaging techniques there is considerable potential for improving the quality and extending the market life of a variety of vegetables and salad products by using MAP. Gas permeability specifications given by the manufacturers are usually under conditions that are different than the high humidity, refrigerated storage conditions of respiring produce. Thus the suitability of a film must be tested with the product under practical conditions (Gorris and Peppelembos, 1992).

The objective of this study was to test the effectiveness of selected polyolefin films in extending the postharvest life of broccoli florets stored at different temperatures.

Materials and Methods

Broccoli (*Brassica oleracea* L. Italica group cv, 'Excelsior') was harvested on a local farm (Corvallis, Oregon) at commercial maturity stage, transported to the laboratory and held overnight at 0°C.

The following morning it was cut, weighed and randomly assigned to treatments and replicates. Florets were trimmed from stalks by hand with a sterile knife. They were surface sterilized by dipping for about 2 minutes in 100 ppm NaOCl and drained on paper towels. Florets (200 g) were then placed into 18 x 26 cm polyolefin film bags. Three polymeric films (manufactured by CRYOVAC) designated as PD-941, PD-961EZ and PD-900 were used as the packaging films. Gas transmission rates of the films used in these experiments, as reported by the manufacturer are presented in Table 3.1. In addition, florets were placed in vented bags (eight 6 - mm holes per bag) comparable to consumer bags in retail grocery stores to serve as controls. Bags, including controls, were sealed with an L-bar sealer (V -300 Fuji Impulse) and immediately transferred to 0°, 5°, or 10°C storage temperatures.

The experiment was a 4 x 3 factorial (4 package types, 3 temperatures) in a completely randomized design. There were three replicates at each time for each combination of packaging type and storage temperature. Samples were evaluated initially and after 8, 12, 21 and 28 days of storage. At each storage period, broccoli florets were evaluated for weight loss and then removed from bags and evaluated for color changes, total chlorophyll concentration and ascorbic acid (vitamin C) content.

Table 3.1. Oxygen, carbon dioxide, and water vapor transmission rates of Cryovac films used in this study.

Film	Oxygen transmission rate ($cc/m^2/24$ hr.)	Carbon dioxide transmission rate ($cc/m^2/24$ hr.)	Water vapor transmission rate ($g/100\ in^2/24$ hr.)
PD - 941	16,544	31,000	5.0
PD - 961EZ	6,000 - 8,000	19,000 - 22,000	0.90 - 1.10
PD - 900	3,000	9,800	0.65

The methods for analyses were the same as those described in Chapter 2. In each treatment, three packages were used for weight loss measurement and for chemical analyses. Color was measured on six florets from each package (n = 18 for each treatment). Each package represented a replicate. Analysis of variance was employed for each storage period and mean values for treatments were separated by Fisher Protected Least Significant Difference (FPLSD). The STATGRAPHICS Statistical Software program was used for analysis.

Headspace Analysis

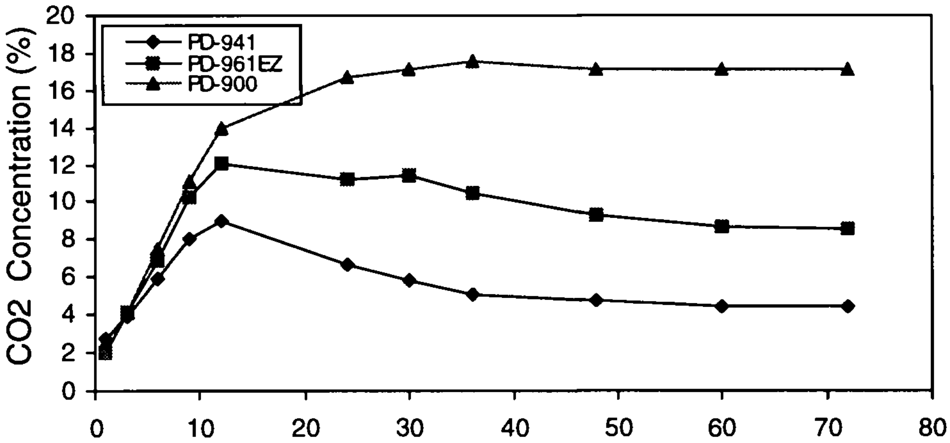
The gaseous composition of the MAP internal atmospheres was analyzed using gas chromatography. Samples (1 ml, three replications) for each treatment were taken at 2, 6, 12, 24, 30,48,72, 144 and 169 hr after storage. Gas samples were withdrawn through the film by using a 1 cc gas-tight syringe and packages were immediately resealed with 2 cm^2 Scotch tape after each needle puncture. CO_2, O_2 , and N_2 measurements of headspace were made by injecting the 1.0 ml gas sample into a Carle Model 311 Gas Chromatograph equipped with a HayeSep R column (2m x 3 mm O.D., 80/100 mesh) and a Molecular Sieve 5A column (2m x 3mm O.D., 80/100 mesh) at 55°C. Separated gases were detected by a thermal conductivity detector (TCD). Helium carrier gas flow was 30 ml/min. TCD Detector responses were quantified by a HP model 3395 digital integrator (Hewlett-Packard Co., USA).

Results and Discussion

Gas Concentrations within the Package

Atmosphere modification inside the MAP packages i.e., CO_2 accumulation and O_2 depletion over time (Figure 3.1), showed that levels of CO_2 inside the packages reached approximately 7, 12 and 17% in PD-941, PD-961EZ and PD-900 films respectively while O_2 level reached approximately 2% in PD-941 bags and 1.5% in PD-961EZ and PD-900 within 12 hr of storage at 10°C. They equilibrated thereafter to about 4.5% CO_2 and 2% O_2 in PD-941 packages, 9% CO_2 and 1.7% O_2 in PD-961EZ packages, and 17% CO_2 and 1.4% O_2 in PD-900 packages during storage. At this time, the differences in atmospheric composition were mainly due to differences in film permeability, since respiration rates would be expected to be similar.

CO_2 accumulation and O_2 reduction inside packages stored at 5°C (Figure 3.2) was slower than at 10°C. During the first 24 hr at 5°C, the CO_2 concentration increased to 6, 8, and 10% and O_2 concentration fell to 6, 5, and 4% inside of PD-941, PD-961EZ, and PD-900 packages respectively. Internal atmospheres of packages equilibrated to about 4, 8, and 13% CO_2 and 2% O_2. No significant differences were found for oxygen concentration relative to film type at 5 and 10°C. A different pattern was found for CO_2 at 5° and 0°C storage



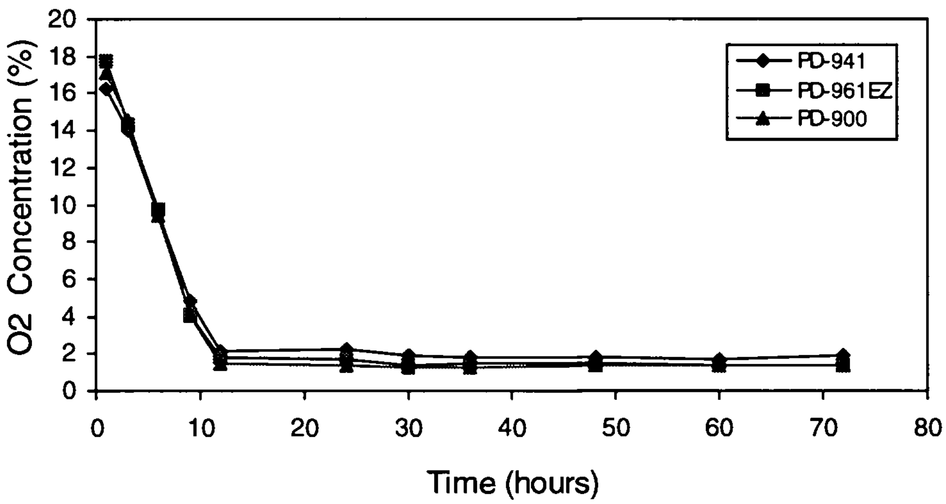


Figure 3.1. Concentrations of CO_2 and O_2 over time in bags of three Cryovac polyolefin films filled with broccoli florets and kept at 10°C.

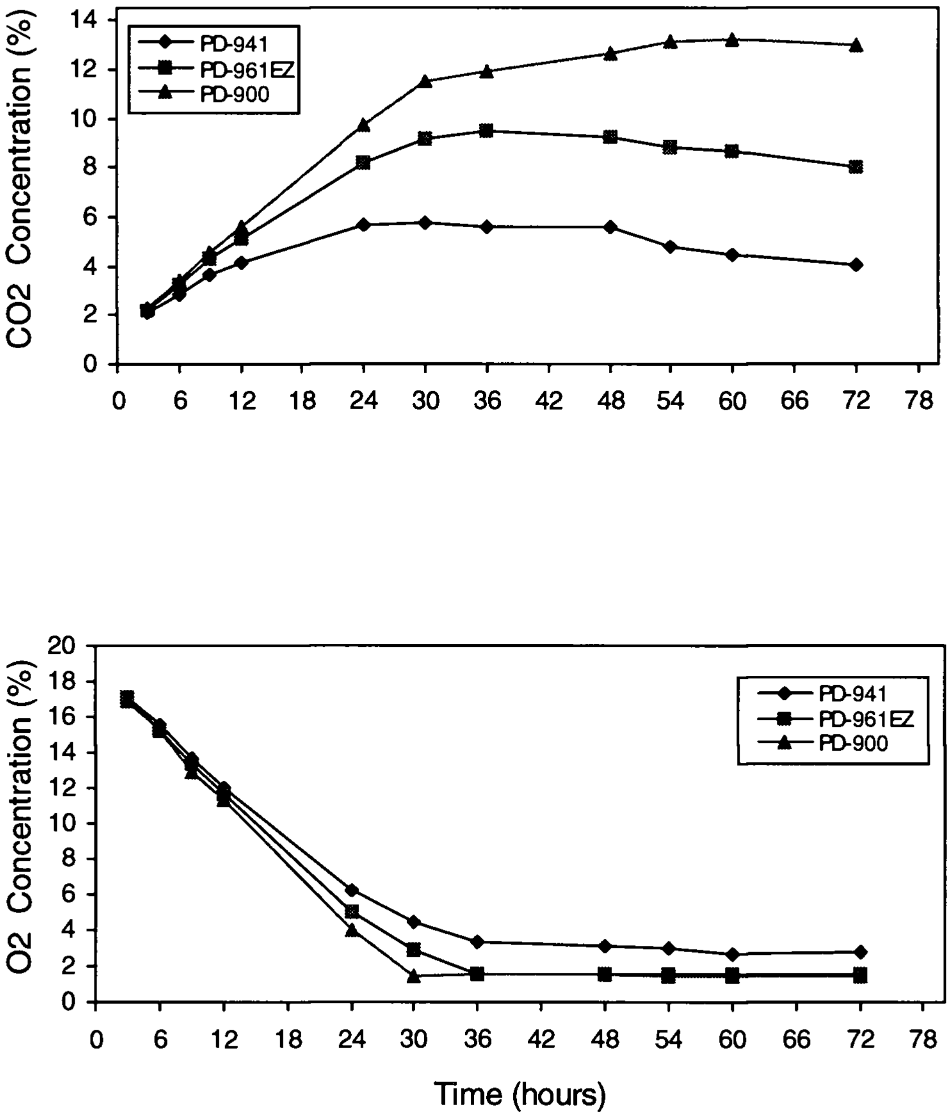


Figure 3.2. Concentrations of CO_2 and O_2 over time in bags of three Cryovac polyolefin films filled with broccoli florets and kept at 5°C.

temperatures and O_2 concentrations did change over storage time (Figures 3.2 and 3.3).

At 0°C storage temperature, oxygen levels dropped to about 5% in PD-941 and 1.8% in PD-961EZ and PD-900 packages within 3 days and the concentration remained almost constant thereafter, implying that O_2 equilibrium was approached by the third day (Figure 3.3). CO_2 concentrations equilibrated to about 4% in PD-941 film, 7% in PD-961EZ film, and 11% in PD-900 film.

