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Discoloration in Raw and Processed Fruits and Vegetables

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Discoloration in fruits and vegetables is reviewed in relation to the chemical and biochemical causes of black, brown, red, yellow, and green discolorations. In raw materials, only a limited understanding has so far been achieved of the internal black and brown discolorations. The biochemical signaling pathways triggered by wounding or chilling-storage, the nature of the enzymes and reactive oxygen species involved, and the identity of the phenolic compounds oxidized are areas where further information is desirable. In processed materials, a greater comprehension is needed of the role of ascorbic acid reactions in the browning of fruits and "pinking" of Brassicaceous vegetables, and more information is desirable on the structure and properties of the discoloring pigments in many products. It is concluded that a greater knowledge of these areas, and of the naturally-occurring constituents that can accelerate or inhibit the causative reactions, would lead to the development of more efficient methods of controlling fruit and vegetable discolorations.

Keywords blackening, browning, reddening, yellowing, greening

INTRODUCTION

Discolorations that bear little resemblance to the expected color of fruits and vegetables have a major impact on saleable quality, yet the reactions leading to these discolorations are often only partly comprehended, or are inferred from other evidence. This is due to the inherent complexity of the reactions involved, to the variability in raw material, and to the practical difficulties in isolating and characterizing the newly-formed pigments.

In this review, the current evidence for the chemical and biochemical causes of selected examples of fruit and vegetable discolorations are described. Only discolorations that arise from reactions between naturally-occurring constituents have been considered, thereby excluding discolorations associated with microbial activity that can occur during the growth of fruits and vegetables, during postharvest storage of raw materials, or during storage after processing.

The review deals, in order, with black, then brown, red, yellow, and green discolorations. For each discoloration, raw fruits and vegetables are considered prior to processed products.

BLACK DISCOLORATIONS

Enzymatic Blackening/Browning in Raw Materials

The blackening/browning reactions that occur in raw fruits and vegetables as a result of abiotic stresses are generally accepted as being due to enzymatic oxidation of phenolic compounds. For most discolorations, however, there is a lack of specific knowledge of the enzymes, of the free radical or other reduced state of oxygen, and of the chemical structure of the phenolic compounds involved. Polyphenol oxidase (PPO) has been reported to be inducible upon biotic or abiotic wounding, though observations of inducible PPO activity have frequently been confounded by failure to distinguish PPO induction from loss of PPO latency, or to distinguish PPO from peroxidase (POD) activity. These problems may be exacerbated by the susceptibility of PPO to "reaction inactivation" due to the quinones formed, giving rise to low measured activities. Evidence for the involvement of POD/H_2O_2 in black or brown discolorations in raw fruits and vegetables has so far been limited. However, it has been demonstrated that POD/H_2O_2 oxidizes dihydroxyphenylalanine to melanin-like compounds in broad bean leaves, and that brown pigments can be formed in aging tobacco leaves and onion scales from the POD/H_2O_2 oxidation of chlorogenic acid and quercetin respectively (Takahama, 2004). It was further proposed that phenoxyl radicals formed by POD-dependent reactions were

reduced by ascorbate in vacuoles. The dehydroascorbate produced could then be transported to the cytoplasm to be reduced by the NADH-dependent glutathione/glutathione reductase system before returning as ascorbate to the vacuoles. This suggested that as long as ascorbate was present in vacuoles, the accumulation of phenolic oxidation products was not significant.

H_2O_2 is the first stable compound among the reactive oxygen species and can be generated during normal cellular metabolism after various abiotic stresses, such as an excess of light, drought, or cold (Dat et al., 2000). Mechanical wounding also stimulates the leaves of several plant species to produce H_2O_2, locally and systemically, due to oligogalacturonides released by a systemically wound-induced polygalacturonase (PG) and to a membrane-bound NADPH oxidase complex (Orozco-Cárdenas and Ryan, 1999; Fry et al., 2001). H_2O_2 can also be formed by reduction of O_2 in vivo by the action of amine oxidases, oxalate oxidases, and related enzymes, and by superoxide dismutase (SOD) from apoplastically formed superoxide ion. POD, besides its main function in H_2O_2 elimination, can catalyze the formation of superoxide ion and H_2O_2 by a complex reaction in which NADH is oxidized using trace amounts of H_2O_2 first produced by non-enzymatic breakdown of NADH. Next the NAD radical formed reduces O_2 to superoxide ion, some of which dismutates to H_2O_2 and O_2 (Lamb and Dixon, 1997). Potential additional sources of H_2O_2 are ascorbic acid (Green and Fry, 2005), and the brown pigments themselves (Takahama, 2004).

Low calcium levels are frequently correlated with internal browning in raw fruits and vegetables for reasons that are not fully understood at present. Symptoms are observed in young expanding leaves, such as in "tipburn" of leafy vegetables, in enclosed tissues, such as in "brown heart" of leafy vegetables or in "black heart" of celery, and in "bitter pit" in apples where tissues are fed principally by the phloem rather than the xylem. Calcium deficiency disorders arise when insufficient calcium is available to developing tissues. It has been generally accepted that this is linked to restricted transpiration. Transpiration is low in young leaves and in enclosed tissues, and in fruit that cannot mobilize the calcium from older tissues and redistribute it via the phloem, the developing tissues rely on the immediate, transpiration-dependent supply in the xylem which therefore limits the available calcium. However, in fleshy fruits, an alternative hypothesis has been proposed that calcium deficiency may be due to hormonal mechanisms developed by plants for the restriction of calcium transport in order to maintain rapid fruit growth (Saure, 2005). High levels of hormones, mainly gibberellins, could be responsible for the decline in calcium uptake during periods of rapid growth. Cytosolic calcium concentration is known to stabilize cell membranes, and is a key regulator of plant defences to such challenges as mechanical perturbation, cooling, heat shock, acute salt stress, hyper-osmotic stress, anoxia, and exposure to oxidative stress (White and Broadley, 2003). Thus, under low calcium conditions, cellular defence regulation may be disrupted and this leads to accumulation of reactive oxygen species that cause oxidation of phenolic compounds and the resulting discoloration.

Phenolic compounds that are closely associated with structural and defense-related functions in plants arise via the phenylpropanoid pathway. On wounding of fruits and vegetables, chemical signals originate at the site of injury that propagate into adjacent tissue where a number of physiological responses are induced including de novo synthesis of phenylalanine ammonia lyase (PAL), the initial rate-controlling enzyme in phenolic synthesis. This leads to the accumulation of phenolic compounds, and subsequent enzymatic oxidation and tissue discoloration. The discoloration is invariably black or brown depending on the structure of the phenolic compounds and the nature of the oxidative enzyme. The phenolic amino acids tyrosine and DOPA are oxidized to melanins, whilst oxidation of non-nitrogenous phenolic compounds, such as the catechins and chlorogenic acid, yields brown pigments that are less chemically defined.

