Preservative Treatments for Fresh-Cut Fruits and Vegetables

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1. Introduction

From the quality standpoint it is desirable to preserve the characteristics of fresh-cut fruits and vegetables at their peak. What the consumer perceives as the most appealing attributes of these products include their fresh-like appearance, taste and flavor, in addition to convenience. Obviously, any food product should be safe for consumption, and fresh-cut products are very sensitive to contamination. Among the limitations to shelf-life of fresh-cut products are: microbial spoilage, desiccation, discoloration or browning, bleaching, textural changes and development of off-flavor or off-odor. Nevertheless, safety aspects are not discussed in this chapter, but were reviewed in Chapter III). The primary quality attributes of a food product include color, texture, flavor and nutritional value. When assessing plant product quality, consumers take product appearance into consideration as a primary criterion, and color is probably the main factor considered (Kays, 1999).

While conventional food processing methods extend the shelf-life of fruits and vegetables, the minimal processing to which fresh-cut fruits and vegetables are submitted renders products highly perishable, requiring chilled storage to ensure a reasonable shelf-life. Preparation steps such as peeling or scrubbing, slicing, shredding, etc remove the natural protection (peel or skin) of fruits and vegetables and cause bruises, rendering them susceptible to desiccation and wilting. This also exposes internal tissues to microbes and potentially deleterious endogenous enzymes. Among the possible consequences of mechanical injuries to produce are increase in respiration rate and ethylene production, accelerated senescence and enzymatic browning (Rosen and Kader, 1989). In conventional types of fruit and vegetable processing, such as canning and freezing, many of these problems are prevented or controlled by heat processing and consequent inactivation of enzymes, by the use of protective packaging materials, or through the application of various additives. In the production of fresh-cut products, the use of heat is avoided in order to prevent cooking of the product, and consequently loss of fresh-like characteristics. Several chemical preservatives can be used, depending on what is to be prevented; often chemical preservatives are applied in the control of enzymatic browning, firmness and decay (Brecht, 1992). Other important applications include the use of controlled modified atmosphere packaging and edible films also have many potential applications.

A survey on consumer perception of convenience products revealed the desire that such products maintain fresh characteristics longer without the use of preservatives (Bruhn, 1994). Unfortunately, depending on the type of quality defect to be prevented or controlled it is not always possible to avoid the use of chemical treatments. One important aspect to consider is the establishment of conditions that allow for quality optimization at a reasonable shelf-life, rather than extending shelf-life at an acceptable quality (Shewfelt, 1994).

In this chapter we review the most common treatments, used to preserve the color and texture of fresh-cut products. Color preservation is, after safety, the most important attribute to be preserved, since frequently a product is selected for its appearance, particularly its color. Color has been considered to have a

key role in food choice, food preference and acceptability, and may even influence taste thresholds, sweetness perception and pleasantness (Clydesdale, 1993). Secondly, texture loss and preservation in fresh-cut products will be discussed, due to its important impact on product appearance and sensory quality.

2. Fresh-cut Products and Color Preservation

Fruits and vegetables are attractive and eye-catching to a large degree because of the richness of pigments that they contain. Preservation of chlorophyll in vegetables, red to purple anthocyanins, yellow, orange and red carotenoids in both fruits and vegetables is of vital importance to maintain quality. Color changes (Figure 1) in fresh-cut fruits and vegetables may have different origins, for example decreased green pigmentation in fresh-cut lettuce may result from senescence, heat exposure or acidification; discoloration or browning of sliced mushrooms and sliced apples and pears is brought about through the action of polyphenol oxidases; white blush development in carrots is initially caused by desiccation, and later lignification. The main focus of this chapter is on prevention of enzyme-catalyzed browning, although some of the other color changes will be briefly discussed.

2.1 Enzymatic Browning

Enzymatic browning is one of the most limiting factors on the shelf-life of fresh-cut products. During the preparation stages, produce is submitted to operations where cells are broken causing enzymes to be liberated from tissues and put in contact with their substrates. Enzymatic browning is the discoloration which results from the action of a group of enzymes called polyphenol oxidases (PPO), which have been reported to occur in all plants, and exist in particularly high amounts in mushroom, banana, apple, pear, potato, avocado and peach. Enzymatic browning must be distinguished from non-enzymatic browning, which results upon heating or storage after processing of foods; types of non-enzymatic browning include the Maillard reaction, caramelization and ascorbic acid oxidation.

Enzymatic browning is a complex process, which can be subdivided in two parts. The first reaction is mediated by PPO (Figure 2) resulting in the formation of *o*-quinones (slightly colored), which through non-enzymatic reactions lead to the formation of complex brown pigments. *o*-Quinones are highly reactive and can rapidly undergo oxidation and polymerization. *o*-Quinones react with other quinone molecules, with other phenolic compounds, and with the amino groups of proteins, peptides and amino acids, with aromatic amines, thiol compounds, ascorbic acid, etc (Whitaker and Lee, 1995; Nicolas *et al.*, 1993). Usually, brown pigments are formed, but in addition, reddish-brown, blue-gray and even black discolorations can be produced on some bruised plant tissues. Color variation in products of enzymatic oxidation is related to the phenolic compounds involved in the reaction (Amiot *et al.*, 1997), and both color intensity

and hue of pigments formed vary widely (Nicolas *et al.*, 1993). Consequences of enzymatic browning are not restricted to discoloration, undesirable tastes can also be produced and loss of nutrient quality may result (Vamos-Vigyazo, 1981). Biochemical details on PPO action were reviewed in Chapter 6. PPO has been considered one of the most damaging enzymes to quality maintenance of fresh produce (Whitaker and Lee, 1995), and the prevention of enzymatic browning has always been considered a challenge to food scientists (Ponting, 1960).

2.1.1 Pre-Harvest Factors

Several parameters may contribute to the development of enzymatic browning. Agricultural practices, soil, fertilizers, climate and harvesting conditions all affect the final quality of fresh-cut products (Ahvenainen, 1996). High nitrogen levels have been related to a greater tendency to brown in potatoes (Mondy *et al.,* 1979).

The selection of raw material for processing needs to be carefully evaluated. The susceptibility to brown may differ from cultivar to cultivar, as exemplified in Tables 1 and 2. Some tissues may have high PPO activity and/or high concentration or types of phenolic PPO substrates which, under appropriate conditions lead to a higher tendency to brown. In pears, it was found that although the phenolic content tended to decrease with delayed harvest time, phenolic levels did not always correlate with the susceptibility to browning (Amiot *et al.,* 1995). In general, high concentrations of phenolic compounds are found in young fruits. While in bananas PPO activity is higher in the pulp than in the peel, in pear and apple, PPO activity is higher in the peel than in the flesh (Macheix *et al.*, 1990). In addition, PPO activity may vary widely between cultivars of the same crop and at different maturity stages. Examples of such variations are shown in Table 3. Ideally, produce varieties with either low levels of PPO or phenolic substrates, or both, should be selected for fresh-cut processing. New varieties with desirable traits for the fresh-cut processing may be developed by conventional breeding techniques, and potentially through biotechnology (Chapter XIII). Nevertheless, it is important to point out that not only PPO activity and concentration of substrates are important; individual phenolics exhibit different degrees of browning and the rate of enzymatic browning is also affected by other polyphenol compounds present in the tissue (Lee, 1992).

Table 1. Enzymatic browning in purées prepared with various apricot cultivars at commercial maturity.

Cultivar	DL*
Henderson	26.3
Moniqui	21.4
Rouge de Roussillon	17.8
Rouge de Fournes	17.8
Polonais	16.8
Canino	16.7
Cafona	11.8
Bebeco	5.3
Precoce de Tyrinthe	3.7

DL* = Difference in lightness between oxidized and non-oxidized apricot purées.
(Adapted from Radi *et al.*, 1997)

Table 2. Susceptibility of potato varieties to enzymatic browning after storage (of whole unpeeled tuber) at 5°C and 75% RH.