CO_2 from respiration accumulated in the three film types during storage and there was a significant storage temperature by time interaction. During the first few hours after sealing, CO_2 concentrations in the packages were increasing and O_2 concentrations were decreasing more rapidly in the 10°C than in the 0°C and 5°C storage temperatures for the three film types. After 6 h, O_2 concentration was 40% to 45% lower in broccoli florets stored at 10°C than 0°C (Figure 3.1 and 3.3). At this time, however, packages of PD-941, PD-961EZ and PD-900 did not differ significantly in O2 concentrations, even though the O_2 transmission rates of PD-941 film is higher than PD-961EZ and PD-900 (Table 3.1). The respiration rate of broccoli is more than 10-fold higher at 10°C than at 0°C (Kader et al., 1989). These higher respiration rates of the florets at higher storage temperature appears to have a greater effect on the modification of the package atmosphere than the film permeation properties. Concentrations of CO_2 were 65% to 75% higher in broccoli stored at 10°C than stored at 0°C and were significantly different between film types. The CO_2 transmission rate for

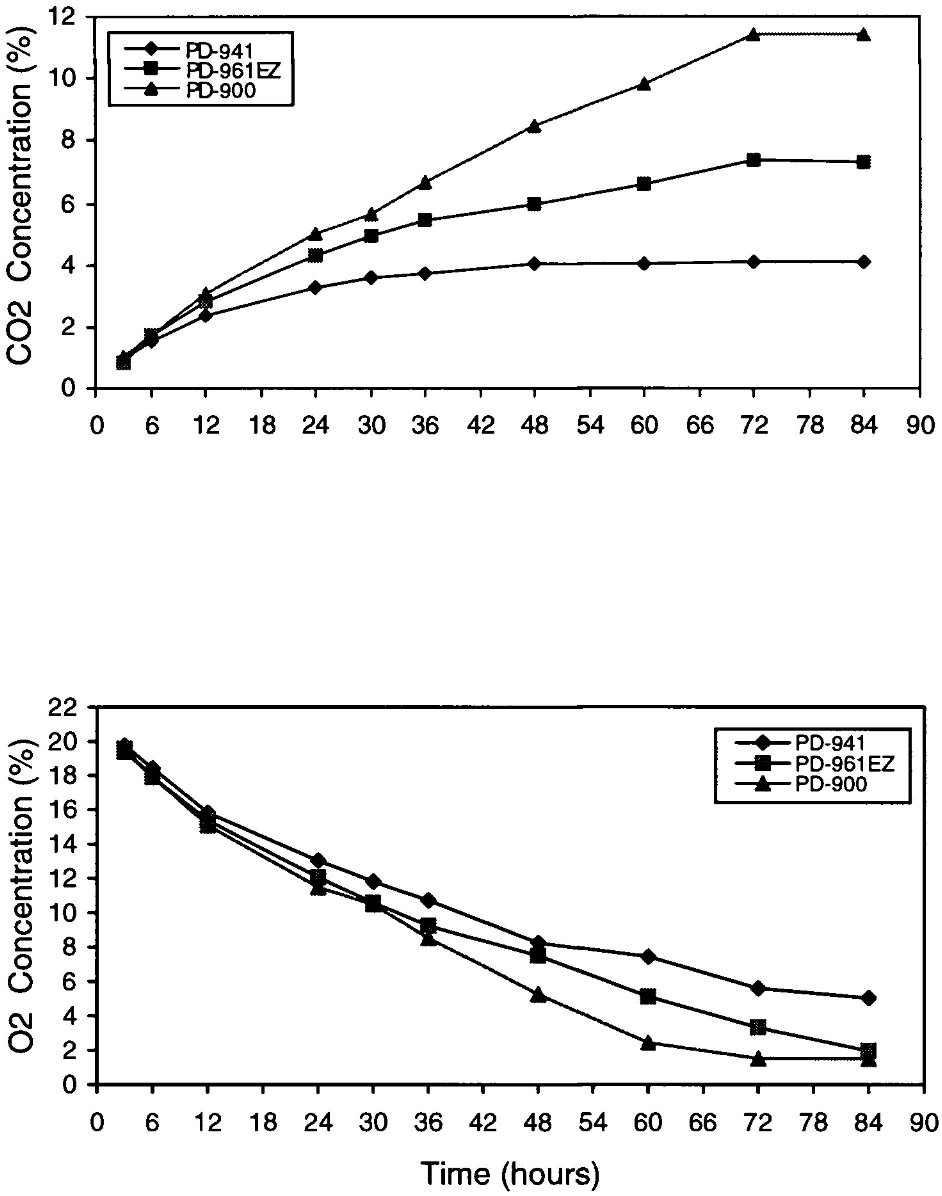


Figure 3.3. Concentrations of CO_2 and O_2 over time in bags of three Cryovac polyolefin films filled with broccoli and kept at 0°C.

PD-941 is higher than the other two film types, which resulted in less CO_2 accumulation during the initial 6 h following sealing.

CO_2 accumulated with time of storage. A higher accumulation of CO_2 occurred in the least permeable film (PD-900), at 10°C (Figure 3.1).

Unpleasant odors (Table 3.2) and symptoms of CO_2 injury were formed by broccoli bagged in PD-900 regardless of the storage temperature. Off-odors have developed in other studies in which broccoli was stored in 10% or higher concentrations of CO_2 in combination with 2.5% or less O_2 (Kasmire et al., 1974; Ballantyne et al., 1988; Makhlouf et al., 1989). It appears that these odors become stronger as CO_2 concentrations increase and storage times increase.

Table 3.2. Development of undesirable odors during storage of broccoli florets at 0°C, 5°C and 10°C packaged in three types of polyolefin films

Film	Treatment Temperature (°C.)	Storage period (days) 12	21
		Undesirable odors*	
PD-941	0	-	-
	5	-	-
	10	-	-
PD-961EZ	0	+	++
	5	+	++
	10	++	++
PD-900	0	++	+++
	5	+++	+++
	10	+++	+++

* -, +, ++, +++ = No undesirable odors, light and dissipating readily after aeration, medium and persisting, strong and persisting, respectively.

Moisture Loss

As expected with wrapped fruits or vegetables, packaging broccoli florets in the three types of polyolefin films significantly reduced weight loss and not many differences were found in terms of type of film and storage temperatures.

A similar trend was observed after 8 and 12 days storage (Figures 3.4 and 3.5). After 21 days a significant interaction between temperature and film type was observed. Broccoli florets at 5°C as well as at 10°C in film PD-961EZ showed the lowest moisture loss value (about 1.2%), followed by florets packaged with PD-941 film and stored at 0°C (1.7%) (Figure 3.6). Finally, in the evaluation at 28 days, (treatments at 10°C were terminated) broccoli florets packaged with PD-961EZ and PD-900 had significantly the lowest weight loss values. No significant differences were found among the rest of the film type treatments (Figure 3.7). Compared with the vented control, all MAP treatments resulted in better moisture retention, but there was a trend for PD-961EZ film packages to retain moisture better than PD-941 and PD-900.

Additionally, for broccoli florets of the same temperature storage, those of film PD-900 showed slightly higher moisture loss than those of film PD-961EZ and PD-941. Sealing broccoli florets in polyethylene bags of the three film types significantly reduced weight loss compared with the controls at all three temperatures evaluated (0, 5 and 10°C). Weight loss from samples sealed in PD-941 PD-961EZ, PD- 900 and perforated treatments (control), was 2.7, 2.4, 4.0 and 12.0% respectively (Figure 3.5) after 12 days in storage at 10°C. Hence,

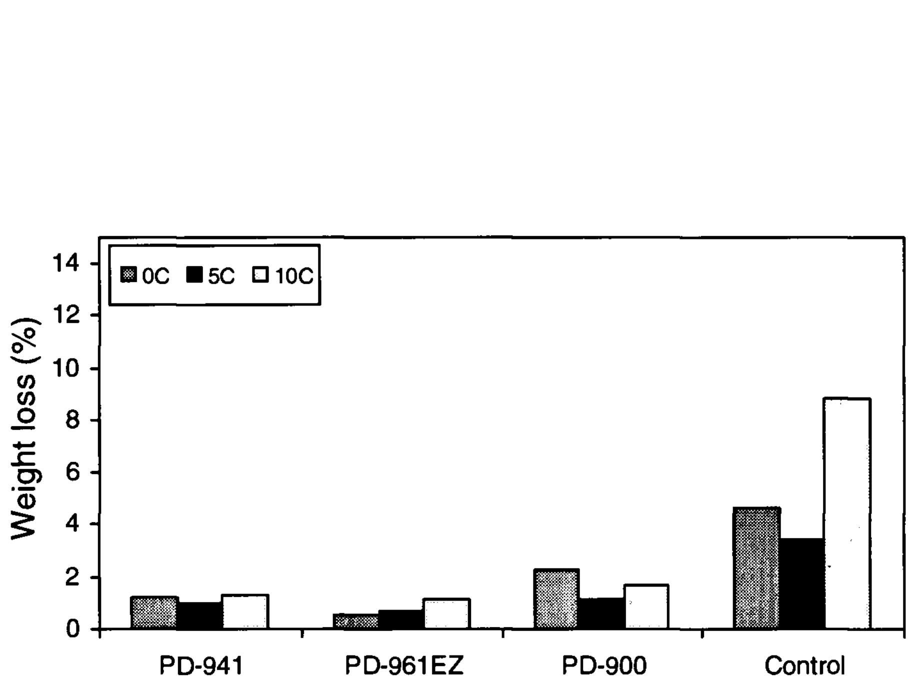


Figure 3.4. Effect of film type and storage temperature on weight loss of broccoli florets after 8 days.

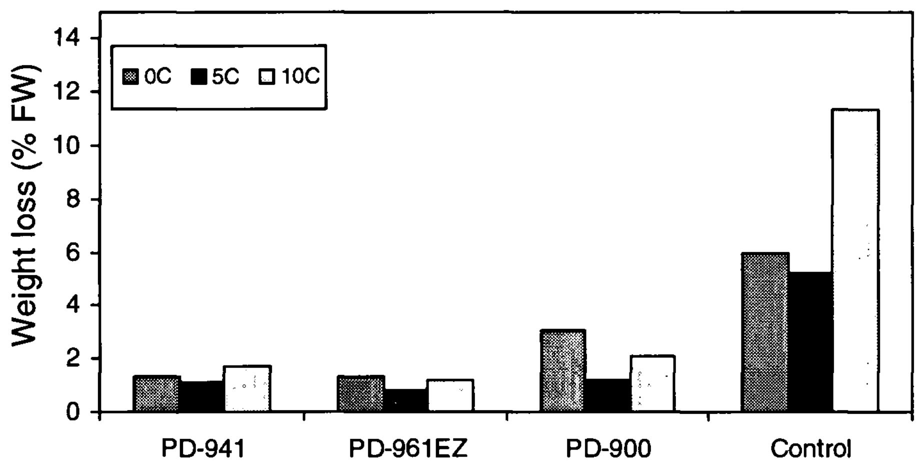


Figure 3.5. Effect of film type and storage temperature on weight loss of broccoli florets after 12 days.

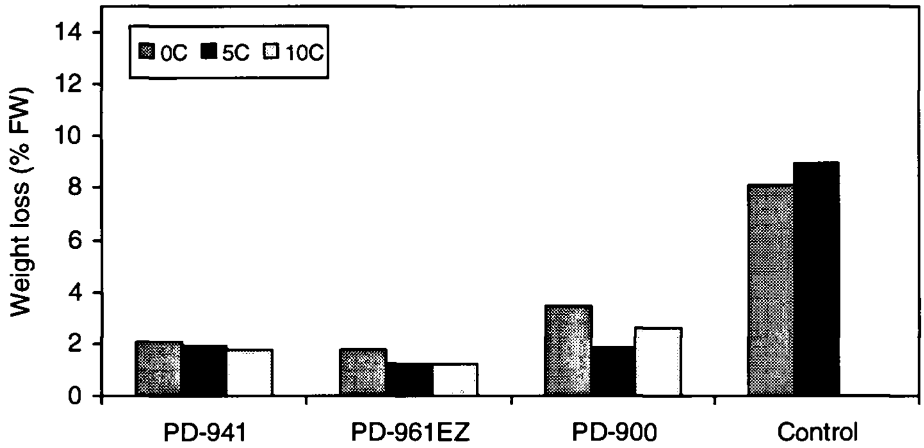


Figure 3.6. Effect of storage temperature and film type on weight loss of broccoli florets after 21 days.

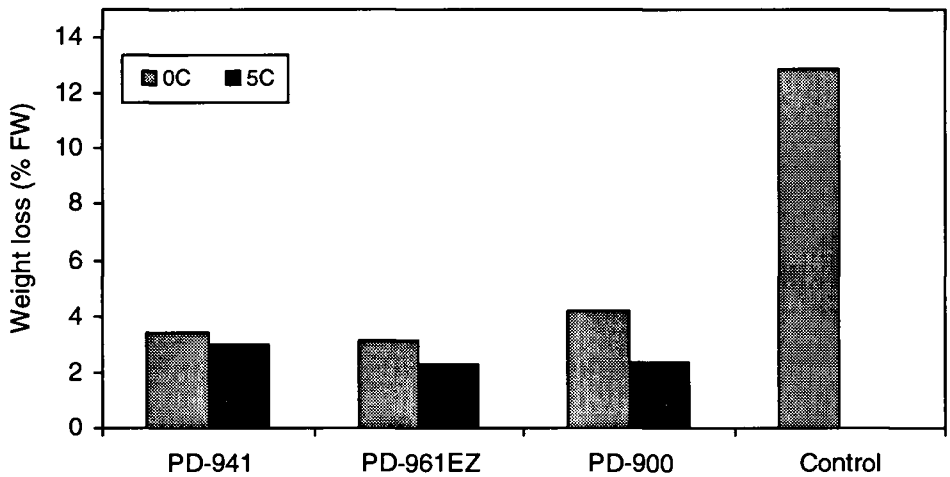


Figure 3.7. Effect of storage temperature and film type on weight loss of broccoli florets after 28 days.

reduction in weight loss obtained with seal-packaging over the control was 88% with film PD-941, 89% with film PD-961EZ, and 82% with film PD-900. Similar results were reported by Kawada and Albrigo (1979) for grapefruit. They reported a reduction in weight loss of grapefruit sealed in 0.015 mm polyvinylchloride and polyethylene films of 58 and 84%, respectively.

Sealed broccoli florets maintained their initial color and firmness longer while perforated treatments (control) shriveled and displayed signs of senescence (yellowing). The perforated control bagged broccoli showed a steady increase in weight loss through the storage period and with increasing temperature from 0° to 10°C (Figures 3.4, 3.5, 3.6, 3.7). However, with the sealed treatments, an increase in temperature was not accompanied by appreciably more weight loss. This was probably due to the moisture-saturated atmosphere maintained in the sealed packages. Plant tissues are in equilibrium with an atmosphere at the same temperature and a relative humidity (RH) of 99% t0 99.5% (Burton, 1982). Any reduction of water vapor pressure in the atmosphere below that in the tissue results in water loss. In entire organs, water in intercellular spaces is not directly exposed to the outside atmosphere. However, cutting a fruit or vegetable exposes interior tissues and drastically increases the water evaporation rate (Brecht, 1995). Although MAP minimized weight loss even at the highest temperature tested, it did not prevent yellowing and deterioration of broccoli at higher (non-optimal) temperatures where the produce became unmarketable after 21 days. This may be due to higher concentrations of ethylene accumulated at higher temperatures (Elkashif et al., 1983) or simply accelerated senescence.