Black Discolorations in Raw Fruits

Greying/Blackening of Avocados. Physiological disorders occur in avocados during storage at low temperatures. The first external signs are dark patches on the skin and a general greyish discoloration of the internal flesh. Other disorders include outer flesh and skin blackening. The fruit is very susceptible during the climacteric rise, especially at the peak of respiratory activity. The presence of ethylene at chill temperatures increases sensitivity to internal greying. Short heat treatment of avocados at 38°C during the ripening period reduced the maximum rate of ethylene production (Florissen et al., 1996). However, this was not correlated with lower levels of 1-aminocyclopropane-1-carboxylic acid (ACC), implying that the ACC degrading enzymes may have been inactivated by the treatment. Treatment of avocados with 1-methylcyclopropene (1-MCP) was shown to reduce internal greying but not the outer black discolorations (Woolf et al., 2005), suggesting that internal greying is linked with senescence-like reactions whilst outer blackening is not. Avocados having low calcium content are especially sensitive to chilling injury. Fruit infiltrated with calcium chloride had an increased storage life and showed a greatly reduced climacteric pattern of ethylene evolution (Wills and Tirmazi, 1982). It has been concluded that POD activity in avocado fruit mesocarp has no role to play in the development of skin blackening (Zauberman et al., 1985). However, this conclusion should be treated with some caution as POD activity in isolated skin tissue was not measured in this study.

Blackening of Raw Peaches and Nectarines. Blackening of the skin of raw peaches is related to abrasion damage during fruit handling that leads specifically to injury of the exocarp tissues, leaving the underlying mesocarp sound and turgid. The damage in combination with iron, copper, or aluminum contamination, feasibly derived from preharvest sprays, are requirements for this discoloration. It has been suggested that the formation of strongly purple ferric complexes with anthocyanins may contribute to the black discoloration of these fruits (Cheng and Crisosto, 1997), although the conclusion that

cyanidin-3-glucoside formed a complex with ferric ion in a model system with an anthocyanin/ferric ion molar ratio of 2/1 conflicted with the evidence presented which suggested a 1/2 complex. The aqueous chemistry of ferric ion was apparently not taken into account in this study and it is feasible that the rapid condensation that occurs between ferric aquo ions at pH >2–3 could have contributed to the high ferric ion titration values and to some of the color changes observed.

Raw Pineapple Blackening. Internal blackening, or "*black-heart*," is an important physiological disorder of pineapples that arises as a result of storage at chilling temperatures. The early symptoms appear as dark spots at the base of fruitlets near the core after about 4 days at ambient temperature following chill storage. Longer storage causes the spots to coalesce and the tissue eventually becomes a dark mass. However, considerable variability in intensity and incidence has been observed due to variations in growing conditions. Internal blackening has even been found at harvest as a result of chilling in the field. It has been linked with high levels of oxygen in chill storage (Paull and Rohrbach, 1985), with the presence of ethylene (Selvarajah et al., 2001), and with calcium concentration and its distribution in the fruit (Hewajulige et al., 2003). PPO activity, an enhanced PAL activity and a reduction in the rate of increase of ascorbate peroxidase activity have also been correlated with the black discoloration (Zhou et al., 2003).

Black Discolorations in Raw Vegetables

"Blackspot" in Potatoes. Internal bruising or "*blackspot*" in healthy potatoes is a blue-grey zone that forms sub-epidermally in the vascular region of the potato tuber, the stem end of the tuber being most sensitive. It occurs after relatively minor impacts with the skin often showing no visible signs of damage. The bruising develops over a period of 1–3 days. Whilst no single factor determines susceptibility, cultivar is generally accepted to be important. "Blackspot" susceptibility tends to be higher in long, hot, and dry growing seasons apparently due to physiologically older tubers being produced that have higher levels of tyrosine (Corsini, 2001). It was enhanced by the use of low potassium fertilizer levels that have been associated with alterations in cell membrane structure and with water relationships within the tuber tissues (McGarry et al., 1996; MacNabnay et al., 1999). "Blackspot" has also been shown to be greater in tubers with higher specific gravities (Baritelle and Hyde, 2003), and strongly increased when tubers were handled under chilled conditions (Corsini et al., 1999).

Conflicting evidence exists for the enzymes and substrates involved in "blackspot" pigment formation. In one study, no evidence was found for any relationship with PPO activity (Schaller and Amberger, 1974), whilst another suggested that POD may be involved (Weaver and Hautala, 1970). Poor correlations have been reported between free tyrosine and "blackspot" across susceptible and resistant cultivars (Mondy and Munshi, 1993) although tyrosine released from protein by a proteinase could be enzymatically oxidised to give the black pigment (Sabba and Dean, 1994). Ascorbic acid and susceptibility to "blackspot" do not appear to be related (Thornton and Workman, 1987; Mondy and Munshi, 1993; Friedman, 1997), a finding that tends to support a pathway that is independent of the activity of both PPO and POD. However, a subcellular redistribution of PPO 12 h after impact has been found and this coincided with a loss of membrane integrity (Partington et al., 1999). The importance of cellular integrity has also been suggested by analysing for cation effluxes and enzyme activities at impact sites and calcium ion was hypothesised as having a crucial role to play (Lærke et al., 2000). In a using two cultivars, one with low and the other with high susceptibility to "blackspot," it has been shown that changes in the color of internal bruises could not be ascribed to changes in discoloration potential during tuber growth and storage (Lærke et al., 2002). These results provided supporting evidence that intracellular compartmentation was the determining factor for "blackspot" susceptibility in potato tubers. The importance of calcium has been further suggested by the finding that increased concentrations in potatoe tissue are linked with a reduction in the severity of "blackspot" (Bamberg and Palta, 2003). Superoxide ion may be generated as a result of wounding potato tubers and it has been proposed that it may be the main PPO substrate involved in the production of melanin in "blackspot" (Johnson et al., 2003). In this study, addition of SOD apparently inhibited pigment synthesis, whilst catalase addition had little effect. Although based on oxidized protein measurements rather than directly on pigment inhibition data, these results were interpreted as showing that superoxide ion was involved in "blackspot" formation and H_2O_2 was not implicated.

Blackspot related pigments have been partially purified from bruised tubers of two commercial potato cultivars (Stevens and Davelaar, 1996). Chemical characterization showed that the partially purified pigments consisted of a protein matrix and a relatively small amount of covalently bound constituents. These polymeric structures absorbed light throughout the visible spectrum without any maximum. Hydriodic acid treatment indicated that they did not contain the classical, black eumelanin pigment. Quinic acid was detectable in hydrolysates of the pigments from only one of the cultivars, which suggested that chlorogenic acid may have been associated with blackspot formation, but was not essential for the discoloration. The results supported the hypothesis that "blackspot" pigments are products of non-regulated reactions between nucleophilic amino acid residues in proteins, and PPO (tyrosinase)-derived quinones. This indicated that "blackspot" formation most probably took place in disintegrated cells. Further studies have suggested that the pigments carry cross-linked 5,6-dihydroxyindole-2-carboxylic acid and benzothiazine units suggesting that both tyrosine and cysteine were incorporated via the PPO (tyrosinase) catalysed pathway of melanogenesis (Stevens et al., 1998).