Storage time	Browning Index		
	var. Bintje	var. Van Gogh	var. Nicola
1 mo.	6	27	44
30 min*	15	40	75
60 min	21	52	88
120 min			
5 mo.			
30 min	16	26	21
60 min	23	56	58
120 min	30	78	98
8 mo.			
30 min	10	28	66
60 min	32	74	112
120 min	62	104	145

Browning evaluation was carried out on 5 mm slices cut from the center of the tubers and left at 23°C for observation at 30 min, 60 min and 120 min after cutting (From Mattila *et al.*, 1993).

Table 3. Relative PPO activity in different apple cultivars.

Cultivar	Relative PPO activity	
	Peel	Cortex
Red Delicious	100	100
Golden Delicious	33	30
McIntosh	46	80
Fuji	57	71
Gala	30	48
Granny Smith	43	73
Jonagold	43	43
Elstar	10	20

(Adapted from Janovitz-Klapp *et al.* 1989)

2.1.2 Post Harvest and Processing Factors

Processing operations such as washing, scrubbing, peeling, trimming, cutting, shredding, etc carried out during the initial stages of fresh-cut preparation cause mechanical injury to the plant tissues. Moreover, even prior to processing, produce manipulation may bring mechanical shocks resulting in cracks and bruises, which can elicit physiological and biochemical responses in the wounded tissue as well as in unwounded distant cells (Saltveit, 1997). Peel removal and loss of tissue integrity with cell breakage facilitate microbial contamination. In addition, exposure to air and release of endogenous enzymes that are put in contact with their substrates, originally in different cell compartments, may lead to detrimental consequences. Living tissues are still physiologically active and respond to wounding. The first responses to mechanical injury relate to respiration rate increase and possibly increased ethylene production (see

Chapter VI) . In general, respiration rates are inversely related to the shelf-life of produce. Quality deterioration may result from increased ethylene production, which may induce higher cellular metabolism, and higher enzymatic activity (Reyes, 1996). Another consequence of wounding is the induction of secondary product synthesis, including a variety of phenolic compounds. Among the enzymes that may have deleterious effects, polyphenol oxidase (PPO), can be the most damaging enzyme with regard to color deterioration of plant foods (Whitaker and Lee, 1995).

During peeling and cutting operations if the equipment used is not in the best condition, for example if dull knives and blades are used, bruising and damage occurs in more tissue layers than intended, thus the sharpness of knife blades can significantly affect product storage life (Bolin *et al.* 1977). An increase of 15% in the respiration rate of hand peeled carrots was detected when compared to unpeeled carrots. In contrast, abrasion peeling, which is more destructive than hand peeling, led to almost doubled respiration rates. For stored carrots, respiration rates increased two- and three-fold when fine-abrasion vs. coarse-abrasion peeling were used, respectively, in comparison with the rates observed for hand peeled carrots. Shredded iceberg lettuce had a 35-40% increase in respiration rate in relation to quartered lettuce heads. The type of equipment used may also affect the physiological response of the tissues; sharp rotating blades gave better results in cutting lettuce (lower respiration and lower microbial count during storage) than sharp stationary blades (O'Beirne, 1995). Evidently, the tissue response to mechanical injury is expected to be more pronounced when extensive wounding is inflicted on the produce, such as the grating of carrots *versus* preparation of carrot sticks. Moreover, the direction of the cut also affects the tissue response to wounding (Zhou *et al.*, 1992).

As a result of cutting there is accumulation of cell fluids on the cut surface, and washing of cut produce may be helpful in minimizing accumulation of potential substrates and enzymes. Removal of cellular fluids (which carry potentially deleterious enzymes such as PPO, peroxidase, etc) released during the cutting operation is important and can be accomplished by simple rinsing procedures. Although washed mushrooms had 15% less soluble phenolics, showed leaching of PPO (two out of four isoforms), and therefore less enzymatic activity, there was also water uptake during washing, and consequently a more rapid deterioration of mushrooms, due to microbial spoilage and mechanical damage (Choi and Sapers, 1994). Other commodities, such as lettuce do not benefit from rinsing. Rinsed and drained shredded lettuce may retain 0.5 – 1% water on the surface, a residual amount that can decrease product quality by facilitating decay; thus de-watering has to be carried out (Bolin *et al.*, 1977). However, centrifugation of lettuce to remove residual cold water may require spin conditions (speed, time) that result in mechanical damage of the produce.

For many fruits and vegetables utilized by the fresh-cut industry, processing is carried out shortly after harvest, but in some instances, the seasonality of harvesting may not allow for this. Potatoes are an example of a vegetable which can be stored before used in the preparation of pre-peeled products. A Finnish study evaluated the tendency to brown of three potato varieties stored stored for different periods (Table 2). Results showed that only

one variety (Bintje) stored for one month would pass the requirements of the local industry, which establishes a maximum browning index of 10 as acceptable for fresh-cut processing (Mattila *et al.*, 1993).

2.1.3 Browning and Enzymes other than Polyphenoloxidase

Mechanical injury (wounding) and ethylene can stimulate phenolic metabolism in the fresh-cut tissue. Wounding and ethylene induce the activity of the enzyme phenylalanine ammonia lyase (PAL), a key enzyme for phenolic bisynthesis. Accumulated phenolic compounds can be used as substrates by PPO, leading to browning. It has been suggested that lettuce storage life is related to the activity of stress-induced PAL (Couture *et al*., 1993; Saltveit, 1997*).* In fresh-cut lettuce browning of pieces is also a major detriment to quality. Different types of browning defects can be observed in lettuce, such as russet spotting (RS), which is characterized by brown spots on the lettuce midribs; browning of cut edges (LEB) and of the leaf surface (LSB). In wounded air-stored lettuce pieces the major defects described are EB and LSB, while RS is most apparent in wounded ethylene-stored samples. A comparison of the response of five types of lettuce (Iceberg, romaine, green leaf, red leaf and butterhead) revealed differences in the maximum level of wound induced-PAL, which were also affected by the storage of the whole lettuce heads before processing. Maximum levels of PAL decreased with increased storage time (Lopez-Galvez *et al.*, 1996). In addition, in harvested lettuce heads the stem tissue near the harvesting cut may develop browning, or so-called butt discoloration, when the cut stem initially becomes yellow, it later develops a reddish-brown color, and finally an intense brown pigmentation. PAL activity is induced by cutting the lettuce stem, with subsequent synthesis and accumulation of soluble phenolic compounds (mainly caffeic acid derivatives), supplying substrates for PPO (Tomas-Barberan *et al*., 1997). PAL activity is believed to be proportional to the extent of wounding.

Peroxidase is an enzyme widely distributed in plants. Changes in peroxidase may be brought about by wounding, physiological stress and infections. Many reactions can be promoted by peroxidase, and in the presence of small amounts of hydrogen peroxide, it can oxidize a number of naturally occurring phenolics. Mono- and diphenols are potential substrates for peroxidase (Robinson, 1991). It is believed that although peroxidase may also contribute to enzymatic browning, its role remains questionable (Nicolas *et al.*, 1993) and limited by hydrogen availability (Amiot *et al.*, 1997).

2.2 Control of Enzymatic Browning

Enzymatic browning may be controlled though the use of both physical and chemical methods, and in most cases both are employed. Physical methods may include reduction of temperature and/or oxygen, use of modified atmosphere packaging or edible coatings, or treatment with gamma-irradiation or high pressure. Chemical methods utilize compounds which act to inhibit the

enzyme, remove its substrates (oxygen and phenolics) or function as preferred substrate. Chemical means of controlling browning will be discussed first.