Color Changes

Modified atmosphere packaging resulted in greater retention of green color in the broccoli tissue, as indicated by CIE hue angle values. Over the first 8 days of storage, there was a significant interaction between films and storage temperatures. Green color retention was greater in samples with PD-900 film at 5°C (hue value = 126.5); although it was not statistically different from samples at 10°C with the same film nor from the samples with films PD-941 and PD-961EZ at the three temperatures evaluated. Control packaged broccoli at 10°C showed the lowest hue value with a mean of 89. Results from CIE hue angle and chroma values (Tables 3.3, 3.4) indicate that green color was better retained in packaged broccoli. These results confirmed previous reports (Leberman et al., 1968; Makhlouf et al., 1989). More CO_2 accumulated in PD-900 (17%) than in PD-941 bags (5%) during 8 days at 10°C. Greater accumulation of CO_2 in the PD-900 bag treatment (Figures. 3.1, 3.2, 3.3) apparently prevented chlorosis by inhibiting ethylene action (Aharoni et al., 1985). However, increased off-odors were also associated with this high CO_2 concentration (about 15 %).

A significant temperature by film interaction was also found after 12 days storage. The best color retention was observed with PD-941 film at 5°C with a

Table 3.3. Effect of package film type and temperature on color change (Hue angle) of stored broccoli florets.

Film type	Temperature (°C)	Storage time (days) 8	12	21	28
		Hue angle ($\tan^{-1}$ b/a)			
PD-941	0	124.5 abc	124.8 bc	125.5 a	125.0 a
	5	126.0 a	126.8 a	124.3 ab	122.2 a
	10	125.0 ab	123.8 cd	114.8 e	--
PD-961EZ	0	121.7 bc	123.2 cd	124.0 ab	124.8 a
	5	125.3 ab	125.7 ab	123.0 abc	125.7 a
	10	125.0 ab	126.0 ab	121.5 bc	--
PD-900	0	126.0 a	125.6 ab	120.4 c	122.9 a
	5	126.5 a	124.6 bc	122.3 abc	122.7 a
	10	121.1 cd	119.2 e	116.7 de	--
Vented bags (control)	0	123.7 abc	122.9 d	120.0 cd	109.7 b
	5	117.7 d	101.1 f	87.2 f	--
	10	89.0 e	87.5 g	--	--

*Means within a column with the same letter are not different, FPLSD, $\alpha = .05$ (each value is the mean of 18 observations)
-- Treatments with florets opening or showing noticeable yellowing were discarded.

Table 3.4. Effect of package film type and temperature on color change (Chroma) of stored broccoli florets.

Film type	Temperature (°C)	Storage time (days) 8	12	21	28
		Chroma $(a^2 + b^2)^{1/2}$			
PD-941	0	18.7 bc	14.7 cd	15.1 c	13.9 b
	5	18.6 bc	14.7 cd	15.5 c	16.9 a
	10	17.9 bc	15.8 c	21.1 b	--
PD-961EZ	0	15.5 c	13.0 d	15.2 c	14.1 b
	5	17.5 bc	14.6 cd	14.7 c	13.5 b
	10	16.1 c	13.1 d	16.5 c	--
PD-900	0	14.9 c	15.3 c	14.3 c	13.7 b
	5	15.9 c	14.6 cd	14.8 c	14.6 ab
	10	14.0 c	13.0 d	14.2 c	--
Vented bags (control)	0	15.8 c	14.6 cd	14.3 c	14.8 ab
	5	21.3 b	30.4 a	30.0 a	--
	10	30..3 a	27.1 b	--	--

*Means within a column with the same letter are not different, FPLSD, $\alpha = .05$ (each value is the mean of 18 observations)

-- Treatments with florets opening or showing noticeable yellowing were discarded

hue value of 126.8. While the lowest color retention was again observed in the control at 10°C with a value of 87.5 and 30.4 for hue angle (Table 3.3) and chroma (Table 3.4). But broccoli packaged in PD-961EZ and PD-900 at 10°C showed the smallest value for chroma (13.0 and 13.0). Treatments with PD-961EZ indicate an increase in hue value as temperature increased; but PD-900 film treatments showed the opposite, hue value and chroma (Tables 3.3 and 3.4) did not change appreciably as storage temperature increased.

The color attributes of broccoli after 21 days of storage indicate that MAP had a great effect during the storage time (Figure 3.8). The hue angle and chroma (Tables 3.3, 3.4) values of broccoli florets in vented bags (control) at 5°C differed significantly from the other treatments. The control at 10°C was discarded before this 21 day evaluation time (florets turned yellow). The highest hue value (125.5) was observed for treatment with PD-941 at 0°C. However, the treatment with the same film type at 10°C showed the smallest hue angle and the highest chroma values respectively (114.8 and 21.1) after the control at 5°C. The smallest value for chroma was found for broccoli in film PD-900 at 10°C ; but this treatment showed a small hue value (116.7).

There were no differences in color between film type treatments after 28 days of storage. However, compared with the control at 0°C, florets bagged in PD-941, PD-961EZ and PD-900 films and stored at 0 and 5°C showed a better color retention. At this evaluation period, all treatments at 10°C and the control at 5°C were already discarded as unmarketable.

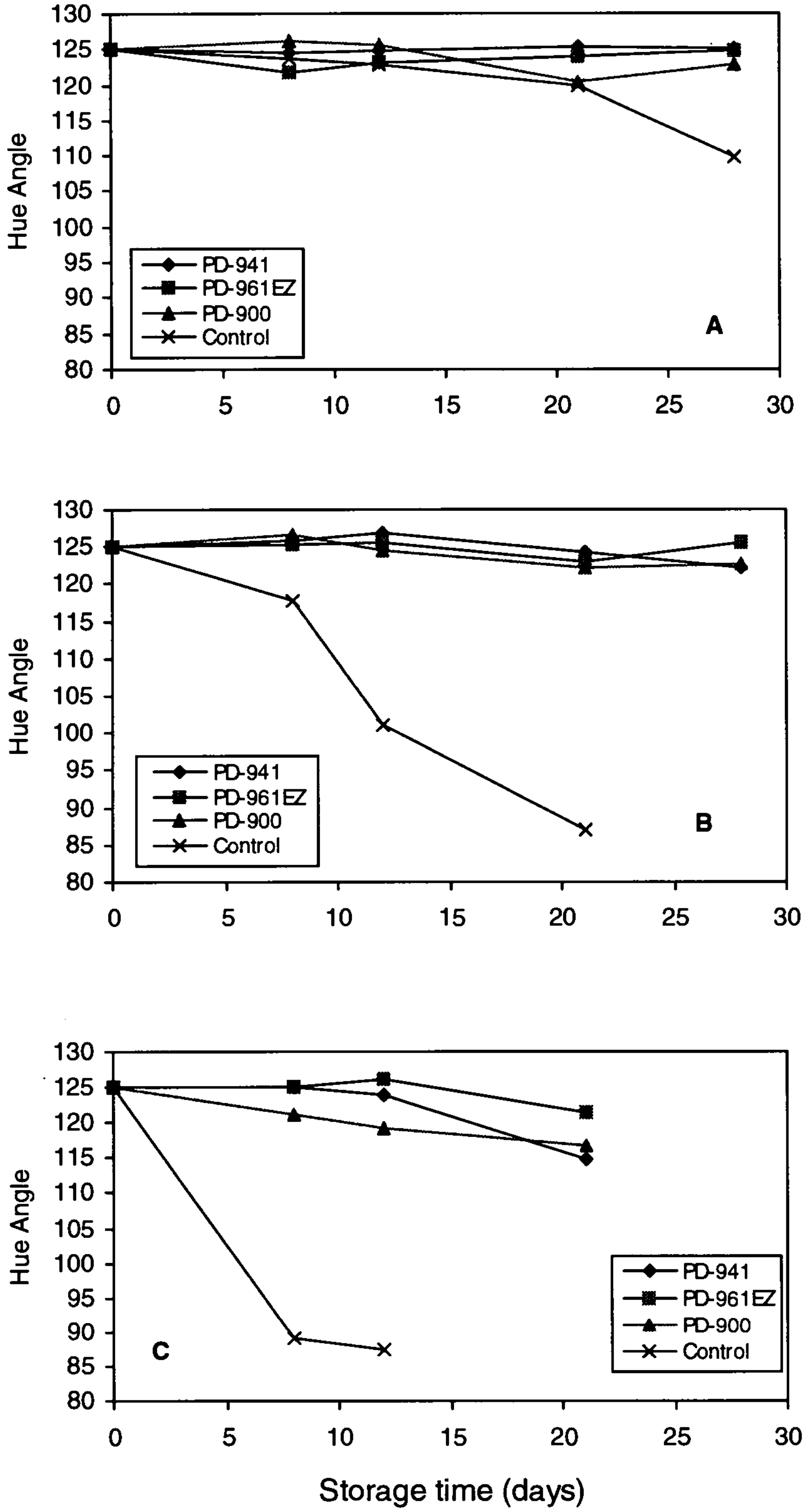


Figure 3.8. Color changes (Hue angle) of broccoli florets over time as affected by package film type and storage at 0°C (A), 5°C (B), and 10°C (C).

Color changes in broccoli at 0°C were not significantly affected by the film type (Figure 3.8). Only slight differences in color were observed during storage at this temperature. These results were confirmed by the visual observation that all broccoli florets maintained a similar color when stored under vented bags and MAP throughout the study up to 21 days of storage. More than 21 days were required for the yellowing process of broccoli of the cultivar tested to occur at 0° C. No consistent changes in hue angle was observed in the bagged florets stored at 0 and 5°C (Figure 3.8), which supported earlier findings (Shewfelt et al, 1983); Brenann and Shewfelt, 1983). More pronounced changes in hue angle were observed in the samples stored at 10°C or in the controls at the three storage temperatures primarily due to less negative a* readings (Appendices Tables A3.2 and A3.).

Broccoli florets bagged in vented bags (control) were the only treatments to show a hue angle lower than 90 due to switch from negative to positive a* readings (Appendix Table A3.2). Florets in control bags stored at 5° and 10°C showed the first evidence of yellowing between 8 and 12 days (Figure 3.8), as indicated by decreasing hue angle, and yellowed markedly between 12 and 21 days.

The change in chroma of broccoli florets was due to the change in sign of a* from - to + as well as decrease in b* (Appendices Tables A3.3 and A3.4)

<u>Chlorophyll Concentration</u>

The data given in Table 3.5 indicate that some chlorophyll degradation has occurred in all samples during the storage period. Temperature and film types greatly influenced the amount of chlorophyll detected in broccoli florets at

Table 3.5. Effect of package film and storage temperature on total chlorophyll concentration of broccoli florets.

Film type	Temperature (°C).	Storage time (days)			
		8	12	21	28
		Chlorophyll, µg/g fresh wt.			
PD-941	0	240.1 a	164.2 ab	122.1 ab	129.9 a*
	5	230.9 ab	200.4 a	121.4 ab	121.2 a
	10	180.6 abcd	152.3 abc	94.5 ab	--
PD-961EZ	0	185.2 abcd	149.6 abc	149.7 a	99.2 ab
	5	195.3 abcd	174.2 ab	157.6 a	77.8 b
	10	188.3 abcd	172.5 ab	90.6 ab	--
PD-900	0	169.3 bcd	128.5 bc	93.8 ab	58.9 b
	5	175.6 abcd	178.7 ab	83.7 b	68.6 b
	10	129.1 d	147.4 abc	93.6 ab	--
Vented bags (control)	0	217.1 abcd	212.3 a	145.6 a	103.5 ab
	5	157.3 cd	82.3 cd	42.6 c	--
	10	44.2 e	33.2 d	--	--

*Mean within a column followed by the same letter are not different, FPLSD, $\alpha = .05$ (each value is the mean of 3 observations)
-- Treatments with florets opening or showing noticeable yellowing were discarded

all time storage intervals. By 8 and 12 days after storage at 10°C, chlorophyll concentration remained 70% to 80% higher in the film packaged florets than in vented controls (Figure 3.9). After 8 days storage the highest chlorophyll concentration was detected in PD-941 packaged florets stored at 0° and 5°C. Chlorophyll amounts after 12 days were higher at 5°C than 0°C for all but the PD-961EZ treatment, which were similar.

Chlorophyll concentration of the florets stored in vented bags (control) remained near the initial level only in the samples stored at 0°C but decreased rapidly at 5° and 10°C storage temperatures after 8 days storage. After 21 days, control samples, stored at 10°C, were withdrawn from the experiment because of tissue yellowing and mold development. However, the chlorophyll concentration of packaged florets at 10°C was retained for 21 days. After 28 days, again chlorophyll content was significantly better in the florets packaged with PD-941 film when stored at 0° and 5°C. Loss of chlorophyll has been slowed in vegetables stored in elevated CO_2 environments (Lieberman and Hardenburg, 1954; Shewfelt et al., 1983). However, another study showed chlorophyll content was maintained in broccoli stored in high CO_2 (Makhlouf et al., 1989). The mode of action of high CO_2 levels on membrane stability is not known. High CO_2 storage of broccoli tissue may result in protection due to direct action of CO_2 on the membrane or by slowing senescence (Kader, 1986). Also, ascorbic acid is part of the anti-oxidant complex in chloroplasts and may maintain chlorophyll in the packaged broccoli tissue by reducing oxidation of chloroplast components (Thompson et al., 1987). Leberman et al. (1968) reported higher chlorophyll retention with increased CO_2 (from 5% to 20%) and reduced O_2. Groeschel et al. (1966) reported chlorophyll losses of green beans stored at 45°F to be higher in air than in CA containing 3% O_2 and 10% CO_2.