Black Discolorations in Processed Vegetables

After-Cooking Blackening of Potatoes

A dark grey to black discoloration develops on holding many varieties of potatoes after cooking. The defect is also found in

blanched potatoes (e.g. frozen french fries) and in canned and dehydrated potato products. This discoloration has been extensively studied and it has been shown that during the cooking of potatoes, a colorless ferrous-chlorogenic acid complex is formed which, on standing, slowly oxidises to the black ferric complex (Hughes and Evans, 1969). The discoloration is inhibited by the presence of citric acid in the potato, and can be reduced by addition of citric acid to the cooking water. Both chlorogenic acid and citric acid vary in their distribution in the tuber, the concentration of the former being higher at the stem end and that of the latter at the bud end. Hence blackening tends to be more prevalent at the stem end of the tuber. After-cooking blackening is influenced by a number of factors such as variety, soils, fertilizers, and season (Wang-Pruski and Nowak, 2004). Bruising, chilling, and storage in light after harvesting can also cause chlorogenic acid levels to increase, and these factors would be expected to increase the incidence of after-cooking blackening, provided that citric acid levels remained constant or decreased. Photo-induced changes in the concentrations of individual chlorogenic acid isomers in potatoes have been shown to have no effect on the development of after-cooking blackening (Griffiths and Bain, 1997).

In addition to agronomic factors, the cooking conditions can have a significant effect on the formation and perception of after-cooking blackening. The pH of the cooking water can affect not only the pigment formation rate but also the final color observed. For example, iron-chlorogenic acid complexes vary in color from green, at pH 5.5, to grey-blue, at pH 6.5–7.0 (Hughes and Swain, 1962). At higher pH values, the complexes have a brown tinge. Cooking water pH values tend to increase when hard water is used and would be expected to depend on the potato/water weight ratio. Increasing pH values also appears to increase the number of chlorogenic acid molecules bound per ferric ion. Model system studies have suggested that one ferric ion formed a complex with either two (Okunev and Pokrovskaya, 1987), or up to three chlorogenic acid molecules (Ameziane et al., 1996). However, little information is available on the aqueous chemistry of ferric ion under these conditions and it is feasible that condensation between aquo species of ferric ion could contribute to color changes.

The correlation of after-cooking blackening with chlorogenic acid levels has been found to break down occasionally (Griffiths et al., 1992) for reasons that continue to be obscure. A possible explanation is that ascorbic acid and other reducing compounds in the potato have inhibited iron oxidation. Although ascorbic acid can also form a dark pigment with iron, similar to the after-cooking pigment in potatoes (Muneta and Kaisaki, 1985), the ascorbate complex with iron (in the ferrous state) is apparently much less stable than the ferric-chlorogenic acid complexes and is, therefore, unlikely to make a significant contribution to after-cooking blackening. Competition with other phenolic compounds for the available ferrous ion may make a contribution to poor correlations, although chlorogenic acid has been found to be the strongest ferrous ion chelator of a number of phenolics (Andjelkovic et al., 2006).

BROWN DISCOLORATION

Enzymatic Browning in Raw Fruits

Raw Apple Browning

Enzymatic browning of raw apple products involving phenolic oxidation reactions catalysed by PPO has been extensively reviewed (Nicolas et al., 1994). This knowledge has led to the development of transgenic material in which the PPO gene has been suppressed and this is expected to lead to varieties that have low browning potentials.

Apple *"superficial scald"* is a severe physiological disorder that appears on the skin after several months in chill storage, during or after removal. It appears as a diffuse browning of the skin, somewhat roughened in severe cases, which becomes more extensive after a few days at room temperature. "Superficial scald" tends to develop mainly on green skinned apples and on the un-blushed areas on red cultivars, and susceptibility is usually higher in less mature fruit. The disorder has been linked with the synthesis and oxidation of α-farnesene in the fruit peel to conjugated to trienols that are toxic to the fruit (Whitaker et al., 1998). Ethylene could be involved in the formation of scald as treatment with 1-MCP has been shown to reduce internal ethylene concentration and scald incidence (Pechous et al., 2005). It also inhibited α-farnesene production, suggesting that ethylene induces transcription of key genes involved in α-farnesene biosynthesis. 1-MCP suppressed the increases in the α-farnesene synthase gene early in storage, although not permanently in a scald-susceptible cultivar. It has been suggested that farnesylation of components of the ethylene pathway may be necessary for the development of "superficial scald" in apples (Haines et al., 2005). However, other studies have found little evidence for a relationship between farnesene, ethylene, and "superficial scald" (Watkins et al., 1993). In addition, studies on the ventilation of Granny Smith apples with chilled humidified air have found a scald reduction without markedly affecting the concentrations of the α-farnesene oxidation products (Matich et al., 1998), and farnesene itself applied as a wipe to apples has been found to reduce scald in regular storage to almost zero (Curry, 2000). The production of transgenic apples with down-regulated genes involved in α-farnesene biosynthesis would be an important step in testing for farnesene involvement in scald development.

PPO activity has been implicated in "superficial scald" in studies with the Granny Smith cultivar which have concluded that disruption of tissue integrity was followed by enzyme activity and polyphenol polymerization, leading to the formation of brown patches on the skin (Piretti et al., 1996). However, when stored under nitrogen, PPO expression was very low in early harvested Granny Smith apples suggesting that the regulation of PPO gene expression was dependent on oxygen (Bauchot et al., 1999). Once the fruit was removed to air, browning symptoms associated with "superficial scald" appeared almost immediately, and therefore probably too rapidly to be due to *de novo* PPO gene expression. POD isoenzymes have also been

implicated in "superficial scald" and using crab apple progeny and commercial apple cultivars has led to a general hypothesis that POD activity may be related to susceptibility (Fernandez-Trujillo et al., 2003). Some exceptions were observed, however, including greater scald incidence and lower POD activity in less mature Golden Delicious than in later harvested fruit, and POD activity was occasionally significantly higher in scald-resistant compared with scald-susceptible cultivars.

Possible involvement of antioxidants with apple "superficial scald" development has been studied (Barden and Bramlage, 1994). In general, antioxidant concentrations at harvest were inversely related to maximum conjugated triene concentrations at the end of storage, and to scald development. It has been suggested that phenolic fatty-acid esters have a potential antioxidant role to play in the natural resistance of apples to scald, although further study was proposed (Whitaker, 1998), and a possible role for antioxidant isoenzymes has been suggested (Kochhar et al., 2003).