Prior to having their GRAS status revoked by the FDA in 1986, due to potential health risks posed to sensitive consumers (Taylor, 1993), sulfites had a widespread application in controlling both enzymatic and non-enzymatic browning. Following their ban for use in fruits and vegetables to be consumed raw, other chemicals have been sought for prevention of enzymatic browning. Regardless of the fact that many different PPO inhibitors have been used in research (Vamos-Vigyazo, 1981; McEvily *et al.*, 1992; Iyengar and McEvily, 1992; Sapers, 1993), in this chapter only inhibitors with potential application for fresh-cut fruits and vegetables will be discussed. It is important to point out that some chemicals used in research may not meet the safety standards, and pose toxic risks, others may impart undesirable sensory effects to foods, and others have shown effectiveness only in fruit juices, but not on cut surfaces.

Traditionally, conventional food processing achieves the prevention of browning through heat inactivation of PPO, as with blanching and cooking. Heat inactivation is an effective method of browning prevention, and PPO is considered an enzyme of low thermostability, although differences in heat stability are reported for different cultivars and PPO isoforms (Zawistowski *et al.*, 1991). Nevertheless, use of heat has also the potential to cause destruction of some food quality attributes, such as texture and flavor, and to result in nutritional losses. It is considered that in fresh-cut products, if heat treatments are applied, they should be minimized and should not cause a cessation of respiration. Rather than, or in addition to the use of heat, the control of enzymatic browning is frequently achieved through the use of different types of chemicals, generally referred to as antibrowning agents.

For an enzymatic browning reaction to occur essential elements are required: the presence of active PPO, oxygen and phenolic substrates. Browning prevention is possible, at least temporarily, through elimination of substrates and/or enzyme inhibition.

2.2.1 Antibrowning Agents

Several types of chemicals are used in the control of browning (Table 4); some act directly as inhibitors of PPO, others by rendering the medium inadequate for the development of the browning reaction, still others act by reacting with the products of the PPO reaction before these can lead to the formation of dark pigments.

Acidulants. While the optimum pH for PPO has been reported as ranging from acid to neutral, in most fruits and vegetables, optimum PPO activity is observed at pH 6.0 to 6.5, while little activity is detected below pH 4.5 (Whitaker, 1994). It has also been reported that irreversible inactivation of PPO can be achieved below pH 3.0 (Richardson and Hyslop, 1985). Nevertheless, it has also been reported that apple PPO is quite tolerant to acidity, and at pH 3.0 it retains 40% of its maximum activity (Nicolas *et al.*, 1994).

The use of chemicals that lower the product pH, or acidulants, finds widespread application in the control of enzymatic browning. The most commonly used acidulant is citric acid. Acidulants are frequently used in combination with other types of antibrowning agents, because it is difficult to achieve efficient browning inhibition solely through pH control. In addition, there are variations in the effect of different acids on PPO; as an example, malic acid has been reported to be more efficient in preventing apple juice browning than citric acid (Ponting, 1960).

Reducing Agents. This type of antibrowning agent causes chemical reduction of colorless *o*-quinones resulting from the PPO reaction back to *o*- diphenols (Iyengar and McEvily, 1992). Reductants are irreversibly oxidized during the reaction, which means that the protection they confer is only temporary since they are consumed in the reaction. When all the reducing agent added is oxidized, the *o*-quinones from the PPO reaction may undergo further oxidation reactions (not involving PPO), and finally rapid polymerization leading to the formation of brown pigments (Figure 2). Due to the oxidative nature of enzymatic browning, reducing agents can also be applied in the prevention of discoloration.

Ascorbic acid is probably the most widely used antibrowning agent, and in addition to its reducing properties, it also slightly lowers pH. Ascorbic acid reduces the *o*-benzoquinones back to *o*-diphenols, and it also has a direct effect on PPO (Whitaker, 1994; Golan-Goldhirsh *et al.* 1992).

Thiol-containing compounds, such as cysteine, are also reducing agents that inhibit enzymatic browning. However, for complete browning control the amount of cysteine required (cysteine to phenol ratios above 1) is often incompatible with product taste (Richard-Forget *et al.*, 1992).

***Chelating Agents*.** By complexing copper from the PPO active site, chelating compounds, such as ethylenediamine tetraacetic acid (EDTA) can inhibit PPO, which is a metalloenzyme containing copper in the active site. Sporix is a powerful chelator, and also an acidulant. Browning prevention in apple juice and cut surfaces was obtained with combinations of Sporix and ascorbic acid (Sapers *et al.*, 1989)

Complexing Agents. This category includes agents capable of entrapping or forming complexes with PPO substrates or reaction products. Examples of this category are cyclodextrins or cyclic non-reducing oligosaccharides of six or more D-glucose residues. In aqueous solution, the central cavity of cyclodextrins can form inclusion complexes with phenolics, consequently depleting PPO substrates. β-Cyclodextrin has the most appropriate cavity size for complexing phenolic compounds, but its water solubility is low (Billaud *et al.*, 1995). β-Cyclodextrin was not effective in controlling browning of diced apples, presumably due to its low diffusion (Sapers and Hicks, 1989). Large variations in the inhibitory properties of cyclodextrins have been found with different phenols tested. β-Cyclodextrin binding strength varies with different phenols. In model systems containing a single phenolic compound, β-cyclodextrin always works as a PPO inhibitor. When mixtures of phenolic compounds were tested the results were variable, and the balance among the PPO substrates present can be modified, resulting in color changes after enzymatic oxidation catalyzed by PPO (Billaud *et al.*, 1995).

Enzyme Inhibitors. One of the antibrowning agents with the most potential for application to fresh-cut products is 4-hexylresorcinol, a chemical that has been safely used in medications for a long time, and has been granted a GRAS status for use in the prevention of shrimp discoloration (melanosis), where it proved to be more effective than sulfite on a weight-to-weight basis (McEvily *et al.,* 1992). Currently, its useon fruit and vegetable products has been delayed while awaiting FDA. The efficiency of 4-hexylresorcinol has been demonstrated in preliminary tests carried out using cut apples and potatoes (McEvily *et al.* 1991).

Other Antibrowning Agents.

Sodium chloride (as other halides) is known to inhibit PPO; its inhibition increases as pH decreases. Chloride is a weak inhibitor; some authors report that the chloride levels required for PPO inhibition are elevated, and may compromise product taste (Mayer and Harel, 1991). Nevertheless, other authors believe that browning control may be possible provided that the dipping solutions are acidic; a pH of at least 3.5 has been suggested (Rouet-Mayer and Philippon, 1986).

Calcium treatments used for tissue firming have also been reported to reduce browning (Drake and Spayd, 1983; Hopfinger *et al.*, 1984; Bolin and Huxsoll, 1989). Although citric acid and/or ascorbic acid dips were not effective in preventing browning of pear, when slices were dipped in 1% $CaCl_2$ and stored for a week at 2.5°C this resulted in lighter color than water-treated control slices (Rosen and Kader, 1989). In fact, this was most likely due to the PPO inhibition by the chloride ion (Table 4).

Honey. Antibrowning activity has been attributed to a small peptide isolated from honey. Browning inhibition (62%) in slices of peeled apples has been achieved by dipping in a 10% honey solution for 30 min. at room temperature. Comparison with a control sucrose solution at the same sugar level as the honey preparation showed only a 23% inhibition of browning (Oszmianski and Lee, 1990).