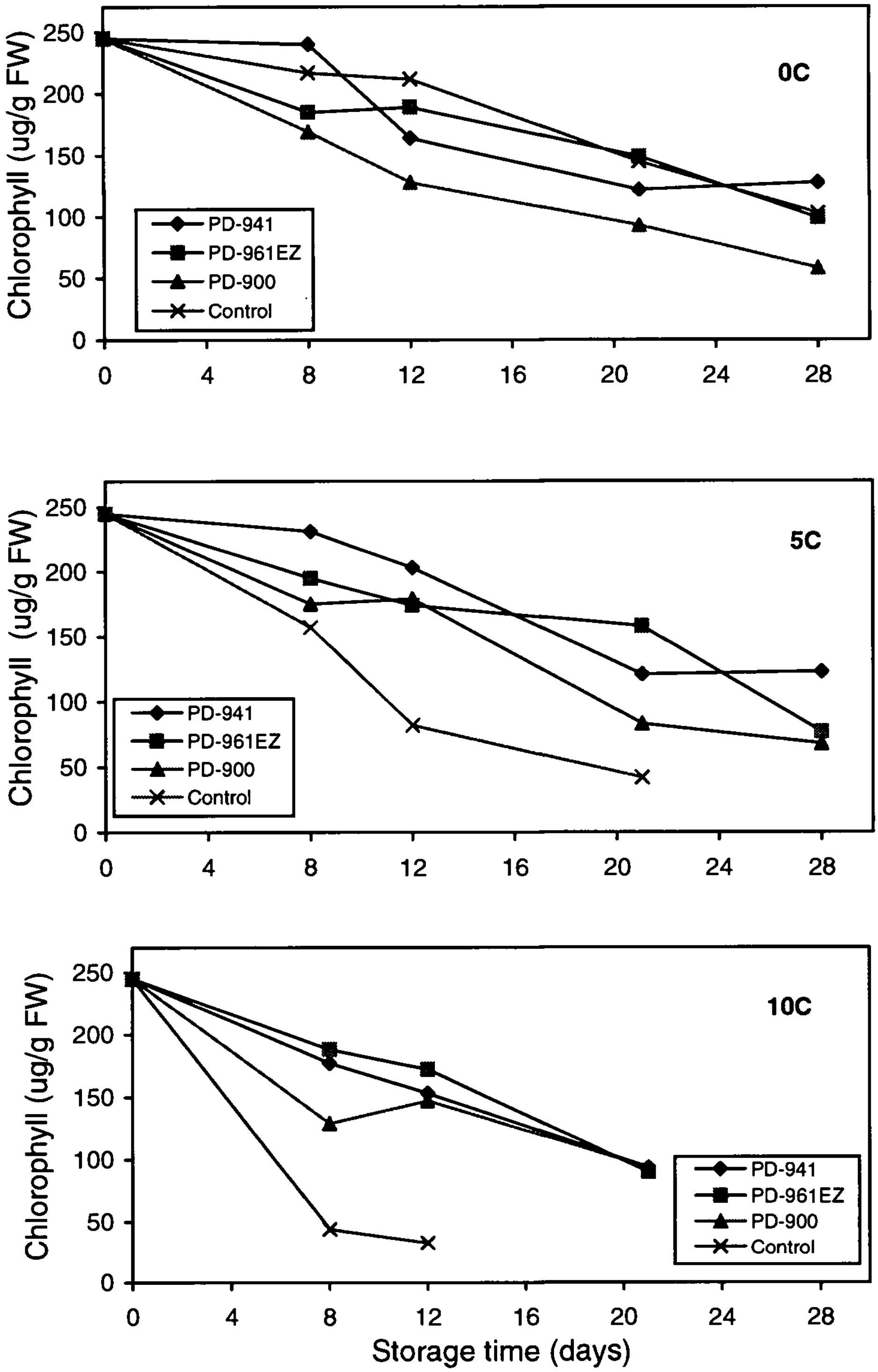


Figure 3.9. Effect of package films on chlorophyll concentration of broccoli florets after storage at 0°, 5°, and 10°C.

On average, the greatest chlorophyll retention over storage time was detected in broccoli florets packaged with PD-941 film at all three storage temperatures (Table 3.5).

At 0°C storage temperature, MAP treatments and vented bag controls resulted in reduced chlorophyll loss by 28 days (Figure 3.9). MAP treatments also reduced chlorophyll loss at 5°C. But a substantial loss in total chlorophyll over 21 days was found in the vented bags samples (42.6 μg/g FW) and these samples were discarded by this time. Further, significant differences among MAP treatments were found. Florets packaged in PD-941 film showed the highest chlorophyll content (129.9 μg/g FW) after 28 days storage. Previous studies have indicated that non-packaged broccoli heads resulted in greater senescence and chlorophyll loss but MAP resulted in better chlorophyll retention in broccoli florets during low temperature storage (Macklouf et al., 1989; Barth et al., 1993).

Ascorbic Acid (vitamin C)

The initial ascorbic acid content of the broccoli florets was 0.91 mg/g fresh tissue. MAP and storage temperature significantly affected the ascorbic acid content of broccoli florets at all time intervals evaluated. Analysis of ascorbic acid content 8 days after packaging indicated that at 0°C ascorbic acid losses were lower in broccoli florets stored in vented bags than in MAP, but at 10°C ascorbic acid degradation was faster in the controls than in MAP florets. Among the MAP treatments, broccoli florets in PD-900 film at 10°C contained the smallest amount of ascorbic acid (0.42 mg/g FW). There was no difference among the other MAP treatments; but they did differ significantly from the mean ascorbic acid of control samples stored at 5° and 10°C (Table 3.6). Burgheimer at al. (1967) also reported a distinct influence of storage temperature on ascorbic acid of spinach stored at 1° or 7°C in CA or air.

Also, there were statistically significant interactions between film types and storage temperatures after 12 and 21 days. Figures 3.10 and 3.11 indicate a decrease in ascorbic acid content as temperature increased from 0° to 10°C; but the amount of decrease was different for the different packaging film types and the vented bags (control). The greatest decrease in ascorbic acid content was found in broccoli florets with film PD-900 at 10°C. Additionally, after 21 days treatments with PD-900 film at the three temperatures studied showed a large decrease in ascorbic acid content. The highest ascorbic acid content was found in broccoli packaged with PD-941 film at 0°C (0.97 mg/g FW); although it was not significantly different from broccoli packaged with the same film at 5°C and with PD-961EZ film at 0 and 5°C, nor did it differ from the control at 0°C.

At the last evaluation time (28 days), ascorbic acid was significantly greater in broccoli with film PD-941 at 0°C while the lowest ascorbic acid content was found in broccoli with PD-900 at 0 and 5°C (Table 3.6). After 28 days, all the treatments at 10°C and the control at 5°C had to be terminated. Samples yellowed completely before this evaluation time. As the CO_2 level in the MAP increased with film PD-900, ascorbic acid losses in broccoli florets increased.

While ascorbic acid in packaged broccoli florets with PD-941 and PD-961EZ films stored at 5°C dropped about 10% from initial values in the first 12 days, losses were minimal throughout the rest of the storage period (Table 3.6). In contrast the degradation of ascorbic acid in vented bags samples (at the same temperature) showed a steady decline over time and decreased 66% by 21 days. Unfavorable storage conditions promote oxidation of ascorbic acid, which is very susceptible to degradation (Liao and Seib, 1987). Postharvest storage of

Table 3.6. Effect of package film type and storage temperature on total ascorbic acid concentration of broccoli florets.

Film type	Temperature (°C).	Storage time (days)			
		8	12	21	28
		Ascorbic acid, mg/g fresh wt.[z]			
PD-941	0	1.00 a	1.01 ab	0.97 a	1.02 a*
	5	0.98 a	0.92 bc	0.91 a	0.84 b
	10	1.08 a	0.84 cd	0.69 b	--
PD-961EZ	0	0.97 a	1.07 a	0.96 a	0.86 b
	5	1.12 a	1.00 ab	0.83 ab	0.87 b
	10	1.00 a	0.95 abc	0.38 c	--
PD-900	0	0.95 a	0.95 abc	0.31 c	0.19 c
	5	1.15 a	0.70 d	0.25 cd	0.16 c
	10	0.43 c	0.33 f	0.14 d	--
Vented bags (control)	0	1.04 a	0.88 bc	0.85a	0.78 b
	5	0.69 b	0.51 e	0.37 c	--
	10	0.27 c	0.37 ef	--	--

*Mean within a column followed by the same letter are not significant, FLSD, α=0.05 (each value is the mean of 3 observations)

[z]The initial value was 1.12 µ/g fresh wt.

-- Treatments with florets opening or showing noticeable yellowing were discarded

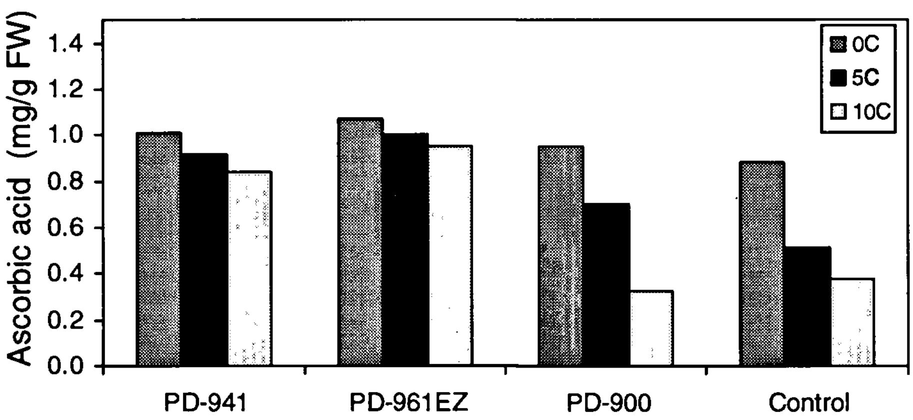


Figure 3.10. Effect of film type and temperature on ascorbic acid concentration of broccoli florets after 12 days storage

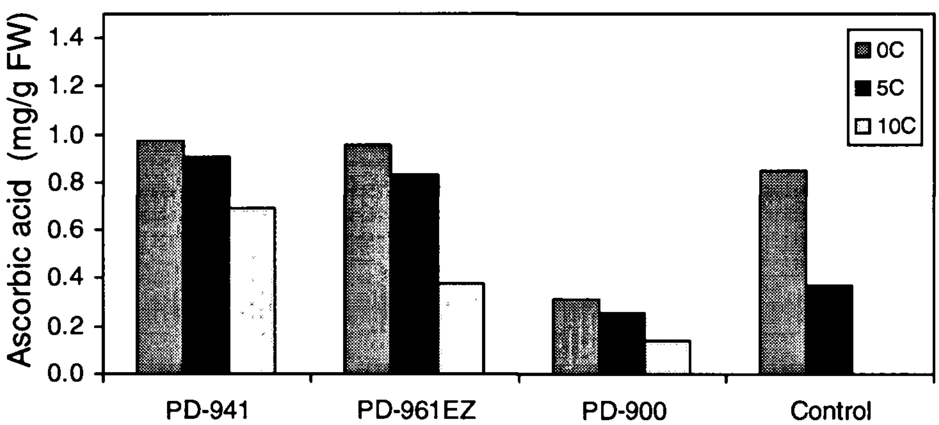


Figure 3.11. Effect of film type and temperature on ascorbic acid concentration of broccoli florets after 21 days storage

broccoli in elevated CO_2 (20%) levels has been shown to result in better maintenance of ascorbic acid compared to broccoli stored in air (Wang, 1979). McGill et al (1966), reported that ascorbic acid losses in spinach kept in 4% O_2 + 9% CO_2 were only about 50% of the losses of spinach stored in air. Also, greater humidity inside the packages possibly served to better preserve vitamin C content in packaged broccoli florets (Ezell and Wilcox, 1959).

Platenius and Jones (1944) reported the influence of decreased O_2 levels on the ascorbic acid content of various vegetables. The lower the O_2 level during storage (1.2-2.1%), the lower were the losses of ascorbic acid in green beans, spinach, kale, broccoli and Brussels sprouts, When a high CO_2 concentration (5.3-19.5%) was added, the results were different. In broccoli and Brussels sprouts, the presence of CO_2 further inhibited losses of ascorbic acid regardless of the temperature at which the experiments were carried out (20°C or 10°C). In contrast, ascorbic acid destruction was accelerated in spinach and asparagus at 20° to 24°C but retarded at 10°C when CO_2 was added to a low O_2 atmosphere.

Conclusions

1. Broccoli florets in perforated bags (controls) showed a typical strong response to low temperatures, the 0°C stored florets survived with fair color for 28 days, even though they had lost about 12% moisture and appeared wilted. At 5°C control florets had fair appearance for 21 days and at 10°C for only 8 days.
2. There were strong temperature effects on time to reach O_2 and CO_2 equilibrium within the MAP film. At 10°C, equilibrium was reached between 12 and 24 hours, whereas at 5° and 0°C, equilibrium times were 36 hours and 12 hours, respectively.
3. Cryovac film PD-941 had superior permeation characteristics which maintained O_2 at 2, 4 and 6% at 10°, 5°, and 0°C, respectively which CO_2 was 6, 5, and 4%. PD-900 and PD-961EZ were less permeable and had higher CO_2 accumulation at higher temperatures.
4. Temperature also had a strong effect on both control and MAP broccoli floret ascorbic acid and green color retention. 0°C strongly maintained ascorbic acid and chlorophyll, except for PD-900 film MAP, which provoked substantial losses of both ascorbic acid and chlorophyll. PD-961EZ was almost as affective as PD-941 for ascorbic acid retention, but PD-961EZ and PD-900 both caused off-odors.
5. Off-odors were noted to begin after only 8 days even at 0°C storage for broccoli in PD-900 film, and became progressively worse as storage time and temperature increased.