In some apple cultivars, a physiological disorder can develop on chill storage resembling "superficial scald" but induced by high CO_2 levels in controlled atmospheres (Burmeister and Dilley, 1995). A free radical oxidation mechanism was proposed.

Internal browning (brown-heart) in apples can range from a small spot of brown flesh to nearly the entire flesh being affected in severe cases. However, even in badly affected fruit, a margin of healthy, white flesh usually remains just below the skin. The browning may include dry cavities resulting from desiccation. Browning develops early in CA storage and may increase in severity with extended storage time. The disorder is associated with high internal CO_2 levels in later-harvested, large, and over-mature fruit. CO_2 is required to make apple ACC oxidase catalytically competent to produce ethylene, but is also known to inhibit ethylene action. High CO_2 levels may feasibly lead to high ethylene production and this may, in turn, overcome the inhibitory action of CO_2. Sensitivity to CO_2 can also depend on cultivar, an effect that may be related to increased NADH oxidase and lower superoxide dismutase activities (Gong and Mattheis, 2003a). Chill storage of apples in a low-oxygen controlled atmosphere caused internal browning that was related to superoxide accumulation due to enhanced activity of xanthine oxidase and NAD(P)H oxidase, and reduced superoxide dismutase activity (Gong and Mattheis, 2003b). H_2O_2 could feasibly have been formed under these conditions, in which case a POD-catalysed oxidation of phenolic compounds may have led to internal browning.

Avocado Browning

Browning in avocados is a characteristic response to chilling, both in the flesh and on the skin suggesting a loss in compartmentation and membrane function in the cell with consequent oxidation of phenolic compounds (Woolf and Ferguson, 2000). Both postharvest heat treatments and exposure on the tree result in a reduction in browning although there is insufficient evidence to know whether such an effect of heat is direct or indirect. Lit-

tle thermal inactivation of browning enzymes might be expected at the temperatures involved. However, it has been found that returning avocados to 20°C from chill store does lead to POD inactivation (Zauberman et al., 1985), presumably as a result of non-thermal effects.

The relative contributions made by PPO and POD remains uncertain. The different rates of browning of avocado cultivars has been correlated with the amount of PPO activity and/or the concentrations of the natural substrates present (Kahn, 1977). However, under restricted ventilation (low oxygen) at 5.5°C, it has been suggested that the discoloration was associated with enhancement of POD activity in the fruit (Van Lelyveld et al., 1984). Higher levels of PPO were also observed although some of this was bound and/or latent enzyme. Increases in PPO and POD activities have also been observed during cold storage of avocado and during shelf life at 20°C and, along with membrane permeability values, have been correlated with brown mesocarp discoloration (Hershkovitz et al., 2005).

Raw Pear Browning

Browning occurs on wounding raw pears due to the enzymatic oxidation of the main phenolic substrates chlorogenic acid and (-)-epicatechin (Amiot et al., 1995). The phenolic content and susceptibility to browning were high in the peel, and appeared to depend on cultivar and to a lesser extent on maturity. As in the case of apples, pears are susceptible to "superficial scald," and pre-storage treatment of susceptible fruits with 1-MCP inhibits the synthesis of α-farnesene. 1-MCP treatment of "d'Anjou" pears has been found to attenuate expression of the gene encoding α-farnesene synthase and this correlated with much lower levels of α-farnesene, conjugated trienol and 'superficial scald' (Gapper et al., 2006). Two apparently different types of *internal brown* discolorations occur in pears. "Core browning" is mainly associated with wet tissue and significant collapse of the pulp whereas "brown heart" is linked with the appearance of dry cavities and may show no symptoms externally. "Brown heart" can occur during the storage of pears under hypoxic conditions, especially in the presence of increased CO_2 partial pressures. Pears subjected to air or CA storage showed a decrease in total ascorbic acid and a significant increase in the oxidized form of ascorbate, especially under CA storage (Larrigaudiere et al., 2001). Total levels of glutathione also decreased after storage for the two different storage atmospheres, but higher levels of the reduced form of glutathione were found in the CA-stored fruits during the same period. These changes corresponded with a sharp burst in ascorbate peroxidase and glutathione reductase activity. A significant increase in SOD activity, higher amounts of H_2O_2, and a late decrease in catalase were also found, particularly in fruits exposed to CA. Internal browning has been shown to depend on the time spent by fruits in low-ascorbate conditions and a threshold level of ascorbic acid has been suggested (Veltman et al., 2000; Zerbini et al., 2002; Franck et al., 2003). It has been proposed that browning is due to damage to cellular membranes caused by a combination of oxygen free radical attack and a

lack of maintenance (ATP) energy (Veltman et al., 2003). By delaying CA storage, pears can become more resistant to internal browning possibly due to increasing their energy status and membrane integrity (Saquet et al., 2003). A genovis model has been proposed that assumes internal browning is caused by an imbalance between oxidative and reductive prosesses due to metobolic gas ingredients inside the fruit, leading to an accumulation of reactive oxygen species (Franck et al., 2007). Tentative evidence has been presented that different metabolic pathways may be involved in "core browning" and "brown heart" although it was concluded that the development of the disorders may be a continuum (Larrigaudiere et al., 2004). PPO, POD and phenolic compounds were not measured in this study, and their involvement in internal pear browning remains uncertain.

Raw Red Fruits Browning

Browning of the pulp and the skin can be a problem in exported table *grapes*. It usually occurs during or after cold storage, and is often only detected once grape consignments have reached their destinations. Browning in raw grapes has been associated with phenolic compounds and PPO activity (Macheix et al., 1991), and with low calcium and other mineral levels (Salisbury and Ross, 1985). Degradation of flavan-3-ols in oxidizing musts has been reported to induce browning both of the must and of the resulting wine (Cheynier et al., 1995). This has been confirmed using grape must model systems, where enzymically generated caffeoyltartaric acid quinones have been found to oxidize catechin to brown polymers, and to degrade the grape anthocyanin, malvidin-3-glucoside.

Postharvest browning of *litchi* fruit pericarp can occur in a few days at ambient temperature after chill storage, and has been associated with dehydration. Concentration of structural calcium in the pericarp has been negatively correlated with fruit deterioration rate and membrane leakage, suggesting that calcium in structural form may influence fruit senescence through its role in tolerance to desication and in maintenance of membrane integrity (Huang et al., 2005). The pH of the litchi pericarp has been shown to increase from 4.3 to 5.3 when storage was at 25°C and 65% RH (Chu et al., 2004). The bright red color of the peel was stable if the pH was maintained at around 4.0, though PPO in the pericarp was potentially active at this pH. Other studies have linked the discoloration with the degradation of anthocyanins caused by anthocyanase and POD or PPO activities (Jiang et al., 2004; Zhang et al., 2005). Cellular localization of visual browning and oxidative activity studies were conducted to determine the relative significance of PPO and POD activities during pericarp browning. Browning was highly localized and restricted to the epicarp and the upper mesocarp. PPO and POD activities were highest in the epicarp, with progressively less activity in both the mesocarp and endocarp. As PPO and POD activities were significantly higher in this tissue and browning was not observed when both enzymes were selectively inhibited, it was postulated that both PPO and POD activities could be associated with litchi pericarp browning. Evidence has been

presented that (-)-epicatechin isolated from litchi is the direct substrate oxidized by isolated pericarp PPO (Sun et al., 2006).