Proteases. Enzymatic treatments with proteases that attack PPO have been suggested as alternative prevention treatments for enzymatic browning. It was presumed that PPO inhibition by proteases was due to proteolysis or to binding at specific sites required for activation. Another possible mechanism of action suggested was related to the presence of sulfhydryl groups (such as cysteine) in the proteases. Enzymatic treatment of PPO could potentially be carried out with bromelain (extracted from pineapple), papain (from papaya) and ficin (from figs). Preliminary tests were done using small pieces of apples and potatoes which were dipped for 5 min. in a 2% enzyme solution in citrate buffer at pH 4.5. Results showed that papain worked best on apples, while ficin worked better on potatoes. Parallel tests on untreated samples and control citrate buffer dipped samples developed comparable discoloration (Labuza, 1992). However, partially purified ficin preparations where the protease was heat inactivated were comparable to preparations containing active ficin as PPO inhibitors (McEvily, 1991). Later it was found that ficin preparations contain, in addition to the protease, other antibrowning agents which are analogues of 4-substituted resorcinol (McEvily *et al.*, 1992). Extracts prepared from papaya contain cysteine

and another 'quinone-trapping' substance identified as a dipeptide cysteine-glutamic acid (Richard-Forget *et al.*, 1998).
Aromatic Carboxylic Acids. Although benzoic and cinnamic acids are PPO inhibitors (Walker, 1975), they have not given prolonged protection as antibrowning agents. When solutions of sodium cinnamate were used to dip apple plugs, browning prevention was obtained on a short term, but over prolonged storage (>24 hr) a severe browning developed (Sapers *et al.*, 1989). It has been suggested that cinnamates and benzoates may undergo a slow but gradual conversion to PPO substrates (Sapers *et al.*, 1989; McEvily *et al.*,1992).

There are consumers who want to avoid any type of food preservative (Bruhn, 1995). It is recognized that the consumer perceives fresh-cut products as a minimally processed product with characteristics close to their raw unprocessed material. These flavor, color and texture characteristics are probably an added appeal of fresh-cut products, and as a consequence, some processors would rather not use chemical additives that could change that perception of a "natural" product. This may be one of the reasons that ascorbic acid, which may be labeled as Vitamin C, is frequently preferred as an antibrowning agent, an added value to the product. Other chemicals of natural origin, or identical to natural compounds are also frequently preferred, an example of which is citric acid. With this in mind some authors have tested the efficiency of other natural products in the control of enzymatic browning, such as pineapple juice. Among the constituents of pineapple juice, antibrowning activity could be attributed to both ascorbic acid and bromelain, but in addition, the juice contains a low molecular weight inhibitor which is as yet uncharacterized (Lozano-de-Gonzalez *et al.*, 1993).

Application of Antibrowning Agents. In general, chemicals used to prevent or control enzymatic browning are used in solutions, frequently as formulations containing one or more compounds, which are used for dipping the fruit or vegetable pieces. It has been reported that with some chemicals, such as ascorbic and erythorbic acid or their salts, limited penetration into the plant tissue is an issue. A comparison of the effect of dipping vs. pressure or vacuum infiltration on the penetration of ascorbic and erythorbic acids, it was found that pressure infiltration was ineffective with potato dice, but extended the shelf-life of potato plugs by 2-4 days when compared to dipping (Sapers *et al.*, 1990). The variation in response of potato plugs and dice to pressure infiltration was attributed to the smaller surface-to-volume ratio in the plugs. The authors of the study suggested that the technique could be applied to larger pieces, even peeled tubers. With apple plugs and dice the pressure infiltration method was superior to dipping, providing an increase of 3 to 7 days in storage life of apple pieces. Nevertheless, infiltrated dice can become water-logged, and require de-watering by centrifugation or partial dehydration to overcome that defect. In addition, if too much pressure is applied, cell rupture occur and lead to loss of textural integrity and perhaps reduced shelf-life.

Combined Treatments. More effective preservation of fresh-cut products can frequently be achieved using a combination of treatments. A common treatment

combination includes ascorbic acid and calcium chloride, such as presented in Table 5 (Ponting *et al.*, 1972). In the case of two apple varieties, e.g. Newton Pippin and Golden Delicious, the highest concentrations of ascorbic acid (1%) and $CaCl_2$ (0.1%) utilized resulted in the lowest loss of reflectance or browning readings. It is interesting that the use of $CaCl_2$ alone caused almost as much inhibition on Newton Pippin apples, this was not so for Golden Delicious. Table 6 shows some results from a study using different combinations of antibrowning agents on slices prepared from three different potato varieties stored varying lengths of time (Mattila *et al.*, 1993). Other combination treatments may include the use of antibrowning agents and physical methods, such as a heat treatment, or controlled atmosphere, such as the combination of 0.5% O_2 and 1% $CaCl_2$, which was effective in minimizing browning in sliced pears (Rosen and Kader, 1989).

Table 5. Effect of treatments with ascorbic acid (AA) and calcium chloride on the prevention of discoloration in apple slices.

Treatment*	Loss of Reflectance (%) compared to freshly sliced apples var. Newton Pippin	var. Golden Delicious
Control – water dip	62.5	60.5
0.05% $CaCl_2$	24.8	58.9
0.1% $CaCl_2$	23.3	51.2
0.5% AA	57.9	59.2
0.5% AA + 0.05% $CaCl_2$	26.9	48.0
0.5% AA + 0.1% $CaCl_2$	24.2	25.6
1% AA	25.5	45.6
1% AA + 0.05% $CaCl_2$	20.5	39.2
1% AA + 0.1% $CaCl_2$	4.2	17.0

Three minute dip in 1L of antibrowning solution, followed by 1 min draining and packaging in plastic bags prior to storage at ~1°C for 11 weeks.
(Adapted from Ponting *et al.*, 1972).

Table 6. Effect of combined treatments on the browning index of potato slices 2h after cutting.

Antibrowning Agent	pH	Browning Index								
		var. Bintje			var. Van Gogh			var. Nicola		
		1mo.	5mo.	8mo.	1mo.	5mo.	8mo.	1mo.	5mo.	8mo.
0.3% AA + 0.5% citric acid	2.4	0	1	1	0	6	2	4	3	3
0.5% AA + 0.5% citric acid	2.4	0	2	0	1	3	2	6	1	2
0.3%AA + 0.3% citric acid + 0.1% $CaCl_2$	2.4	0	2	2	6	5	2	4	2	4
0.3% AA + 0.3% citric acid + 0.2% K sorbate	3.2	0	3	3	4	4	4	9	3	3
0.5% AA + 0.5% citric acid + 0.2% K sorbate	2.8	0	2	1	1	2	2	6	2	2
0.1% AA + 0.1% citric acid + 0.1% Na benzoate	3.5	0	4	2	2	7	3	4	8	6
0.5% citric acid + 0.005% 4-hexylresorcinol	2.6	0	1	2	0	3	2	5	3	3
Water	5.7	1	4	6	10	22	9	67	39	13

Dipping solution was applied at 5°C for 1 min in a ratio of 2 L of solution/kg of potato slices; slices were drained for 1 min and then kept for 2h at 23°C prior to browning evaluation.
(Adapted from Mattila *et al.*, 1993)

2.2.2 Physical Treatments and Browning Control

One of the most commonly used approaches to controlling enzymatic activity in fresh-cut products is the use of low temperature during handling, processing and storage. At low temperatures, not only is enzymatic activity reduced, but general metabolic rates are also lower, which assists in extending product shelf-life. Some of the physical methods suggested for application in post-harvest handling of fruits and vegetables have also been proposed for fresh-cut products. These include the use of modified/controlled atmospheres and gamma-irradiation. Non-thermal methods currently being investigated by food processors which may have application for fresh-cut products include treatment with high pressure treatments or high electric field pulses (Ohlsson, 1994).

2.2.2.a Reducing Oxygen Availability

It is important to consider that as a requirement of living tissues, fresh-cut products cannot be exposed to environments with complete removal of oxygen. Nevertheless, enzymatic browning can be delayed (in the presence of active enzyme and phenolic substrates) if oxygen is not available for the reaction to take place. In fruits and vegetables used for either conventional or fresh-cut processing it is a common practice to hold pre-prepared produce (already peeled, cut, etc) immersed in water, brine or syrup to retard diffusion of oxygen. However, tissue will brown when it is re-exposed to air. In addition, during the time the tissue is held, osmotic equilibrium may result in loss of solutes and imbibition of the storage solution.

Modified atmospheres are frequently used in packaging and/or storage of fruits and vegetables. These conditions as well as edible coatings can also be successfully adapted to fresh-cut fruits and vegetables (see Chapter X).