Broccoli in PD-961 film had slight off-odors only in the 10°C storage, but it became noticeable at 0°, 5°, and 10°C after 12 days.

Broccoli in PD-941 film developed no off-odors during 28 days, and this occurred at all three storage temperature (0°, 5°, 10°C).

Chapter 4

Storage Characteristics and Quality of Seven Broccoli Cultivars.

Abstract

This study assessed the influence of cultivar under MAP on quality maintenance of broccoli (*Brassica oleracea* L Group Italica) florets during storage. Florets of seven broccoli cultivars were packaged using Cryovac PD-941 polyolefin film and evaluated initially at harvest and simulated marketing temperatures after 8, and 21 days of storage at 4°C plus additional 3 and 5 days at 15°C. Gas concentrations within packages monitored by gas chromatography (measured and reported in an earlier experiment) were about 5% CO_2 and 2% O_2. Quality evaluations indicated that the ascorbic acid content varied between 0.84 and 1.15 mg/g fresh wt initially and between 0.83 and 1.01 mg/g fresh wt after 21 days. Cultivars with initial high levels of ascorbic acid did not necessarily remain high during storage. There were significant differences in chlorophyll concentration and color measurements among the seven cultivars after storage. Chroma values corresponded more closely to chlorophyll concentration than hue angle. MAP restricted weight loss and no differences among cultivars were found. However the rate of water loss was higher after exposure to 15°C.

Introduction

Broccoli is an important vegetable in the American diet and per capita consumption is increasing. The high levels of nutrients may account to some degree for the increasing popularity of broccoli in this health conscious era. The major nutrients contributed by broccoli include ascorbic acid, vitamin A, riboflavin, thiamine, calcium, phosphorus and iron. The popularity of broccoli continues to increase by 8-9% annually, particularly as a fresh vegetable but also as the frozen product. The annual per capita consumption in the United States has risen from 0.64 kg fresh equivalent in 1968 to more than 1.3 kg in 1989 (Nonnecke, 1989).

Broccoli has been shown to be responsive to modified atmosphere and low temperature. The critical level of O_2 or CO_2 in modified atmosphere for storage of broccoli may be influenced by cultivar, cultural methods and season (Lougheed, 1986). Research on other vegetables such as crisphead lettuce has shown that the effects of modified atmosphere is highly temperature and cultivar dependent (Brecht et al., 1973). The quality of carrots stored under controlled or modified atmosphere usually depends on cultivars and cultural conditions (Isenberg, 1979).

The increasing importance of broccoli to the fresh market and frozen food industry has led to the development of specialized growing and marketing systems. Breeding has to accommodate the demands of specialist growers, producers and market requirements, and new cultivars have been developed to suit different production systems and to accommodate changing specifications. The majority of presently available cultivars are F_1 hybrids with greater uniformity. Current efforts of breeding programs focus on improved F_1 hybrids for earlier maturity, excellent blue-green color, deeply branched heads, desirable bud type and resistance to downy mildew (Baggett and Kean, 1985; Nonnecke, 1989).

Fruit and vegetable cultivars are routinely screened and selected for specific functional properties. Although the trade and the consumer have provided incentives for lightly processed fruit and vegetables, no cultivar has been deliberately developed with processing and distribution in mind. To maintain a focus on providing a consumer product, the definition of minimal processing is extended to processing that, when combined with the optimization of all other steps of production, preparation, and distribution of produce to the consumer, will yield products of extended shelf life with fresh-like quality. This process starts with the selection of the most suitable variety (Romig, 1995).

Within a specific crop, the diverse cultivars and genotypes vary in physical attributes, color, flavor, firmness, pest resistance, response to stress, etc. (Romig, 1995). Lightly processed fruits and vegetables are always more perishable than their counterparts; therefore, selecting cultivars with enhanced shelf life characteristics is important (Brecht, 1995). The objective of this study was to evaluate the storage characteristics and quality of florets of seven broccoli cultivars after storage in sealed polyolefin bags of a single type.

Materials and Methods

Broccoli cultivars ('Claudia', 'Caravel', 'Emerald City', 'Barbados', 'Patriot', 'ATX 91-307' and 'Arcadia') were obtained from a cultivar trial at the Oregon State University Vegetable Research Farm located near Corvallis, Oregon. Characteristics of the cultivars, from the unpublished observations of Baggett (1994) were as follows: 'Emerald City' has a head diameter of ≈ 20 cm., medium florets, fair head stem color, fair exsertion, yellow undercolor, yellow florets and a general score of 3.0 (1-5 scale, 5 = best). 'Claudia' has a head diameter of ≈25 cm., fine even florets, poor head stem color, poor exsertion, big solid heads, good yield and a general score of 3.0. 'Barbados' has a head diameter of ≈25 cm, medium florets, fair head stem color, fair exsertion, some variation in floret color from blue-green to yellow-green, and a general score of 3.0. 'Arcadia' has a head diameter of 15-20 cm, fine florets, fair head stem color, fair exsertion, yellow rosettes, yellow undercolor, and a general score of 3.0. 'Caravel' had a head diameter of ≈18-20 cm, fine even florets, fair head stem color, fair exsertion, segmented dome head shape and a general score of 3.5. 'ATX 91-307' has a head diameter of ≈15-20 cm, fine slight uneven florets, good head stem color, fair exsertion, good blue-green color, compact dome, and a general score of 3.5. Finally, 'Patriot' has a head diameter of ≈15-20 cm, fine florets, poor head stem color, poor exsertion, very compact dome, and a general score of 3.0.

Broccoli floret samples were prepared in the same way as indicated in Chapter 2. Twenty samples (200g each) from each cultivar were obtained randomly. The samples were packaged in low density polyolefin film designated as PD-941 by the manufacturer CRYOVAC (75 gauge; oxygen permeability of 16,544 cc/m^2/24 hrs; CO2 permeability 31,000 cc/m^2/24 hrs, water vapor transmission rate of 5.0 g/100 in^2/24 hrs.) and sealed with a heat impulse sealer. The PD-941 film was selected as near optimum based on the results from the earlier study (Chapter 3). Four replicates of each cultivar were used for quality evaluation the day of harvest. All other samples (16 for each cultivar) were stored at 4°C for 21 days, then 8 samples of each cultivar were transferred to a room at 15°C and stored for another 5 days. For each cultivar, four samples were removed for quality evaluation at days 8 and 21 of storage at 4°C and after 3 and 5 more days of storage at 15°C. Cultivar samples were assessed for weight loss, color changes, chlorophyll and ascorbic acid concentrations. Samples were weighed and the percentage of weight loss was determined. Chemical analyses for chlorophyll determinations were carried out using a spectrophotometric assay (Anderson and Boardman 1964) with a Bausch & Lomb 2000 UV-visible spectrophotometer, as adapted by Barth et al.,(1992). Ascorbic acid was determined by the titrimetric assay described by Pelletier (1985). The methodology was the same as described previously in Chapter 2. Colorimetric measurements were recorded using a Minolta Chroma Meter series CR-200 after calibration with a white standard tile (L = 97.78, a = -0.69, b = 2.31). Tristimulus L*, a*, and b* values, were measured at the center of each floret on 6 florets for each sample. The L*a*b* readings were used to compute the hue angle and chroma (Gnanasekharan et al., 1992). Since the tristimulus a* value was less than 0 in green vegetables, the hue angle was computed by $180° + \tan^{-1} b/a$ (Minolta, 1988). The L*a*b* color space was selected based on its successful use in quantifying color changes in green vegetables (Shewfelt et al., 1988; Gnanasekharan et al., 1992).

After each evaluation time, data were collected and statistically analyzed for analysis of variance (ANOVA) and Fisher Protected LSD tests (STATGRAPHICS Software).

Results and Discussion

Moisture Loss

As shown previously with film packaged fresh produce, weight loss was very low in all samples and only small differences were found among the cultivars tested. There was no a large increase in moisture loss even 5 days after transfer of the samples to 15°C storage room. The highest value (only 2.05%) was found for 'Caravel' (Figure 4.1).

While statistical differences in moisture loss were detected among cultivars after 8 days storage where the values ranged from 0.87% to 1.12% but these small differences have little practical significance. By 21 days storage (Figure 4.1), a statistical differences in moisture loss were also observed among the seven cultivars. Cultivars 'Caravel' and 'ATX' showed the lowest moisture loss (about 1.06%). The greatest amount of moisture loss was found in 'Patriot' (1.25%) followed by 'Barbados' (1.16%). Although the differences in water loss rates were statistically significant at the different times intervals, they were so low that they would be minimally important in practical applications. Cohen and Hicks (1985) reported that broccoli heads exhibited significant differences in weight loss between cultivars during a simulated roadside market display study, which had no protective packaging.

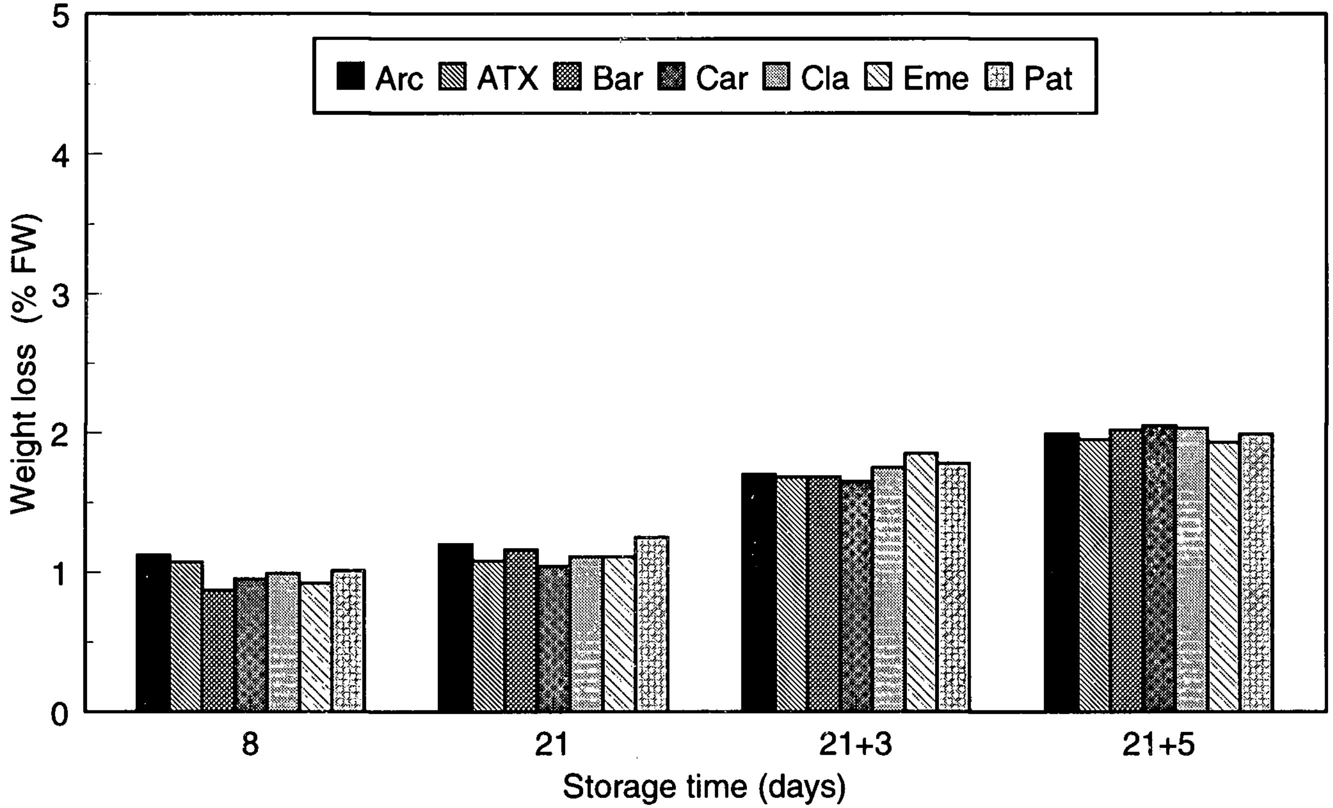


Figure 4.1. Weight loss of seven broccoli (florets) cultivars after storage at 4°C. 21+3: 21 days at 4°C + 3 days at 15°C; 21+5: 21 days at 4°C + 5 days at 15°C.

Weight loss remained low even after 21 days storage at 4°C plus 3 and 5 days at 15°C. No differences were found among cultivars (Appendix Table A4.1) after 3 and 5 days exposure to 15°C where the values ranged from 1.65% to 1.85% and from 1.93% to 2.05%. In this experiment using the same MAP, weight loss was similar in all cultivars. However, had the cultivars been evaluated on perforated or nonpackaged conditions, larger differences might have been observed.

Moisture loss during storage is very dependent on relative humidity and heat of respiration. For most products, the greatest benefit of packaging is greater relative humidity around the product, leading to reduced water loss. Package environments generally have a relative humidity that is very close to saturation, and this probably more than CO_2 and O_2 modification, helps preserve food quality (Beaudry and Lakakul, 1994). Ben-Yehoshua (1985) reported that using a Polyethylene shrink wrap to produce a water-saturated atmosphere and decrease transpirational water loss has been an effective method of increasing postharvest life of some tree-fruits, particularly citrus. Significant cultivar differences in postharvest water loss of nine pepper cultivars stored for 14 days at 8°, 14°, or 20°C has been reported. But water loss rates for packaged fruits were very low (Lownds et al., 1994).