Enzymatic Browning in Raw Vegetables

Raw Artichoke Browning

Globe artichoke heads harvested in winter months can be stored in good condition at 4–20°C, but the shelf-life has been found to diminish markedly for spring-harvested material, due to internal browning (Lattanzio et al., 1989). The heads initially increased in phenolic compounds, followed by a decrease that depended on the storage temperature and time of harvest. The PPO activity remained relatively constant during storage. It has been suggested that non-enzymatic reactions may be a major factor in the browning of cold-stored, non-mechanically damaged, artichoke heads (Lattanzio et al., 1994). The proposed mechanism involved the phenolic-induced release of iron from ferritin, the formation of ferric iron complexes with the phenolic compounds, and conversion of these complexes to brown pigments.

Raw Jicama (Yam Bean) Browning

Storage of jicama roots for 1 week at 10°C causes external decay and a chill-induced brown discoloration of the flesh that is independent of cultivar. At lower temperatures, the pulp can take on a translucent appearance but not necessarily develop brown discoloration; these roots probably also exhibit external decay. At 13°C, the roots retain their internal quality for 5 months. The discoloration was associated with an increased level of soluble phenolic compounds and PAL activity (Cantwell et al., 2002). It has been suggested that browning of cut jicama at 20°C is related to the process of lignification in which POD plays an important role (Aquino-Bolanos and Mercado-Silva, 2004).

Raw Lettuce Browning

Numerous types of browning can occur in raw lettuce either during growth, or in storage of whole heads or cut leaves. "Tipburn" refers to browning of leaf margins that occurs in the field and has been linked with low levels of calcium and other mineral distribution within the plant; "russet spotting" is associated with exposure to ethylene leading to brown spots on the midrib of the lettuce head in chill storage; "brown stain" is associated with sunken oval or irregular spots with dark brown borders on or near the midrib due to exposure to raised levels of CO_2, before transferring to air, and such exposure can also lead to internal browning ("heart-leaf injury"); "wound-induced browning" refers to browning of the tissue due to cutting or bruising. Wounding appears to induce phenolic metabolism and browning of mechanically injured lettuce by different mechanisms to ethylene as 1-MCP treatment, either before or after tissue excision, did not interfere with the wound-induced increase in phenolics (Saltveit, 2004). Exposing mid-rib leaf

tissue to vapors or aqueous solutions of n-alcohols has been found to partially inhibit wound-induced browning if the treatment is carried out within 2 h of excision (Choi et al., 2005). On the basis that 1-butanol specifically inhibited phospholipase D, these results have been interpreted as suggesting that this enzyme and the oxylipin pathway that culminates in the production of jasmonic acid, may be involved in producing the wound signal responsible for increased phenolic metabolism and subsequent enzymatic browning. However, it is feasible that a more general denaturation of enzymes by the n-alcohols occurred, and this contributed to browning inhibition during the first 2 h after excision. Treatment with the alcohols at later times may have been less effective due to the synthesis of phenolic compounds and to browning reactions being more advanced.

Wounding of whole heads or leaves of lettuce can induce *de novo* synthesis of specific proteins, including PAL (Saltveit, 2000; Campos-Vargas and Saltveit, 2002). This can be inhibited by mild heat-shock that leads to re-direction of synthesis to "heat-shock proteins." PAL formation can be prevented by heat treatments at temperatures between $40°C$ and $60°C$ and the heat-treated lettuce did not show any browning after being held for approximately 15 days in air at $5°C$ (Saltveit and Loaiza-Velarde, 2000). Further evidence is required, however, on the effect of such heat treatments on the activity of PPO and POD. These enzymes can be unstable, depending on their structures and on micro-environmental factors (Adams, 1991a). If significant inactivation had occurred, as has been shown in one study by washing cut tissues of iceberg lettuce at $47°C$ (Fukumoto et al., 2002), browning could also be suppressed as a result of the low enzyme activity. In the same study, phenolic levels were reduced by warm washing, and this would be expected to contribute to less browning. It was also found that chlorine significantly reduced phenolics and browning in lettuce washed at $4°C$ though whether this was due to suppression of enzyme formation, or to enzyme inhibition, or other cause, was undetermined.

In the case of "russet spot," the involvement of POD in brown pigment formation has been suggested (Ke and Saltveit, 1989). It was found that, in one cultivar, the increase in ionically bound POD and indole acetic acid oxidase activity, but not PPO activity, was associated with the increase in the discoloration during plant development. Results obtained using PAL inhibitors have indicated that the formation of "russet spot" lesions and their browning may be temporally separated, suggesting that an increase in PAL activity and the production of phenolic compounds are secondary effects of lesion formation (Peiser et al., 1998).

Raw Potato Browning

Brown discoloration in raw potatoes that occurs during handling and cutting prior to processing is generally accepted as being caused by PPO activity. Evidence has been presented that the highest level of PPO resides just below the skin (Thygesen et al., 1995). Genetically engineered potato varieties with less PPO had less tendency to brown (Bachem et al., 1994), and the wild species, *Solanum hjertingii*, with low

levels of certain PPO isoenzymes, did not exhibit enzymatic browning (Sim et al., 1997). It has been suggested that enzymatic browning of potatoes is correlated with tyrosine turnover, which depends on the concentrations of PPO, tyrosine, chlorogenic acid, and ascorbic acid, rather than with any single parameter (Matheis and Belitz, 1978). Tyrosine liberated from proteins as a result of the activity of a protease may be important (Sabba and Dean, 1994). It is feasible that the constant levels of tyrosine found during chill storage of fresh-cut strips of potatoes (Cantos et al., 2002) was due to the rate of formation from proteins being equal to the rate of consumption in browning reactions, whilst the increase in chlorogenic acid found during storage was due to its synthesis being more rapid than its browning-related consumption. In studies using vacuum-packed peeled raw potatoes, where less wounding may have taken place, tyrosine and chlorogenic acid were formed faster than they were consumed in browning reactions (Thybo et al., 2006). In tyrosine model systems, mushroom PPO (tyrosinase) led to the formation of oligomers of 5,6-dihydroxyindole, 5,6-dihydroxyindole-2-carboxylic acid and adducts of the two (Bertazzo et al., 1999). The solution rapidly turned dark red and a small quantity of dark-brown precipitate was observed. In contrast, horseradish POD/H_2O_2 converted the tyrosine into a yellow product containing a tyrosine-based skeleton, and a pale-brown precipitate slowly formed.