Modified Atmosphere Packaging. Among other benefits the use of modified or controlled atmospheres retards senescence and consequently extends storage life of products. Modified or controlled atmospheres should be seen as a supplement to an adequate management of temperature and controlled humidity (Kader, 1992).

Modified atmosphere packaging aims at the creation of an ideal gas composition in the package, which can be achieved through: (1) Commodity-generated modified atmosphere in the package, and (2) through the establishment of an active modified atmosphere in the package. However, it is important to avoid damaging low levels of oxygen or high levels of carbon dioxide, which lead to anaerobic respiration, resulting in the development of off-flavors and odors and increasing susceptibility to decay. Appropriate gas composition of modified atmosphere need to be experimentally determined for each particular product (Wills *et al.*, 1998). Using a moderate vacuum packaging with polyethylene (80 μm) for the storage of shredded Iceberg lettuce at 5°C, browning was inhibited over a 10 day period (Heimdal *et al.*, 1995). Browning of

commercially prepared cut lettuce was retarded in packaged product, where the atmosphere was altered by the respiring product. Visual quality of the cut lettuce packaged in sealed bags received an original score of 9 (excellent), after storage for 2 weeks at 2.8°C the score dropped to 7 (good), while samples stored in unsealed package received a score of 3 (poor). Modified atmosphere packaging was also efficient in controlling microbial buildup during storage (King *et al.*, 1991).

Edible Coatings. Shelf-life extension has also been investigated by enrobing fresh-cut products in edible coatings. Such thin layers of protective materials are applied to the surface of the fruit or vegetable as a replacement for the natural protective tissue (epidermis, peel). Edible coatings are used as a semipermeable barrier that helps reduce respiration, retard water loss and color changes, improve texture and mechanical integrity, improve handling characteristics, help retain volative flavor compounds and reduce microbial growth. It is possible to create a modified atmosphere enrobing fresh-cut produce in edible coating. (Baldwin *et al.,* 1995; Nisperos-Carriedo and Baldwin, 1996, Baldwin, 1996). Detailed information on edible coatings is presented in several reviews (Krochta *et al.,* 1994: Baldwin *et al.*, 1995a,b; Nisperos-Carriedo *et al.*, 1995).

Basically, edible coatings are comprised of one or more major component (polysaccharides, proteins, resins, waxes or oils), which may be improved by the addition of plasticizers, surfactants and emulsifiers. A propriate selection of edible coatings is important due to the hydrophilic nature of cut surfaces of many fresh-cut products. Some coatings may not adhere to such surfaces, others may offer good adherence, but may be poor barriers to moisture, or not resist to water vapor diffusion (Baldwin *et al*., 1995a,b). Lipid components confer important water-barrier characteristics to some coatings, however, they may present a drawback, because they may give a waxy or gummy mouthfeel to the product (Wong *et al.,* 1994). On the other hand, hydrophilic polymers (such as carboxymethyl cellulose) do not work well in reducing water loss of coated products, due to their poor moisture barrier characteristics (Baldwin *et al,* 1996). Emulsion coatings containing mixed components seem to have a better performance, such as coatings of casein and acetylated monoglyceride; when the pH is adequately adjusted, a tight matrix is formed trapping the lipid molecules (Krochta *et al.*, 1994). In addition some lipid components (such as acetylated monoglyceride) are solid at room temperature, and without an emulsifier (such as calcium caseinate) could not be used as a coating for fresh fruits and vegetables (Avena-Bustillos *et al.*, 1997). In the application of some coatings it is possible to induce the formation of cross-links between pectin molecules of the fresh-cut product surface and the coating (Wong *et al.,* 1994). Interestingly, different food additives can be incorporated into coating formulation, such as coatings with antioxidants (Baldwin *et al.*, 1995). The efficiency of ascorbic acid in delaying enzymatic browning in cut apple and potato was improved when incorporated in an edible coating formulation in comparison to dipping. A carboxymethylcellulose-based coating did not control enzymatic browning of cut apples and potatoes, but when such a coating was combined

with additives (antioxidant, acidulant and preservative), browning control was superior than dipping the fresh-cut produce in solutions with the same additives (Baldwin *et al.*, 1996). Examples of browning inhibition of apple slices have been described with different edible coatings, such as formulations containing casein and lipid (Avena-Bustillos and Krochta, 1993), or soybean protein (Kinzel, 1992).

2.2.2.b Reducing Temperature

Temperature management during handling is essential in minimizing the damaging effects of mechanical injury, because of the ability to low temperature to reduce metabolic reactions. Temperature has a tremendous effect on respiration rates, moreover it affects permeability of gases through the packaging films and also slows microbial growth. Fresh-cut products generally have higher respiration rates than the same intact produce, the respiration increase may vary from a few percent to over 100%. Moreover, the degree of respiration increase varies with temperature and commodity (Watada *et al.*, 1996). Storage temperature is a critical parameter in achieving maximum shelf-life of products. Refrigeration throughout the production chain up to consumption is of fundamental importance in extending the shelf-life of fresh-cut products. To ensure high quality products it is recommended that fresh-cut products are kept at temperatures just above freezing; nevertheless temperature needs to be adequately chosen in order to avoid damage such as chilling injury in sensitive commodities. A common practice in the preparation of fresh-cut products is rinsing the peeled and/or cut product in cold water, which helps lower the temperature in addition to removing cellular exudates released during the peeling and/or cutting of produce. De-watering of rinsed products is normally required to control decay. This is done commercially through centrifugation, but can also be achieved with forced air.

Although emphasis is normally placed on the use of low temperatures, there are examples of benefits of some heat treatments on browning control. Heat shock treatment (45°C for 105 min) of whole apples later used for preparing slices resulted in product with less browning and firmer texture than product prepared from non-heated fruit (Kim *et al.*, 1993). In conventional food processing, the most widely used methods for enzyme inactivation rely on heat application. Optimum PPO activity has been reported to vary with the source of the enzyme and reaction conditions (pH, substrate, etc). PPO from several plant sources exhibits maximum activity in the temperature range of 20 - 35°C. Many factors affect PPO heat stability, among them enzyme source, plant cultivar, molecular form (isozyme), and heat penetration into the tissue (Vamos-Vigyazo, 1981). PPO is not a very heat-stable enzyme; thermal inactivation occurs at temperatures higher than 40°C. Temperature stability of PPO depends on the source of the enzyme. Moreover, PPO thermostability is also influenced by cultivar, growing location and pH (Vamos-Vigyazo, 1981; Nicolas *et al.* 1994). Banana PPO is inactivated in 15 min at 80°C (Galeazzi and Sgarbieri, 1978), while green pea PPO required 29 min at 80°C, or 2.5 min at 90°C, and only 1 min at 95°C (Krotov *et al.*, 1971). Low temperature blanching may be effective in

preventing or controlling enzymatic activity in fresh-cut products. Blanching (95°C for 3 min) of ready-to-use pear cubes under aseptic conditions resulted in complete inhibition of enzymatic browning, with an acceptable texture reduction, as judged by a trained panel (Pittia *et al.*, 1999). Recently, heat shock treatment has been suggested as a new way to control browning in fresh-cut products. The mechanical injury caused by tissue wounding induces synthesis of enzymes, such as phenylalanine ammonia lyase (PAL), involved in phenolic metabolism leading to accumulation of phenolic compounds, which in turn can be potential substrates for PPO. Within 24 hr of cutting, iceberg lettuce cut into 2 x 2 cm pieces showed a 6- to 12-fold increase in PAL activity. A heat shock treatment on cut iceberg lettuce for 90 seconds at 45°C prevented such increase in PAL activity, which might offer a new alternative to control browning in fresh-cut products (Saltveit, 2000).