Ascorbic Acid Concentration

The results of the ascorbic acid analyses of seven broccoli cultivars initially and after 8 and 21 days under the same storage conditions are shown in Appendix Table A4.2 and figure 4.2. After 8 days storage, concentration of ascorbic acid ranged from 0.92 to 1.13 mg/g fresh weight (FW) in the seven cultivars of broccoli. 'ATX' and 'Barbados' were statistically higher in ascorbic acid concentration than the other five cultivars. The same trend was observed after 21 days storage, however, by this time, the 'Claudia' cultivar was significantly lower in ascorbic acid (0.83 mg/g FW) than all the other cultivars. Ascorbic acid concentration ranged from 0.83 to 1.01 mg/g FW.

Moisture loss and wilting affect the nutritional value of vegetables, especially the vitamin content (Weichmann, 1986). Ascorbic acid loss in broccoli florets appeared to follow the loss of weight (mainly considered moisture loss) during storage. The MAP apparently greatly reduced the weight loss in all cultivars tested and thus the average losses of ascorbic acid were minimal during storage at 4°C. Low temperature and the maintenance of the turgidity of the tissues delay its degradation in fresh vegetables (Ezell and Wilcox, 1959).

Initial high levels of ascorbic acid in some of the cultivars examined (e .g 'Claudia' and 'Caravel' with a concentration of 1.15 and 1.09 mg/g FW respectively) did not necessarily remain high during storage (Appendix Table A4.2). Storage losses did not exceed 10 percent in any case. 'Caravel' and 'Claudia' contained more ascorbic acid initially but lost it at a somewhat faster rate in storage (Figure 4.2).

All broccoli cultivars showed good retention of ascorbic acid after storage at 4°C for 21 days; however, after exposure to 15°C for 3 days there was a decrease in ascorbic acid concentration in the seven cultivars. 'Barbados'

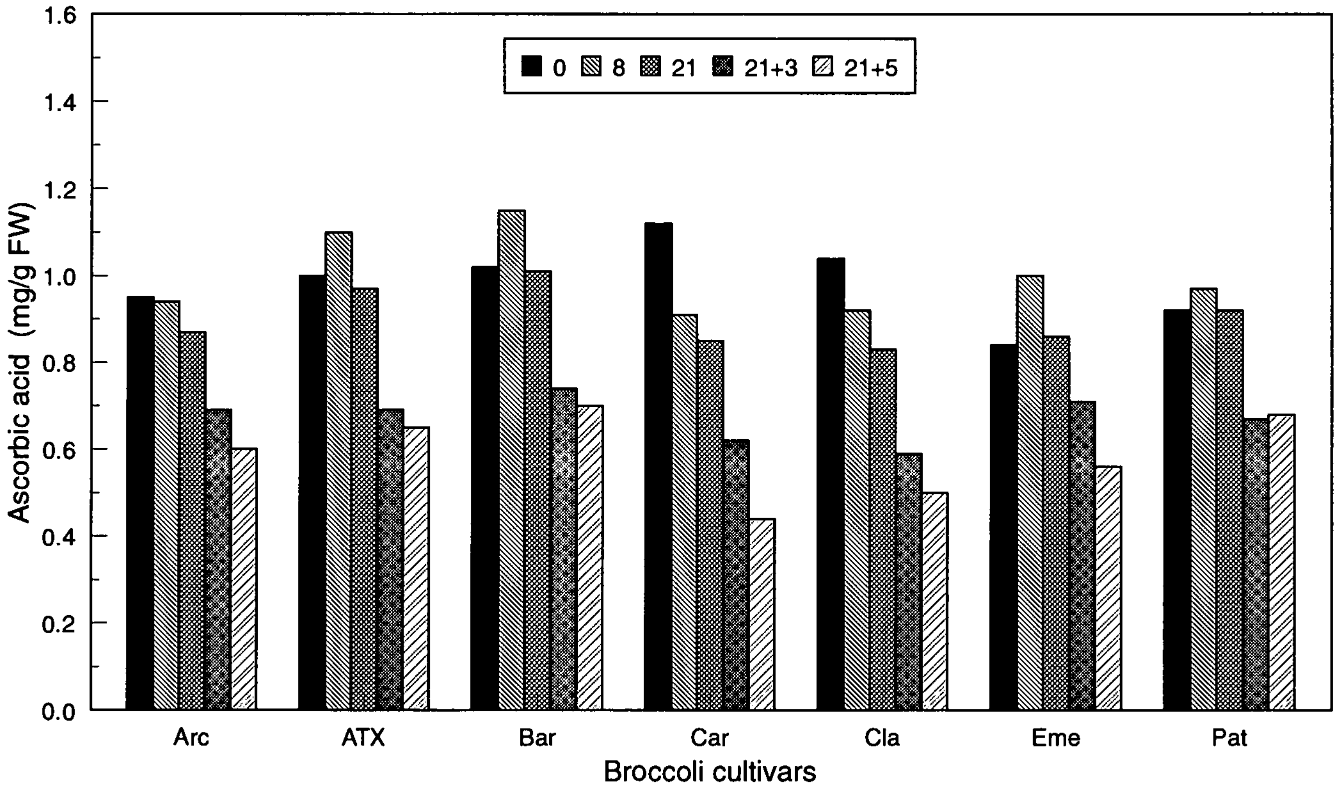


Figure 4.2. Storage effects on florets ascorbic acid concentration of seven broccoli cultivars.

retained significantly higher ascorbic acid concentration (0.74 mg/g FW) than any of the others. Also after exposure to 15°C for 5 days 'Barbados' was significantly higher in ascorbic acid than the other six cultivars followed by 'ATX' and again the lowest ascorbic acid concentration (0.44 mg/g FW) was found in 'Caravel'.

Significant differences were found among all cultivars, and ascorbic acid was lower in 'Claudia' and 'Caravel' than in the other cultivars after storage. Similarly, Albrecht et al. (1990) reported that the ascorbic acid concentration varied significantly among six cultivars of broccoli initially and after 3 weeks storage in air at 2°C. They reported that retention of ascorbic acid ranged from 56-98% in six cultivars of broccoli. This was a much greater range of loss than experienced in this study involving MAP storage. Watada et al.(1976) reported the influence of cultivar on ascorbic acid concentration in tomatoes. Cultivar differences were greater in ripened compared to mature green fruit. Cultivar variations in cabbage have also been reported. Cabbage ascorbic acid concentration varied between 0.480 and 1.809 milligrams per gram of fresh weight among 31 cultivars (Burrel et al., 1940).

Chlorophyll Concentration

The initial chlorophyll concentration of broccoli cultivars was variable, ranging from 123 to 222 µg/g FW. 'Claudia' and 'ATX' cultivars contained significantly more chlorophyll in the fresh state (initial evaluation) than the other 5 cultivars (Appendix Table A4.3). However, 8 days after storage, 'Claudia' lost chlorophyll at a somewhat faster rate while 'ATX' retained significantly greater chlorophyll concentration than the others. Lowest chlorophyll was found in the Patriot cultivar (Figure 4.3). By day 8, The total chlorophyll concentration of

Claudia broccoli had decreased more than 30%. Samples of cultivars ATX and Patriot had lost 14 and 12% respectively. However, 'Emerald' had only a small decrease below initial chlorophyll level by day 8.

During 21 days of 4°C storage, there was a small further decrease in chlorophyll concentration for most of the cultivars compared with the decrease during the first 8 days (Figure 4.4). The greatest chlorophyll concentration was found in 'ATX' with 195.4 μg/g FW, which was significantly different from the others. There were no significant differences between the other 6 cultivars. However, during 21 days of 4°C storage, 'Emerald' and 'Patriot' showed the lowest chlorophyll loss (only ≈7 and 5%) compared to their initial values (Figure 4.4).

After exposure of the packaged florets to 15°C for 3 days, the cultivar ATX still showed the greatest chlorophyll concentration (193.5 μg/g FW). The lowest chlorophyll concentration was found in the cultivar Claudia which differed significantly only from 'Emerald' cultivar by day 3 after exposure to 15°C.

Rapid chlorophyll degradation apparently occurred between 3 and 5 days of exposure to 15°C. A substantial reduction in the total chlorophyll over 21 days at 4°C plus 5 days at 15°C was found for all samples, ranging from 41.8 to 127.1 μg/g FW by day 5 (Figure 4.5). Again, the cultivar ATX had the most chlorophyll although it did not differ significantly from cultivar Barbados. The greatest loss of chlorophyll (≈ 40%) between 3 and 5 days of exposure to 15°C was found in 'Emerald' (Figure 4.5). Eheart,(1969) reported differences in chlorophyll concentration among three cultivars of broccoli (Waltham-29, Prime, and E-4200).

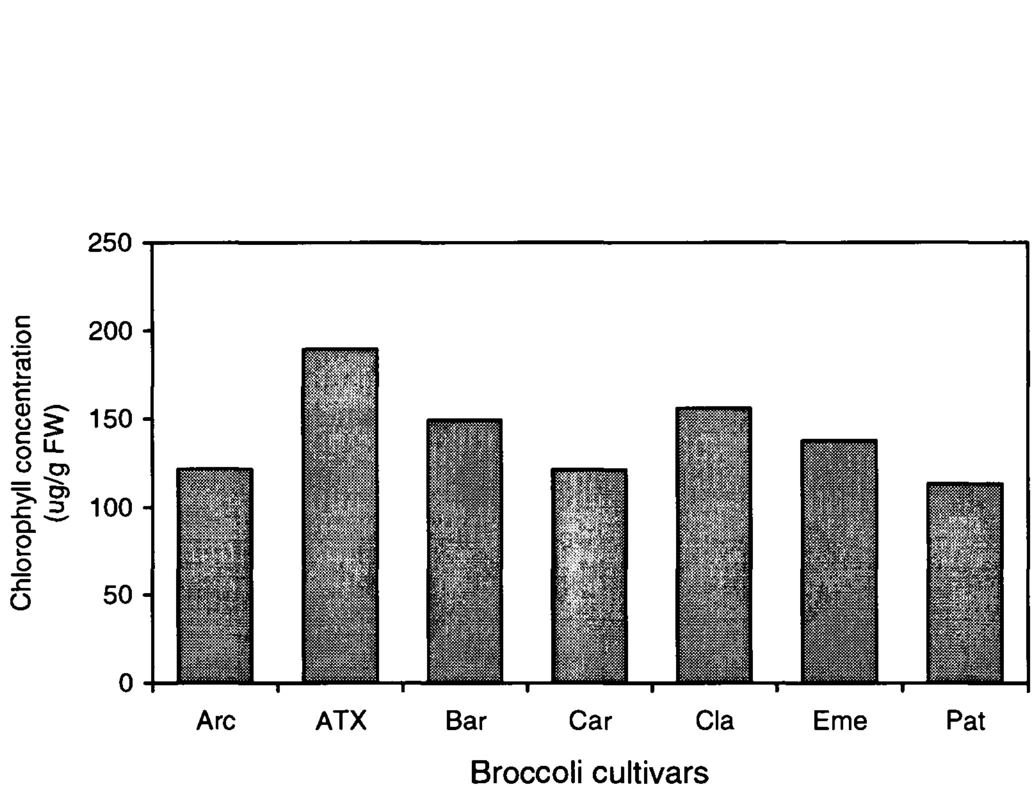


Figure 4.3. Florets chlorophyll concentration of seven broccoli cultivars after 8 days storage at 4°C.

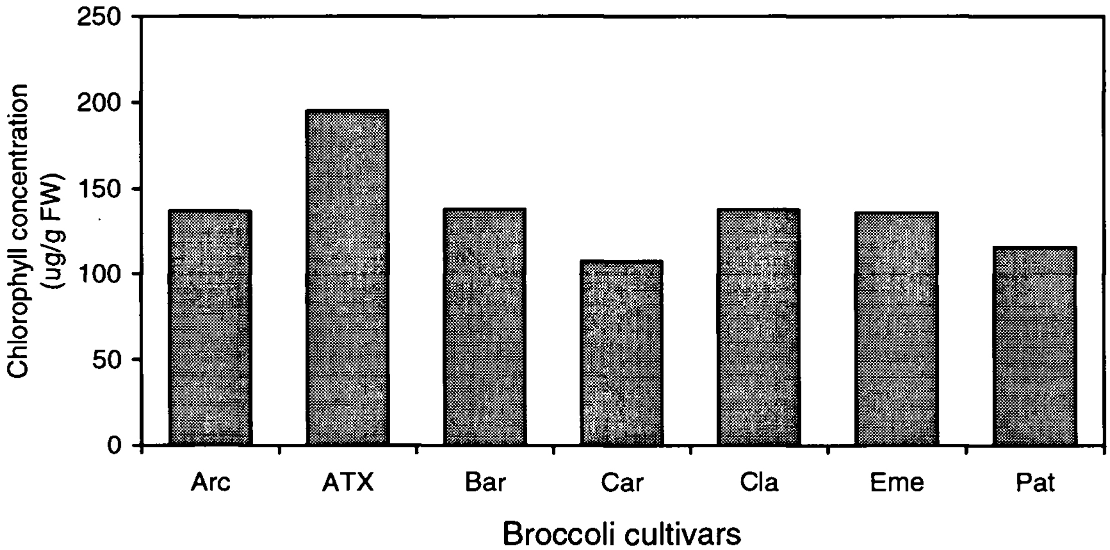


Figure 4.4. Floret chlorophyll concentration of seven broccoli cultivars after 21 days storage at 4°C.