Browning potential in potatoes has been correlated with depressed ascorbic acid levels caused by chill storage and by chemical alternatives to chilling, such as treatment with chlorophenyl isopropyl carbamate (Munshi, 1994). Reconditioning of chill-stored potatoes can further lower the ascorbate levels and thereby increase browning potential. However, despite causing a decrease in ascorbic acid levels, the freezing of potato tubers has been found to cause a reduction in enzymatic discoloration (Mondy and Chandra, 1979). This reduced discoloration was feasibly due to the lower phenolic levels that were observed. It has been proposed that inhibition of browning in bruised potatoes could be associated with *de novo* synthesis of ascorbic acid and to the absence of ascorbic acid oxidase (Fukuda et al., 1995).

Raw Yam Browning

In tubers of yam, brown discoloration is most intense at the stem end where there is a high concentration of phenolic compounds (Onayemi, 1986). The most economically important yam, the white yam, apparently shows less tendency to brown because of the low substrate concentration, particularly (+)-catechin. Storage leads to accumulation of phenolic compounds, although this is counterbalanced by loss of PPO activity. However, some cultivars of yams show browning that is poorly related to PPO activity (Omidiji and Okpusor, 1996). POD activity could feasibly be involved in the browning of fresh yam, and has been implicated, along with total phenolics, in the browning of yam flour and paste derived from it (Akissoé et al., 2003) POD tended to be more stable after long times at lower balancing temperatures although initially less stable than PPO under all

blanching conditions (Akissoé et al., 2005).

Non-Enzymatic Browning

Non-Enzymatic Browning in Fruit Products

Processed Citrus Fruits Browning. Browning in processed citrus fruits has been associated with the degradation of ascorbic acid and sugars. Lemon juice browning is a major problem during processing and storage in the presence of air. Using model systems, evidence has been provided that browning occurs as a result of ascorbic acid decomposition to α,β-unsaturated carbonyls in the presence of organic acids, such as citric acid (Clegg and Morton, 1965). Browning was maximal at pH 4.5 and was further enhanced by amino acids. Citric acid was involved in the formation of brown pigments though it probably did not act as a catalyst or as a source of carbonyl compounds (Clegg, 1966; Kurata et al., 1973). Currently, the role of citric acid in brown pigment formation remains uncertain. Furfural, a degradation product of ascorbic acid, was formed but apparently did not contribute to browning. The concentration of hydroxymethylfurfural (HMF) has been found to have a high correlation with the level of browning in lemon juice and this suggested that sugar degradation can also play an important role in the formation of brown pigments (Robertson and Samaniego, 1986).

Orange juice slowly turns brown on storage, for example in enamelled cans but not plain cans (Blundstone, Woodman and Adams, 1971), as a fruit concentrate or dehydrated powder (Shaw et al., 1977), or when stored in plastic films with different barrier properties (Zerdin et al., 2003). At 35°C, storage of pasteurized single strength orange juice led to degradation of ascorbic acid and to significant browning (Naim et al., 1997). In orange juice model systems, at 50°C, it has been suggested that browning is due to ascorbic acid degradation in the early stages and thereafter to the breakdown of sugars (Murata et al., 2002). Arginine and proline promoted browning.

Processed Pineapple Browning. Pineapple color change occurred simultaneously with ascorbic acid loss in flexible, retortable pouches (Salunkhe et al., 1978). In juice processed in glass tubes, HMF and brown pigments have been shown to increase linearly with heating time following zero order reaction kinetics (Rattanathanalerk et al., 2005). Browning is known to occur to a much lesser extent when pineapple is processed in plain tin cans, feasibly due to the ascorbic acid being held in a stable, reduced form as a result of can corrosion reactions.

Processed Red Fruit Browning. The anthocyanin pigments of processed red fruits lose their color and become involved in undesirable browning reactions during storage (Markakis, 1974). The naturally occurring anthocyanins themselves are unstable as shown by their degradation in model systems to colorless degradation products that turn brown in the presence of oxygen (Adams, 1973). The presence of sugars is known to increase the rate of anthocyanin breakdown with fructose, arabinose, lactose, and sorbose having a stronger effect than sucrose. This is feasibly due to reactions between furfural, HMF

and hydrolyzed anthocyanins (anthocyanidins) that produce brown pigments (Debicki-Pošpiil et al., 1983). In juices of the blood orange, evidence has been presented that polymers are formed between the anthocyanins and degradation products of sugar and ascorbic acid (Krifi et al., 2000). There is a complex relationship between anthocyanin breakdown and ascorbic acid degradation. In particular, the hydroxyl and glycosyl substitution in the anthocyanin, and the presence of oxygen, have significant effects on the reactions with ascorbic acid. The products of ascorbic acid oxidation, or direct condensation of the anthocyanin with ascorbic acid, may both be involved in the color loss mechanism (García-Viguera and Bridle, 1999). Flavanols are known to influence color degradation in anthocyanin–ascorbic acid model systems (Poei-Langston and Wrolstad, 1981), and metal ions probably play a vital role as catalysts of ascorbic acid oxidation, or in ascorbic acid-metal ion-anthocyanin complexes (Sarma et al., 1997).

Non-Enzymatic Browning in Vegetable Products

Processed Potato Browning. Occasionally, unacceptably high levels or non-uniform browning can take place in the manufacture of fried potato products (Jankowski et al., 1997). This has long been established as being due to the breakdown of starch to glucose and fructose on storage of potatoes at chill temperatures (Adams, 1991b). Excessive Maillard browning can then occur on frying. Considerable variation between different cultivars is due to the chilling susceptibility being largely inherited, although the accumulated sugar levels can vary with growing conditions within a single variety. The glycolytic breakdown of hexose phosphate is restricted at low temperatures, due to the cold-lability of key regulatory enzymes such as phosphofructokinase or fructose-6-phosphate phosphotransferase. In addition, exposure to low temperatures leads to enhanced synthesis of invertase and destruction of an invertase inhibitor. Other factors which may be involved in starch breakdown include the resistance of the amyloplast membrane to enzymatic degradation, and the starch granule composition as determined by the relative amounts of amylose and amylopectin.