2.2.2.c Applying Gamma Radiation

Application of gamma radiation to fruits and vegetables has been used for insect and disease disinfestation, as well as to retard ripening and sprouting. Irradiation applied to fresh-cut carrots stored in microporous plastic bags, resulted in limited respiration increase due to wounding, and ethylene production was also reduced. Treatment was considered to increase shelf-life of the product (Chervin *et al.*, 1992). Nevertheless, the application of irradiation may bring about undesirable biochemical changes. In fact, enzymatic browning may be aggravated by irradiation treatments, which may alter the permeability of cell compartments favoring contact between PPO and its substrates (Mayer and Harel, 1991). Apples and pears irradiated as a quarantine treatment showed decreased firmness, which was cultivar dependent, and change in internal color of Gala and Granny Smith apples (Drake *et al.*, 1999). Endive samples that were irradiated revealed longitudinal internal pink-brown lines, which progressed to the entire vegetable piece becoming pink-brown. In contrast, the cut control discolored only on cut surfaces (Hanotel *et al.*, 1995). Such alterations may be an indication of cell damage, release of PPO and browning in the irradiated endive.

2.2.2.d Use of other Non-Thermal Technologies

High Pressure Technology. High pressure processing has applications in food preservation due to its potential effect on microorganisms and enzymes. Inactivation of deleterious enzymes has been achieved through application of high pressure technology (Hendrickx *et al., 1998; Seyderhelm et al., 1996; Weemaes et al.,* 1994). An important advantage of this new technology is that high pressure treatments at low temperatures have either no effect or a minimal effect on flavor and nutritional value of foods. However, high pressure processing may create new textures or tastes (Messens *et al.*, 1997).

While bacterial spores are highly resistant to pressure treatment, and over 1200 MPa is required for their inactivation, yeasts, molds, vegetative cells are pressure sensitive and can be inactivated by milder treatments at ~ 300-600 MPa. When aiming at enzyme inactivation, pressure requirements vary depending on the enzyme; some enzymes are resistant to 1000 MPa, others can be inactivated by a few hundred MPa at room temperature. High pressure has been considered as an alternative for irreversible inactivation of PPO (Hendrickx *et al.*, 1998). It has been observed that the application of low pressure results in pressure-induced membrane damage with consequent decompartmentalization and enzyme activation. In fact, pear PPO (cell-free extracts) was activated after pressure treatment at 400 MPa for 10 min at 25°C (Asaka and Hayashi, 1991). PPO activation was also described in low pressure treatments of crude carrot and apple extracts (Anese *et al.*, 1995).

PPO sensitivity to pressure varies with the enzyme source; while apricot PPO has been inactivated at ~100 MPa, and strawberry PPO at 400 MPa, potato and mushroom PPO required much higher pressures (~800 – 900 MPa). In addition, PPO inactivation by pressure is affected by pH (Anese *et al.* 1995).

Many studies have been carried out in model systems, cell-free or crude extracts, not real foods. Experiments carried out on whole foods revealed that high pressure treatment caused browning of mushrooms, apples and potatoes (Gomes and Ledward, 1996). It is known that food ingredients have a protective effect on enzyme pressure stability, and the efficiency of the pressure treatment depends on pH, temperature and treatment. When comparing the barostability of different food enzymes, PPO was second, after only peroxidase, as the most tolerant to pressure treatment duration (Seyderhelm *et al.*, 1996). Due to the poor effectiveness of lower pressure treatments on PPO activity, it has been suggested that its inhibition would require a combination of pressure treatment with one or more additional methods, such as blanching, modified atmospheres, and/or refrigeration (Anese *et al.*, 1995). Complete inactivation of enzymes is not expected with application of hydrostatic pressures compatible with maintenance of food tissue integrity (Whitaker, 1996).

Pulsed Electric Fields. The use of high intensity pulsed electric fields is a new technology that has been suggested to inactivate microorganisms and enzymes with minimal resultant temperature increase (Qin *et al.,* 1996). Application of high intensity electric field pulses on a culture of potato cells increased the release of PPO into the medium, with both increased intensity and duration of treatment (Knorr and Angersback, 1998). Preliminary results using model systems (enzyme solutions) resulted in large variations among enzymes. Although a reduction of 88% in pectinesterase activity has been reported in treated orange juice (Hye, W.Y. *et al.*, 2000),
a moderate activity reduction of 30-40% was described for PPO and peroxidase treated in buffer solutions (Ho *et al.*, 1997). Although this technology seems to offer potential applications to liquid foods, it still seems premature to recommend its use in fresh-cut products.

2.3 Other Color Changes

2.3.1 White Blush in Carrots

The bright orange color of fresh carrots can disappear in stored fresh-cut products, particularly when abrasion peeling is used. Carrots may develop "white blush", also known as "white bloom", a discoloration defect which results in the formation of a white layer of material on the surface of peeled carrots, giving a poor appearance to the product. Upon peeling, the protective superficial layer (epidermis) of carrots is removed, generally by abrasion, leaving cell debris and an irregular surface, which while moist presents the natural orange color of carrots. Once the carrots are exposed to air, they easily dehydrate, and the dried cell debris acquires a whitish color forming a white layer on the carrot surface. The disruption of surface tissues followed by dehydration in white blush formation was confirmed by scanning electron microscopy, when comparing carrots peeled with a knife and a razor sharp blade. Knife-peeled carrot surfaces appeared severely damaged, compressed, sloughed and separated from underlying tissue, therefore prone to dehydration. Razor-peeled carrot surfaces were cleaner and apparently only a thin layer of cells had been removed, resulting in a product that upon drying did not acquire the whitish appearance (Tatsumi *et al.,* 1991). At this stage, the quality defect can be reversed by dipping the carrots in water and allowing for rehydration (Cisneros-Zevallos *et al.*, 1995).

It has been suggested that with time, phenolic metabolism may be activated, inducing increases in lignin, phenolic compounds and phenylalanine ammonia lyase activity, and irreversible color change takes place (Cisneros-Zevallos *et al.*, 1995; Howard and Griffin, 1993). A positive test for lignin was described in the white abraded material. The severity of the lignification will depend on the harshness of the peeling process (coarse sand paper > fine sand paper > stainless steel pad). Hand peeling of carrots with a razor blade resulted in no development of "white blush". As the lignification process is enzyme-mediated, some dipping treatments directed to inactivate the responsible enzymes have been tested. A successful result was obtained with a treatment combining heat inactivation and an acidic environment. Carrots peeled with coarse sand paper and dipped for 20 – 30 sec in a 2% citric acid solution at 70°C did not develop the defect for at least 5 weeks in cold storage; product taste was not affected by the treatment (Bolin and Huxsoll, 1991). Edible films have also been shown to protect carrots from this quality defect (Sargent *et al.*, 1994). Sensory results showed preference for carrots coated with an edible cellulose-based coating, due to a fresh appearance (Howard and Dewi, 1995), since consumers perceive white blush carrots as not fresh or aged. Losses of carotenes have been described in fresh-cut carrots; with the application of an edible coating a 50% retention of β-carotene was obtained after 28 days of storage, compared to 33% retention in the control (Li and Barth, 1998). Edible coating emulsions containing caseinate-stearic acid were effective in reducing the white blush defect of carrots (Avena-Bustillos *et al.*, 1994).

2.3.2 Yellowing or Degreening

Reduction of green pigmentation and therefore the predominance of yellow pigments is a normal process in ripening or senescence of many fruits and vegetables; such changes can be accelerated by ethylene. In fresh-cut products, the stress imparted by wounding results in increased respiration, ethylene production, and other alterations. In fact, degreening is also observed during storage of leafy and other green fresh-cut products. Shredded Iceberg lettuce darkens during storage, particularly at high temperatures. Simultaneously, loss of green pigmentation was observed (Bolin *et al.*, 1977; Bolin and Huxsoll, 1991). Studying the susceptibility of fresh-cut Baby and Romaine lettuces to browning, it was observed that samples of photosynthetic tissue became lighter during storage. In fact, while there is mid-rib discoloration, the photosynthetic tissues also develop browning *and* loss of green pigments (Castañer *et al.*, 1999). In a study on coleslaw color, changes went noticed over a period of cold storage; changes were from green to a lighter white color suggesting chlorophyll degradation resulting in colorless compounds (Heaton *et al.*, 1996). It is still unclear what are the reactions involved in the loss of chlorophyll in green fresh-cut products. During the preparation steps of fresh-cut products, there is release of acids and enzymes, and both could be involved in the loss of green pigmentation.