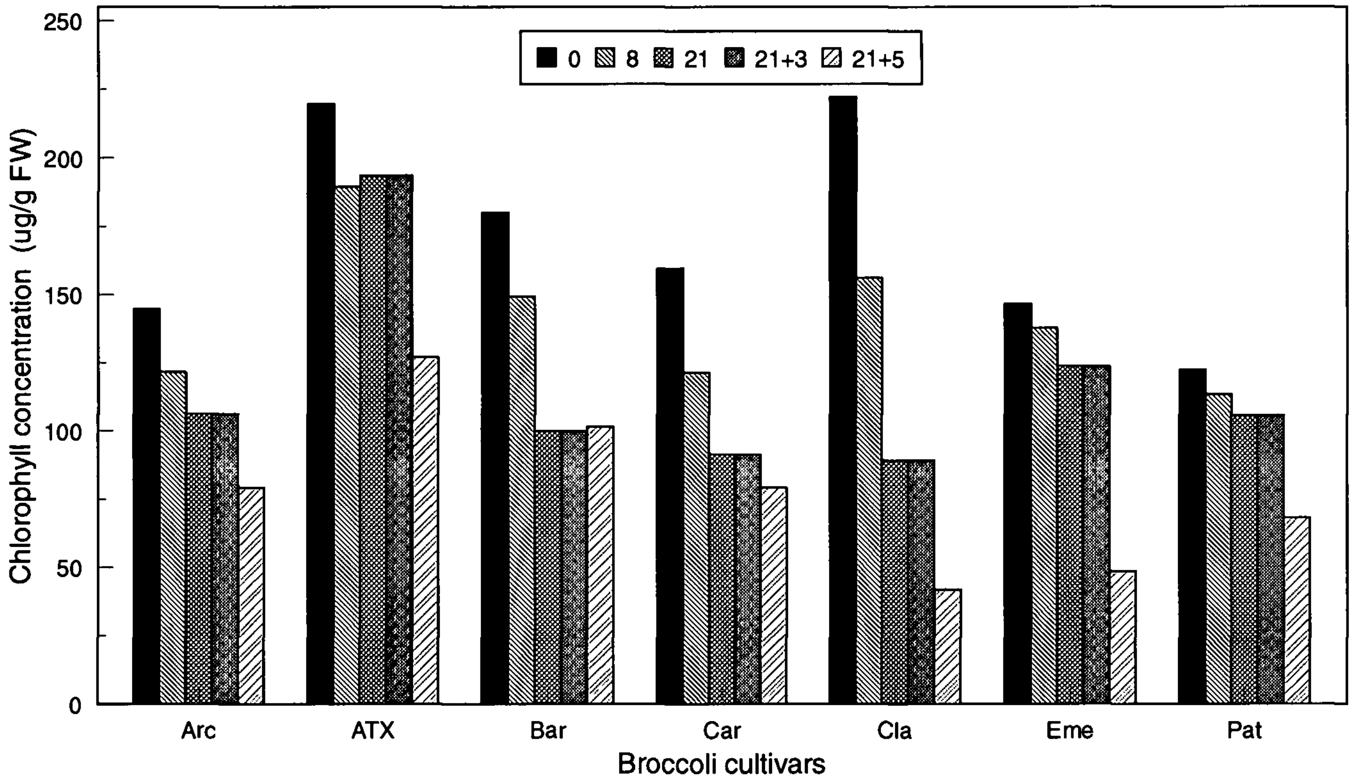


Figure 4.5. Storage effects on florets chlorophyll concentration of seven broccoli cultivars.

The desirable green color of broccoli is due to chlorophyll. These results suggest that the rate of chlorophyll breakdown of broccoli florets is significantly influenced by cultivar. The temperature during the three week storage period (4° C) was low enough to retard chlorophyll degradation in most of the cultivars, yet additional short periods of storage (5 days) at high temperatures (15°C) led to significant degradation of chlorophyll.

Extensive variability in chlorophyll concentration in individual samples within a cultivar was observed which could be attributed to the superposition of environmental factors (i. e., light exposure and temperature) on ripeness differences.

Color Analysis

Differences in instrumental color derived functions hue angle ($\tan^{-1}$ b/a) and chroma $(a^2 + b^2)^{1/2}$ for the florets of the different broccoli cultivars are shown in Tables 4.1 and 4.2. Color values from initial evaluation ranged from 124.1 to 129.3 and from 12.7 to 20.1 for hue angle and chroma respectively.

After 8 days storage, the highest hue angle value was detected in samples from the cultivar Barbados (130.1) although that was significantly different only from cultivar Arcadia (Table 4.1). With respect to chroma, the 'Patriot' and 'Arcadia' with the lower hue angle values, showed the highest values for chroma after 8 days storage (Table 4.2). Furthermore, only these two cultivars differed significantly from the others. The lowest chroma value was detected in samples from 'ATX' after 8 days storage.

Table 4.1. Color change (Hue angle) of broccoli cultivars (florets) packaged in Cryovac PD-941 film after storage at 4°C plus 5 days at 15°C.

	Hue angle (tan^{-1} b/a)				
Cultivar	0 d[x]	8 d at 4°C	21 d at 4°C	21 d at 4°C + 3 d at 15°C	21 d at 4°C + 5 d at 15°C
Arcadia	123.5	125.6 b	125.7 de	126.1 b	122.2 a
ATX 91-307	127.5	129.4 a	132.0 a	126.5 b	120.7 a
Barbados	126.2	130.1 a	129.1 b	127.4 b	121.1 a
Caravel	124.1	127.8 ab	127.0 cd	124.4 b	105.2 bc
Claudia	129.3	129.1 a	125.2 e	118.2 a	99.3 c
Emerald City	126.4	128.8 a	128.2 bc	126.7 b	110.1 b
Patriot	128.8	127.7 ab	128.5 bc	124.9 b	118.4 a

*=Means within a column followed by the same letter are not different, FPLSD, $\alpha = 0.05$ (each value is the mean of 24 observations)
d[x]= day

Table 4.2. Color change (Chroma) of broccoli cultivars (florets) packaged in Cryovac PD-941 film after storage at 4°C plus 5 days at 15°C.

	Chroma $(a^2 + b^2)^{1/2}$				
Cultivar	0 d^x	8 d at 4°C	21 d at 4°C	21 d at 4°C + 3 d at 15°C	21 d at 4°C + 5 d at 15°C
Arcadia	16.3	17.6 a	16.9 a	18.2 bc	21.8 c*
ATX 91-307	14.9	13.0 b	13.3 c	14.1 d	20.3 c
Barbados	13.0	14.9b	13.9 c	17.0 cd	20.4 c
Caravel	15.3	14.6b	15.2 b	19.8 bc	25.4 b
Claudia	12.7	15.0b	17.2 a	23.4 a	34.6 a
Emerald City	20.1	13.8 b	15.3 b	17.4 cd	27.2 b
Patriot	16.7	18.6 a	16.8 a	21.1 ab	23.7 bc

*=Means within a column followed by the same letter are not different, FPLSD, $\alpha = 0.05$ (each value is the mean of 24 observations)

d^x= day

A similar trend was observed after 21 days of storage. The higher hue angle values (132.0 and 129.1) and the lower chroma values were detected in the cultivars ATX and Barbados respectively (Tables 4.1, 4.2). In contrast, the lowest hue angle and the highest chroma values were found for 'Claudia' but these did not differ significantly from 'Arcadia'.

After exposure of the packaged florets to 15° for 3 days there was a large change in color in all broccoli cultivars as judged by both the hue angle and chroma values (Figures 4.6 and 4.7). Color values ranged from 118.2 to 127.3 for hue angle and from 14.1 to 23.4 for chroma after exposure to 15° for 3 days (Tables 4.1 and 4.2). The highest hue angle value (127.4) was in cultivar Barbados but it differed significantly only from 'Claudia'. With respect to chroma, the lowest value was found in the cultivar ATX, but it did not differ significantly from 'Barbados' and 'Emerald' after 3 days of exposure to 15°C.

All samples markedly changed color between 3 and 5 days after exposure to 15°C (Figures 4.6 and 4.7). There were significant differences in hue angle and chroma values among the seven cultivars. 'Arcadia' showed the highest hue angle although not significantly different from the cultivars Barbados, ATX and Patriot. The lowest chroma value was detected in 'ATX' and was not significantly different from the cultivars Barbados, Arcadia and Patriot after five days of exposure to 15°C.

Cultivar differences in color have been reported for other vegetables. Lownds et al. (1994) noted that color development in seven pepper cultivars stored at 8, 14, or 20°C, was cultivar and packaging dependent, and the ratings increased with the storage temperature.

All broccoli cultivars ('Claudia', 'Caravel', 'ATX', 'Emerald', 'Arcadia', 'Barbados' and 'Patriot') were still green after storage at 4°C for 21 days and after 3 days exposure to 15°C. The samples started yellowing between 3 and 5

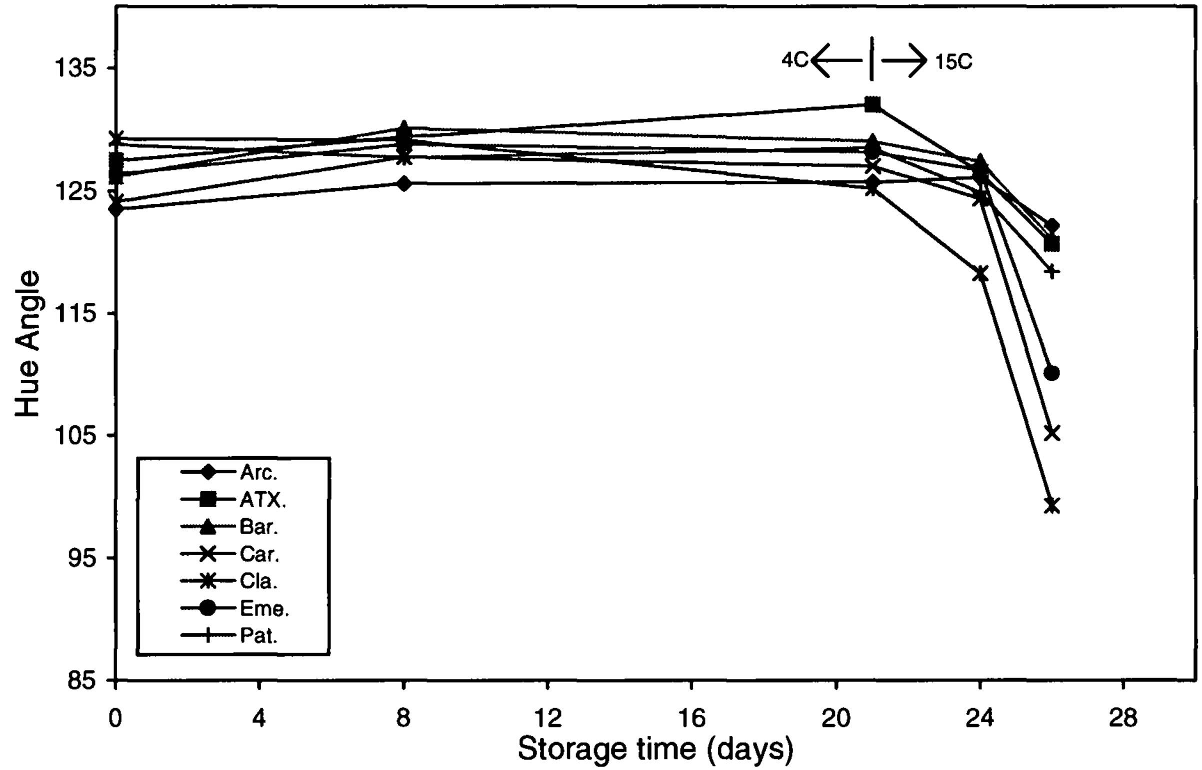


Figure 4. 6. Color change (hue angle) of seven broccoli cultivars after MAP storage (Cryovac PD-941 film) at 4°C and 15°C.