RED DISCOLORATIONS

Red Discoloration in Raw Vegetables

Red-Purple Discoloration of Mushrooms

During the storage of washed fresh mushrooms, a red-purple discoloration can sometimes occur on the cap surface at a relatively late stage (Choi and Sapers, 1994). When L-DOPA solution was applied to the surface of a mushroom, an orange-red color developed immediately that slowly turned purple on absorption by the tissue. This suggested that the L-DOPA was undergoing an initial PPO catalysed oxidation followed by reaction of the products with phenolic compounds in the hyphae. Glutaminyl-4-hydroxybenzene (GHB), the major substrate of PPO in mushroom sporophores was tested along with various

phenolic acids. Using model systems, it was found that gallic, ferulic, and sinapic acids induced a purple color in the oxidation of L-DOPA by PPO with the sinapic acid pigment being the most stable. The enzymatic oxidation of GHB in the presence of sinapic acid resulted in the formation of a red color and it was concluded that this would eventually lead to browning. The evidence indicated that the red-purple pigment formation may be due to oxidation of L-DOPA to indole-5,6-quinone followed by non-enzymatic polymerization of the quinone with radical products of sinapic acid oxidation. Chemical oxidation of L-DOPA and/or sinapic acid, as could arise from the presence of hypochlorite in water used to wash mushrooms, was considered as an unlikely source of purpling though the potential for the purely chemical formation of purple pigments was demonstrated using sodium periodate as an oxidant. Structural characterisation of the purple pigments formed in model systems proved difficult due to their instability, and for similar reasons, it was not possible to isolate, purify and characterize the purple pigments produced in washed mushrooms.

Pinking of Heat Processed Fruit and Fruit Products

Pink-purple discolorations can occur in fruits such as apples, bananas, gooseberries, guavas, peaches, and pears when heat-processed in unlacquered tin cans. The "pinking" varies with raw material and growing conditions, whilst the main processing factors leading to increased discoloration are excessive heating and delayed cooling of the cans. Evidence has been presented that "pinking" in canned pears occurs when the fruit has a high leucocyanidin concentration (Chandler and Clegg, 1970). It has been proposed that the first step is the oxidation of leucocyanidin to the corresponding quinone methine. Heating could then convert this compound to the red cyanidin via the conjugated anhydrobase. In the absence of reducing agents, the conjugated anhydrobase would be expected to chelate stannous ions, forming a purple-pink complex (Figure 1). Stannous ions were found to inhibit discoloration in canned pear purée putatively because they were able to reduce the quinone methines back to the leucocyanidins. Whether stannous ions acted to promote or inhibit discoloration in pears was probably determined not only by the amount of tin present, but also by the time at which it became available to react with the products of leucocyani-
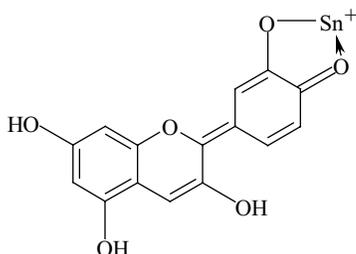
din breakdown. Other studies have tentatively suggested that heat processing of banana and bean leucoanthocyanidins caused the formation of colored phlobaphene-like polymers containing quinonoid rings and xanthylium nuclei (Ranganna and Parpia, 1974). Stannic chloride was found to precipitate the leucoanthocyanidins; heat processing of the precipitate led to a purple pigment.

Pinking of Processed Vegetables

Pinking of Processed Brassicae. A pink discoloration has been observed in frozen Brussels sprouts (Lindquist et al., 1951) and in canned cabbage (Mahadeviah et al., 1965). Heat processing can cause "pinking" of cauliflower florets apparently due to leucoanthocyanin conversion and to non-enzymatic browning reactions (Strachan and Nonnecke, 1961). The "pinking" of processed cauliflower can be inhibited by ascorbic acid treatment (Uylaser and Sahin, 2002) and the pink pigment is not therefore formed as a result of ascorbic acid degradation. In contrast, the pink discoloration that has been found in dried cabbage was attributed to the non-enzymatic interaction of the products of ascorbic acid and amino acid degradation (Ranganna and Setty, 1968). The amino acids were thought to form aldehydes, via the Strecker degradation, which then reacted with dehydroascorbic (DHA) and 2,3-diketogulonic acid. The discoloration was intensified by blanching which enhance rapid oxidation of ascorbic acid. DHA is known to react with amino acids to yield scorbamic acid that can then form a red pigment by reacting with a second molecule of DHA (Kurata et al., 1973) (Figure 2). This pigment could feasibly be the pigment that caused the ascorbic acid-related pink discoloration in processed Brassicae.

Pinking of Processed Onions

The pink discoloration of processed Allium species, dehydrated onion, is a long-standing problem (Schwimmer, 1981). The reaction mechanism was proposed to involve the action of endogenous alliinase on alk (en) yl-L- cysteine sulphoxides to form thiopropanal-S-oxide which then reacted non-enzymatically with onion amino acids to yield a "pigment precursor." The latter then reacted with carbonyl components in a rate-limiting step to produce the discoloring pigments. The

Figure 1 Potential structure of the purple-pink pigment that causes discoloration in canned pears (Chandler and Clegg, 1970).
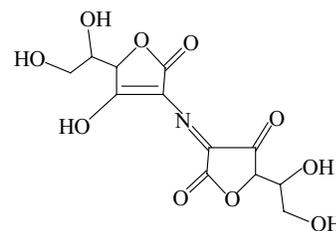

Figure 2 Proposed structure of the red pigment formed from ascorbic acid and amino acids (Kurata et al., 1973).

pigments absorbed maximally between 490 and 540 nm, depending on the source of onion and on experimental conditions. In extracts of yellow onion, "pinking" has been found to be greatest at pH 6.1, and added S-methyl-L-cysteine sulphoxide led to small increases in the discoloration and in the levels of thiosulfinate degradation products (Lee and Parkin, 1998). S-(1-propenyl)-L-cysteine sulphoxide (isoalliin) has been shown to be the primary precursor for *Allium* discoloration, and the importance of the 1-propenyl thiosulfinate degradation products of S-alk(en)yl-L-cysteine sulphoxides, relative to the thiopropanal-S-oxide breakdown product, as pigment precursors was confirmed (Kubec et al., 2004). The propyl, 1-propenyl and methyl thiosulfinates that occur in onions formed pink, pink-red and magenta pigments. Model system studies using acetate buffer, pH 5.6, have suggested a discoloration mechanism involving alliinase-catalysed degradation of isoalliin to a thiosulphinate 'colour developer' that can then react with amino acids to give substituted pyrrole 'pigment precursors' (Imai et al., 2006). Heating near boiling point of the 'pigment precursors' with the thiosulphinate, allicin, formed from the alliinase degradation of S-(2-propenyl)-L-cysteine sulphoxide (alliin), caused two of the substituted pyrrole molecules to react with the allyl moiety of the allicin to yield reddish-purple dipyrrole pigments.