The visual quality of broccoli is lost when florets turn yellow; retention of green color has been attained with the use of modified atmosphere packaging and storage at 10°C (Barth *et al.*, 1993). These authors found that within 48 hr the carbon dioxide concentration inside broccoli packages reached equilibrium at ~8% and oxygen content was 10%, causing a reduction of respiration rate. Modified atmosphere packaging contributed to significantly higher retention of green color in broccoli, as indicated by the total chlorophyll levels and color determination (hue angle). In contrast, in less than 72 hr non-packaged samples lost about 20% of initial chlorophyll content. Although there was ethylene accumulation during storage, it is suggested that the elevated carbon dioxide atmosphere counteracted ethylene effects, thus preventing chlorophyll degradation. Furthermore, packaging of broccoli spears resulted in improved retention of vitamin C.

In a study of texture improvement, calcium chloride treated fresh-cut green pepper and non-treated control stored at 10°C had significant losses in green color after 4 days of storage. Calcium-treated samples stored at 5°C were significantly better in all sensory attributes by day 4 and their superiority was maintained throughout the 8-day storage period (Barrett, unpublished).

3. Prevention of Texture Loss in Fresh-cut Products

Appearance of a food product plays an important role on consumer's evaluation; it has been estimated that 95% of American consumers take appearance into account in their purchases of fruits and vegetables (Shewfelt, 1994). As mentioned earlier, color has a great impact on appearance

consideration, but quality loss is also observed with changes in texture (Figure 1), another important quality criteria for many fruit and vegetable products.

While genetic background is the major contributor to the texture of a plant food, other factors, such as morphology, cell wall-middle lamella structure, cell turgor, water content, and biochemical components, all affect texture (Harker *et al.*, 1997). In addition, texture is also affected by growing conditions, including environmental factors and production practices (Sams, 1999). After harvesting it is important to store fruits and vegetables at the appropriate temperature and relative humidity to preserve their quality. Storage temperature has a major effect on water, weight loss and metabolic activity.

In general, perishability of intact fruits and vegetables correlates well with respiration rates; produce with a high respiration rate tends to be more perishable. In fresh-cut products, as a result of wounding, respiration is elevated compared to the intact produce. Moreover, the extent of wounding also affects the shelf-life of products. Hand tearing of lettuce has been shown to be less damaging than slicing with rotating knives, and reduction of lettuce piece size shortens shelf-life (Bolin and Huxsoll, 1991).

3.1 Fruit and Vegetable Tissue Firming

During fruit ripening one of the most notable changes is softening, which is related to biochemical alterations at the cell wall, middle lamella and membrane levels. Although pectic enzymes, polygalacturonase and pectin methylesterase, have been attributed to a significant role in the softening process, the precise mechanism is still unclear.

3.1.1 Calcium and/or Heat Treatments

It is well known that calcium is involved in maintaining the textural quality of produce. Calcium ions form cross-links, or bridges between free carboxyl groups of the pectin chains, resulting in strenghtening of the cell wall. A common treatment used to improve tissue firmness is to dip fruit or vegetable pieces in calcium solutions, as described for strawberries (Main *et al.,* 1986), pears and strawberries (Rosen and Kader, 1989), and shredded carrots (Izumi and Watada, 1994), among others. In contrast, calcium treatment was not effective in carrot slices and sticks, a fact attributed to insufficient calcium absorption by the tissue, since the levels of calcium were two and three times higher in shredded carrots than in sticks and slices, respectively. In addition, increasing the concentration of $CaCl_2$ in the dip solution (0.5% or 1%) brought an increase in the tissue calcium content of treated samples, without a subsequent correlation with product texture (Izumi and Watada, 1994).

A combined treatment associating low temperature blanching to activate the enzyme pectinesterase (PE) prior to the calcium dip is helpful in preserving fruit texture. PE brings about the de-esterification of pectin, thus increasing the number of calcium binding sites. To such mechanism has been attributed the firming effect observed in apple slices kept at 38°C for 6 days immediately after harvest, and sliced and dipped in calcium solution after 6 months of cold storage

(Lidster *et al.*, 1979). In fresh-cut melon cylinders dipped in calcium chloride solutions at different temperatures (Luna-Guzman *et al.* 1999), texture was firmer in samples treated at 60°C (77% improvement in firmness), than at 40°C (58% improvement) and 20°C (45% improvement).

Frequently, calcium chloride has been used as a firming agent, however, it may confer undesirable bitterness to the product. Fresh-cut cantaloupe cylinders dipped in calcium lactate solutions resulted in a textural improvement similar to calcium chloride treated fruit cylinders. Sensory evaluation indicated that results were better, e.g. bitterness and a more detectable melon flavor was perceived. Fresh-cut cantaloupe cylinders treated by a combination of heat treatment (60°C) and calcium lactate dip were not significantly different either in bitterness or firmness, in relation to fruit treated at 25°C (Luna-Guzman and Barrett, 2000).

Heat treatment alone has been shown to have the potential to benefit product texture. In a comparison of 11 apple cultivars, heat treatment of whole fruit resulted in firmer products when compared with non-heated fruit; the best firmness improvement was obtained with Golden Delicious and Delicious apples (Kim *et al.*, 1993). Heat treatment of whole apples improved apple slice firmness, but the storage temperature of whole fruit after heating had a significant effect on product firmness; except for fruit of the cultivar McIntosh. Heat-treated apples stored at 2°C were firmer than products from fruit kept at 10, 18 and 25°C for 7 days. Slices prepared from heat-treated apples showed increased firmness during storage of up to 7 days for Golden Delicious (firmness 34% higher than on day zero of storage) and up to 14 days for Delicious apple (48% higher firmness than at the beginning of storage). With longer storage times there was a decrease in firmness for both cultivars (Kim *et al.*, 1994).

3.1.2 Use of Modified Atmosphere Packaging

Controlled atmospheres retard senescence, lower respiration rates and slow the rate of tissue softening (Kader, 1992). Texture loss has been reported to decrease in controlled atmosphere packaging of fruit. Strawberry slices kept under controlled atmosphere for a week had comparable firmness to whole and to freshly-sliced fruit (Rosen and Kader, 1989). The authors suggest that the effect of controlled atmosphere on firmness appeared to be cultivar-dependent.

Storage stability of halved fruits was evaluated in a combination treatment which included chemical dips to prevent browning and retard texture loss, complemented by different storage conditions. Table 7 presents the texture results for peach halves stored in: (1) sealed packages where oxygen was being consumed with accumulation of carbon dioxide, and (2) sealed packages containing an oxygen scavenger; the latter treatment gave better results. Interestingly, a peach cultivar with soft texture, Suncrest, even showed an increase (+0.5 N/wk) in fruit firmness during the first few weeks of storage with oxygen scavengers. Similar storage conditions were not successful in the treatment of pears. The authors suggest as an optimum treatment for halved peaches and apricots a combination of a dip in 2% calcium chloride and 1% zinc

chloride, followed by packaging with an oxygen escavenger and storage at 0 - 2°C (Bolin and Huxsoll, 1989).

Table 7. Texture loss in fresh-cut freestone peach halves stored for 7 weeks at 2°C.