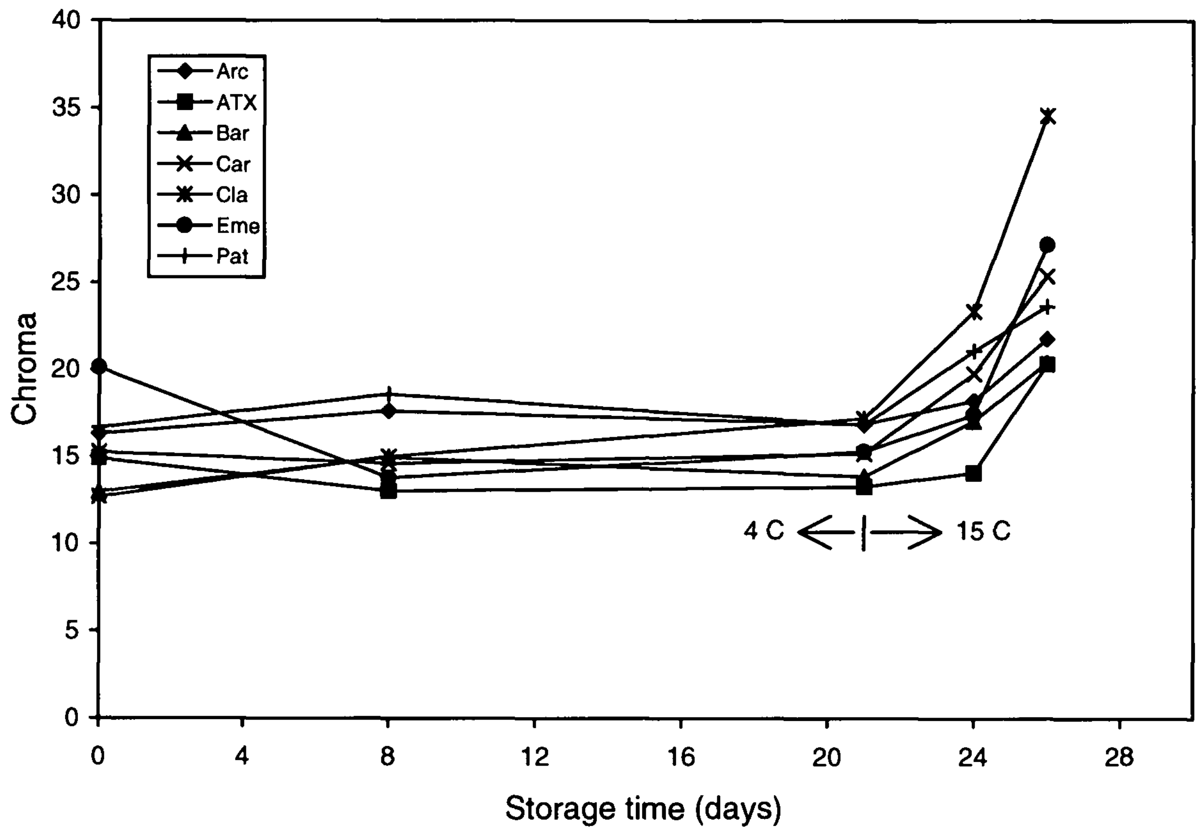


Figure 4.7 Color change (chroma) of seven broccoli cultivars after MAP storage (Cryovac PD-941 film) at 4°C and 15°C.

days exposure to 15°C. The color score of 'Claudia' was poorer than the other six cultivars (Tables 4.1 and 4.2). One possible explanation may be that 'Claudia' might have a higher respiration rate and produce more ethylene, but no measures of these were taken. Storage of broccoli cultivars in polyolefin bags retarded yellowing and hence improved quality, possibly because of the high CO_2 and low O_2 concentrations in the sealed bags. Modified atmospheres in the storage environment have been reported to maintain broccoli quality (Lipton and Harris, 1974; Makhlouf et al., 1989), even at 24°C (Lieberman and Hardenburg, 1954).

Conclusions

1. MAP broccoli cultivars during 21 days at 4°C retained green color, chlorophyll, ascorbic acid and fresh weight very well. Upon transfer to 15°C, more rapid losses occurred.
2. While quality characteristics at harvest differed greatly between cultivars, they lost these qualities at different rates.
3. Broccoli cv. 'ATX91-307' was superior to 6 other cultivars packaged in PD-941 MAP during 21 days at 4°C plus up to 5 days at 15°C.

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Appendices

Table A2.1. Effect of N fertilization rates and storage time at 0°C on weight loss of broccoli florets sealed in B-900 Cryovac film.

Storage time (days)	Nitrogen fertilization rates (Kg/ha)	Percentage of weight loss
8	0	0.59 a*
	90	2.78 c
	180	1.93 b
	270	3.00 c
12	0	1.49 a
	90	3.66 c
	180	2.83 b
	270	3.78 c
28	0	2.64 a
	90	5.28 c
	180	4.24 b
	270	5.95 c

*Means within a column in each storage time followed by the same letter are not different, FPLSD, $\alpha = .05$ (each value is the mean of 4 observations)

Table A2.2. Effect of nitrogen fertilization rates and 0°C storage time on chlorophyll concentration of broccoli florets sealed in B-900 Cryovac film.

Storage time (days)	Nitrogen fertilization rates (Kg /ha)	Chlorophyll concentration (µg/g fresh wt)
0	0	124.1 c
	90	139.5 bc
	180	142.0 b
	270	171.6 a
8	0	102.1 b*
	90	108.2 b
	180	112.3 b
	270	135.8 a
12	0	84.3 b
	90	115.4 a
	180	124.3 a
	270	126.6 a
28	0	65.9 a
	90	58.2 a
	180	57.4 a
	270	67.8 a

*Means within a column in each storage time followed by the same letter are not different, FPLSD, $\alpha = .05$ (each value is the mean of 4 observations)

Table A3.1. Effects of package film type and storage temperature on weight loss in broccoli florets.

Film type	Temperature (°C).	Storage time (days)			
		8	12	21	28
		Loss of weight (% FW)			
PD-941	0	1.21 ab	1.34 ab	2.03 ab	3.39 a*
	5	0.99 ab	1.09 ab	1.90 a	3.02 a
	10	1.33 abc	1.73 ab	1.73 a	--
PD-961EZ	0	0.56 a	1.38 ab	1.74 a	3.13 a
	5	0.71 ab	0.85 a	1.21 a	2.27 a
	10	1.18 ab	1.18 ab	1.19 a	--
PD-900	0	2.29 c	3.06 c	3.42 b	4.21 a
	5	1.14 ab	1.19 ab	1.86 a	2.38 a
	10	1.67 bc	2.09 bc	2.59 ab	--
Vented bags (control)	0	4.68 e	5.96 d	8.14 c	12.86 b
	5	3.41 d	5.25 d	8.93 c	--
	10	8.82 f	11.47 e	--	--

*Means within a column with the same letter are not significantly different from each other ($p \leq 0.05$). (each value is the mean of 3 observations)
-- Treatments with florets opening or showing noticeable yellowing were discarded

Table A3.2. Effect of package film type and temperature on instrumental color values of stored broccoli florets.

Film type	Temperature (°C)	Storage time (days) 8	12	21	28
			L values		
PD-941	0	52.8	41.4	42.2	41.1
	5	53.2	42.1	42.1	43.8
	10	53.5	42.8	46.4	--
PD-961EZ	0	52.9	42.6	42.1	41.9
	5	53.3	41.1	42.1	41.5
	10	53.1	40.5	40.7	--
PD-900	0	46.8	41.3	44.5	42.2
	5	47.7	40.5	39.5	42.2
	10	47.0	39.8	40.7	--
Vented bags (control)	0	48.3	41.6	42.8	43.9
	5	51.0	53.4	55.0	--
	10	62.1	47.6	--	--

-- Treatments with florets opening or showing noticeable yellowing were discarded (each value is the mean of 18 observations)

Table A3.3. Effect of package film type and temperature on instrumental color values of stored broccoli florets.

Film type	Temperature (°C)	Storage time (days) 8	12	21	28
		a* values			
PD-941	0	-10.6	-8.4	-8.7	-8.0
	5	-10.8	-8.7	-8.7	-8.7
	10	-10.3	-8.8	-8.3	--
PD-961EZ	0	-8.2	-7.2	-8.5	-8.0
	5	-10.1	-8.5	-8.0	-7.9
	10	-9.2	-7.7	-8.6	--
PD-900	0	-8.8	-8.9	-7.4	-7.4
	5	-9.4	-8.2	-7.9	-7.8
	10	-7.1	-6.3	-6.3	--
Vented bags (control)	0	-8.7	-7.9	-6.7	-5.4
	5	-9.8	-6.1	1.5	--
	10	0.2	0.7	--	--

-- Treatments with florets opening or showing noticeable yellowing were discarded (each value is the mean of 18 observations)

Table A3.4. Effect of package film type and temperature on instrumental color values of stored broccoli florets.

		Storage time (days)			
Film type	Temperature (°C)	8	12	21	28
		b* values			
PD-941	0	15.3	12.0	12.3	11.4
	5	15.1	11.8	12.8	14.0
	10	14.6	13.1	19.1	--
PD-961EZ	0	13.0	10.8	12.6	11.5
	5	14.2	11.8	12.3	11.0
	10	13.1	10.5	14.1	--
PD-900	0	12.0	12.5	12.1	11.5
	5	12.8	12.1	12.5	12.2
	10	12.0	11.4	12.7	--
Vented bags (control)	0	13.1	12.3	11.8	13.5
	5	18.7	29.5	29.9	--
	10	33.5	23.2	--	--

-- Treatments with florets opening or showing noticeable yellowing were discarded (each value is the mean of 18 observations)

Table A4.1. Weight loss of broccoli cultivars (florets) packaged in Cryovac PD-941 film after storage at 4°C plus 5 days at 15°C.

	Weight loss (% FW)			
Cultivar	8 d^x at 4°C	21 d at 4°C	21 d at 4°C + 3 d at 15°C	21 d at 4°C + 5 d at 15°C
Arcadia	1.12 c	1.20 bc	1.70 a	1.99 a*
ATX 91-307	1.07 bc	1.08 ab	1.68 a	1.95 a
Barbados	0.87 a	1.16 abc	1.68 a	2.02 a
Caravel	0.95 ab	1.04 a	1.65 a	2.05 a
Claudia	0.99 abc	1.11 ab	1.75 a	2.03 a
Emerald City	0.92 ab	1.11 ab	1.85 a	1.93 a
Patriot	1.01 abc	1.25 c	1.78 a	1.99 a

*=Means within a column followed by the same letter are not different, FPLSD, $\alpha = 0.05$. (each value is the mean of 4 observations)
d^x= day

Table A4.2. Ascorbic acid concentration of broccoli cultivars (florets) packaged in Cryovac PD-941 film after storage at 4°C plus 5 days at 15°C.

	Ascorbic acid (mg/g F.W.)				
Cultivar	0 d^x	8 d at 4°C	21 d at 4°C	21 d at 4°C + 3 d at 15°C	21 d at 4°C + 5 d at 15°C
Arcadia	0.95 cd*	0.94 b*	0.87 bc*	0.69 ab*	0.60 bc*
ATX 91-307	1.00 bc	1.10 a	0.97 a	0.69 ab	0.65 ab
Barbados	1.02 bc	1.15 a	1.01 a	0.74 a	0.70 a
Caravel	1.12 a	0.91 b	0.85 bc	0.62 ab	0.44 e
Claudia	1.04 b	0.92 b	0.83 c	0.59 b	0.50 de
Emerald City	0.84 e	1.00 b	0.86 bc	0.71 ab	0.56 cd
Patriot	0.92 de	0.97 b	0.92 ab	0.67 ab	0.68 ab

*=Means within a column followed by the same letter are not different, FPLSD, $\alpha = 0.05$ (each value is the mean of 4 observations)
d^x= day

Table A4.3. Chlorophyll concentration of broccoli cultivars (florets) packaged in Cryovac PD-941 film after storage at 4°C plus 5 days at 15°C.

	Chlorophyll (μg/g F.W.)				
Cultivar	0 d^x	8 d at 4°C	21 d at 4°C	21 d at 4°C + 3 d at 15°C	21 d at 4°C + 5 d at 15°C
Arcadia	144.8 bc	121.7 cd	136.9 b	106.2 bc	79.3 bc
ATX 91-307	219.6 a	189.4 a	195.3 a	193.5 a	127.1 a
Barbados	180.0 ab	149.3 bc	137.7 b	99.8 bc	101.4 ab
Caravel	159.5 bc	121.3 cd	107.5 b	91.3 c	79.2 bc
Claudia	222.2 a	156.0 ab	137.5 b	89.1 c	41.8 d
Emerald City	146.6 bc	137.7 bcd	135.7 b	123.7 b	48.6 d
Patriot	122.3 c	113.4 d	115.5 b	105.7 bc	68.2 cd

*=Means within a column followed by the same letter are not different, FPLSD, $\alpha = 0.05$ (each value is the mean of 4 observations)
d^x= day

Table A4.4. Instrumental color values (L*) of broccoli (florets) cultivars packaged in Cryovac PD-941 film after storage at 4°C plus 5 days at 15°C.

	L* values				
Cultivar	0 d	8 d, 4°C	21 d, 4°C	21 d, 4°C + 3 d, 15°C	21 d, 4°C + 3 d, 15°C
Arcadia	43.3	42.6	43.6	44.9	47.1
ATX 91-307	40.5	40.2	41.0	43.1	45.6
Barbados	39.9	41.5	42.1	43.4	46.8
Caravel	43.7	42.6	42.3	44.3	48.7
Claudia	40.3	40.7	43.3	46.4	56.7
Emerald City	44.0	42.1	43.1	43.7	52.3
Patriot	42.2	43.5	42.9	45.3	48.1

(each value is the mean of 24 observations)

Table A4.5. Instrumental color values (a*) of broccoli (florets) cultivars packaged in Cryovac PD-941 film after storage at 4°C plus 5 days at 15°C.

	a* values				
Cultivar	0 d	8 d, 4°C	21 d, 4°C	21 d, 4°C + 3 d, 15°C	21 d, 4°C + 3 d, 15°C
Arcadia	-9.0	-10.2	-9.8	-10.6	-11.5
ATX 91-307	-9.1	-10.1	-8.8	-8.2	-10.3
Barbados	-7.7	-9.5	-8.7	-10.3	-9.7
Caravel	-8.6	-8.8	-9.1	-11.0	-5.9
Claudia	-8.0	-9.4	-9.8	-10.7	-5.5
Emerald City	-11.7	-8.6	-9.4	-10.2	-8.5
Patriot	-10.2	-11.3	13.6	-11.9	-10.5

(each value is the mean of 24 observations)

Table A4.6. Instrumental color values (b*) of broccoli (florets) cultivars packaged in Cryovac PD-941 film after storage at 4°C plus 5 days at 15°C.

	b* values				
Cultivar	0 d	8 d, 4°C	21 d, 4°C	21 d, 4°C + 3 d, 15°C	21 d, 4°C + 3 d, 15°C
Arcadia	13.6	14.3	13.7	14.8	18.5
ATX 91-307	11.8	10.1	9.9	11.4	17.5
Barbados	10.4	11.5	10.8	13.6	17.7
Caravel	12.6	11.6	12.1	16.4	23.5
Claudia	9.8	11.7	14.1	20.6	33.9
Emerald City	16.3	10.7	12.0	14.0	25.4
Patriot	13.2	15.0	13.6	17.4	20.8

(each value is the mean of 24 observations)