Peach variety	Initial Texture (N)	Modified Atmosphere		With O_2 scavenger	
		Texture (N)	Rate of loss (N/week)	Texture (N)	Rate of loss (N/week)
Fairmont	>21	8.0	1.7	17.0	0.5
Suncrest	7	4.0	0.5	10.5	+0.5
Flamecrest	21	3.9	2.4	7.6	1.9

Texture measurement units in Newtons (N). Adapted from Bolin and Huxsoll, 1989

3.3 Water Loss Prevention

After harvest, produce must utilize internal moisture solely; water lost through transpiration cannot be replaced. Although plant tissues are mainly composed of water, even small changes in water content may have a large impact on produce quality, causing losses, which may result in a few hours under dry and warm conditions. Water losses of 3 and 5% in spinach and apple, respectively, render these commodities unmarketable (Sams, 1999). Crispness of fresh produce is related to turgor pressure, whose loss may also contribute to softening. Leafy vegetables are particularly susceptible to desiccation because of their large surface-to-volume ratios; moreover loose leaves, such as spinach, are more prone to desiccation than a compact head, such as a whole lettuce head (Salunkhe and Desai, 1984). As a consequence of water loss, appearance changes such as wilting, reduced crispness may occur.

Fresh-cut products tend to be more vulnerable to water losses because they are no longer intact after peeling and cutting or shredding, slicing, etc. Peel or skin is a very important barrier to loss of turgor and desiccation; many commodities have a protective waxy coating, highly resistant to water loss. Evidently, peel removal renders commodities more perishable. The mechanical injury brought on by cutting and the method used, directly expose the internal tissues to the atmosphere, promoting desiccation. The shredding or slicing operations result in increased surface area, an additional problem. Moreover, mechanical injury brings about physiological responses, such as respiration increase and potentially ethylene production, responses that shorten the life of a commodity. When rinsing of products is done after cutting, this is frequently followed by centrifugation. If accelerated centrifugation speed or long centrifugation times are applied increased desiccation can result, as reported for

cut lettuce (Bolin and Huxsoll, 1989). Appropriate handling techniques including temperature and relative humidity control can help minimize the rate of water loss. Reduction of water loss can be achieved basically by decreasing the capacity of the surrounding air to hold water, which can be obtained by lowering the temperature and/or increasing the relative humidity. To reduce the rate of water loss in cool storage, it is also important to restrict the air movement around the commodities (Wills *et al.*, 1998). The primary parameter affecting celery quality is water loss; small reductions in moisture (2.5 – 5%) may lead to flaccidity, shriveling, wrinkling and pithiness. Significant increase in moisture retention by celery sticks were described with the application of a caseinate-acetylated monoglyceride coating (Avena-Bustillos *et al.*, 1997). Additionally, it is important to point out that appropriate packaging is of enormous importance in preserving fresh-cut products.

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Appendix. Evaluation of Enzymatic Browning

Different authors have used somewhat different methods to measure the intensity of enzymatic browning, and some tried to establish correlations of browning with PPO activity and/or phenolic substrates content. Frequently browning evaluation is based on reflectance measurements on exposed surfaces, such as cut fruits and vegetables or produce homogenates. Laboratory assays commonly involve extraction of browning products and measurement of absorbance at particular wavelengths. Nevertheless, as not all PPO products are soluble, some authors have developed methods that also evaluate the insoluble colored products.

Following we present a brief description of some of the methods that were used by authors whose results are displayed in tables included in this chapter. It is also included a more complete approach for assessing susceptibility to browning, and at last we present a visual assay that can be used in assessing produce varieties tendency to discoloration.

Reflectance measurements. Table1 presents results in DL*; decrease in L* indicates darkening of the samples.

Homogenates (purees) of previously dried apricots were poured in small petri dishes and reflectance measurements (L* lightness; a* green/red; b* blue/yellow chromaticity) were taken with a Minolta CR300 chromameter. The authors determined the difference between measurements taken from oxidized and non-oxidized (added of enzyme inhibitors) samples, and expressed the results in DL*, Da* and Db*.

Radi, M., Mahrouz, M., Jaoud, A., Tacchini, M., Aubert, S. and Amiot, M.J. (1997) Phenolic composition, browning susceptibility, and carotenoid content of several apricot cultivars at maturity. *HortScience* 32(6):1087-1091.

Browning Index results were expressed on Tables 2 and 6.

Evaluation based on sensory evaluation by a trained panel.

Twenty potato slices (5 mm thick) were left to stand at 23°c for 30, 60 and 120 min, and discoloration was evaluated by comparison with slices that had just been cut. Results were scored by browning grades from “0” (no color change) to “3” (strong change). To each browning grade, a coefficient was attributed, as follows: “0” browning grade $\Rightarrow$ coefficient 0; “1” browning grade $\Rightarrow$ 1; “2” browning grade $\Rightarrow$ 5; “3” browning grade $\Rightarrow$ 10.

Example of a Browning Index calculation. Considering that fro the 20 potato slices evaluated, 10 received a grade “0”, 5 a grade 1, 3 a grade 2 and 2 a grade 3, the final Browning Index would be:

$$(10 \times 0) + (5 \times 1) + (3 \times 5) + (2 \times 10) = 40$$

Mattila, M., Ahvenainen, R. and Hurme, E. (!993) Prevention of browning of pre-peeled potato. In COST 94, Proc. Workshop “Systems and operations for post-harvest quality”, J. De Baerdemaker et al., eds, Leuven, Belgium.

Loss of Reflectance data were presented on Table 5. Reflectance measurements were made by reading total reflectance from apple slices rotated to 3 positions (~120° apart), and then averaging these readings to obtain the final reflectance value. Such result was compared to readings from fresh cut apple

slices to calculate percent reflectance loss. The authors found that for apple slices loss of reflectance correlated better with the subjective evaluation of color than the “a” or “b” values.

Ponting, J.D., Jackson, R. and Watters, G. (1972) Refrigerated apple slices: preservative effects of ascorbic acid, calcium and sulfites. *J. Food Sci.* 37:434-436.

Estimate of apple susceptibility to browning. Amiot *et a.* (1992) measured:
(1) Absorbance at 400 nm of an apple extract containing soluble pigment formed during the browning reaction. For details on the methods used, please, refer to the paper cited below.
(2) Lightness (L*) of the pellets obtained after centrifugation during the preparation of the soluble pigments extract. L* results were related to the insoluble brown pigments.

The authors suggested that the normalized sum of A_{400} and L* be used to express the degree of browning.

Amiot, M.J., Tacchini, M., Aubert, S. and Nicolas, J. (1992) Phenolic composition and browning susceptibility of various apple cultivars at maturity. *J. Food Sci*. 57(4):958-962.

Note: Visual observation of browning is poorly correlated with measurements of absorbance at only one wavelength (Nicolas *et al.*, 1993). Depending on the pigments formed during browning, there may be a wide variation (360 – 500 nm) of maximum optical absorption (Amiot *et al.*, 1997). For a detailed discussion on measuments of browning refer to Nicolas *et al.* (1993) and Macheix *et al.* (1990).

For a visual evaluation of browning potential, which can be helpful in selecting cultivars with lower browning tendency, a quick assay was described by Kader and Chordas (1984).
(A)PPO activity evaluation: To slices (3-4 cm diameter) of fruit or vegetable add one drop of a freshly prepared 0.1 *M* solution of catechol in 0.1 *M* citric acid-phosphate buffer pH 6.2 (PPO substrate). Let rest for 6 min and then compare the samples, and score the browning intensity. The discoloration is rated on a progressive 1-to-5 scale.

According to the authors, the natural PPO substrate content does not interferewith the test within the 6 min duration of the assay.
(B)PPO substrates evaluation: To slices (3-4 cm diameter) of fruit or vegetable add one drop of each of the following solutions in succession: a 10% sodium nitrite, 20% ureas and 10% acetic acid. Let rest for 4 min and then apply 2 drops of 8% sodium hydroxide solution.

This test is based on a color reaction developed by endogenous phenolic compounds with the reactives added to the fruit or vegetable slice. The intensity of the deep cherry-red color developed during the reaction depends on the amount of phenolic compounds present in the tissue. The result is rated on a 1-to-5 scale, from the less colored to the most intensely colored sample, according to the chart presented in the cited paper.

Kader, A.A. and Chordas, A. (1984) Evaluating the browning potential of peaches. *California Agriculture* (3/4):14-15.