YELLOW DISCOLORATIONS

Yellow Discoloration in Raw Green Vegetables

Raw green vegetables lose color during short-term storage in the raw state, and over a longer time-scale in frozen storage, developing unattractive yellow or olive-brown hues. Yellowing of raw broccoli due to chlorophyll destruction has been linked with lipid peroxidation (Zhuang et al., 1995), whilst variation in chlorophyll loss between broccoli cultivars has been associated with differences in antioxidant enzyme activity, and especially in the ratios of SOD to POD (Toivonen and Sweeney, 1998). Broccoli yellowing was mediated by ethylene action and inhibited by treatment with 1-MCP (Xuetong-Fan and Mattheis, 2000). External application of ethylene (as ethephon) has been shown to accelerate chlorophyll degradation, putatively via chlorophyllase, Mg dechelatase, and POD activities, whilst cytokinin (applied as 6-benzylaminopurine) reduced the rate of chlorophyll breakdown (Costa et al., 2005). Heat treatment at 50°C has also been suggested to control the chlorophyll loss in broccoli by suppressing an increase in chlorophyll-degrading POD in the microsomes and the cytosol (Funamoto et al., 2003). In POD-mediated chlorophyll degradation, the enzyme oxidizes the phenolic compounds using H_2O_2 and forms phenoxy radical. The phenoxy radical then oxidizes chlorophyll and its derivatives to colorless low molecular weight compounds through the formation of C13-hydroxychlorophyll *a*, a fluorescent cchlorophyll atabolite and a bilirubin-like compound as an intermediate (Yamauchi et al., 2004). In addition to the phenoxy radical, superoxide anion, formed in the POD-catalyzed reaction, might be
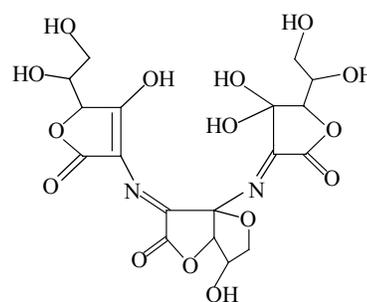

Figure 3 Proposed structure of the yellow pigment formed from ascorbic acid and amino acids (Hayashi et al., 1983).

involved in chlorophyll oxidation. Elevated levels of H_2O_2 have been found in heat-treated broccoli florets (Shigenaga et al., 2005).

Yellow Discoloration in Processed Fruits and Vegetables

In model system studies, and potentially in processed fruits and vegetables, it has been shown that a relatively stable yellow product can be formed during reaction between DHA and an amino acid (Hayashi et al., 1983) (Figure 3). It was proposed that the red pigment formed via scorbamic acid (Figure 2) could react with a second molecule of scorbamic acid to yield the yellow pigment. Heating the yellow pigment alone in phosphate buffer at pH 7 showed a high degree of browning which suggested that it was an important intermediate in the browning reaction of DHA with amino acids.

Yellowing of Salted Radish Pickles

Salted winter radish root, *Takuan-zuke*, is a traditional Japanese food that naturally acquires a bright yellow color during salting and fermentation. However, the yellow dye is unstable under fluorescent lighting and this leads to a partial loss of color on retail display. This non-uniformity, coupled with the consumer perception that the color is artificial, has led to a desire by manufacturers to inhibit yellowing. Using model systems, it was found that the conditions for yellow pigment formation were the same as for thioglucosidase action (Maeda et al., 1982). Yellow pigment formation was enhanced with increasing pH in the range 4.5–7.2 and in the presence of small amounts of ferrous or cuprous ions (Ozawa et al., 1993). Ascorbic acid stimulated the production of the yellow pigment in the presence of radish enzymes, due to activation of thioglucosidase, whereas it inhibited pigment formation in the absence of the enzymes. Evidence has been presented that the yellow pigment is produced as a result of the reaction between 4-methylthio-3-butenyl isothiocyanate, from the thioglucosidase-catalysed hydrolysis of the major radish glucosinolate, and tryptophan formed during the fermentation (Matsuoka et al., 2002). The pigment was identified as 2-[3-(2-thioxopyrrolidin-3-ylidene) methyl]-tryptophan using IR, MS, ^{1}H-, and ^{13}C-NMR spectroscopy (Figure 4). The
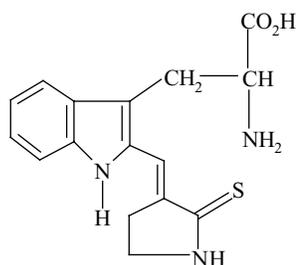
Figure 4 Yellow pigment formed in salted radish roots (Matsuoka et al., 2002).

same compound was also shown to be a major component of the yellow-colored fraction isolated from salted radish extracts.

GREEN DISCOLORATIONS

Greening of Processed Fruits

Green Staining in Preserved Olives

Green staining can occur during the lactic fermentation stage of processing Spanish-style green table olives. Pigment analysis of fruits classified according to the surface area affected showed a progressive increase in the concentration of all copper-pheophytin complexes with the course of the discoloration (Gallardo-Guerrero et al., 1999). In green-stained olives of cv. Gordal, novel derivatives of oxidised chlorophylls with copper have been observed (Gandul-Rojas et al., 1999). The source of the copper was not established although pectins were suggested as a possible reservoir (Gallardo-Guerrero et al., 2002). It has been proposed that the green staining defect was due to loss of cell integrity and the accumulation of the copper complexes of oxidised chlorophylls (Gallardo-Guerrero et al., 2003).

Greening of Processed Vegetables

Greening of Garlic Products

Garlic products, such as purees and juices, and bottled garlic in vinegar or oil, can develop a blue/green discoloration unless a blanching step is interposed during production. In the traditional, home-made Chinese product ("Laba" garlic) where development of green color is desirable, the garlic requires around 4 months storage under chill conditions before it can turn green during pickling in vinegar. Evidence has been presented that dormancy termination is linked with the greening of chopped garlic (Bae and Lee, 1990). When prepared from dormancy-terminated bulbs, the chopped material turned green during the first week of storage and then brown after that. Greening appears to require alliinase activity and therefore to be analogous to the pinking that occurs in processed onions and in leeks. It has been suggested that isoalliin is enzymatically cleaved to 1-propenyl-containing thiosulfinates in the disrupted tissue and that these compounds reacted with amino acids to produce the pigments

(Kubec et al., 2004). At pH 5.0 and at 45°C, glycine model systems containing the allyl derivatives that occur in garlic gave rise to blue products. Evidence has been presented that thiosulfinate conversion in "Laba" garlic pickling solution is proportional to the formation of the pigments (Bai et al., 2005), and an increase in the permeability of the garlic intracellular tonoplast to acetic and other monocarboxylic acids has been linked with "Laba" garlic greening (Bai et al., 2006). Observation of a "near isosbestic point" led to a proposal that the pigments were a mixture of yellow and blue species with the former being more stable and both having similar structures (Bai et al., 2005). Using ground-up chill-stored garlic cloves, a single species of green pigment has been isolated and purified with absorption maxima in the yellow and blue spectral regions (Lee et al., 2007). It was suggested that this pigment contained sulphur, though elucidation of its structure proved problematical due to pigment instability. Blue/green pigments have also been shown to be produced from the reddish-purple dipyrroles formed in onion/garlic model systems (Imai et al., 2006).

Greening During Cooking of New Potatoes

A strong green to blue-green discoloration can sometimes be formed during the cooking of new potatoes. This occurs as a result of boiling the potatoes in hard water, when the pH value can rise to pH 8–9. Using potato cooking water, it has been demonstrated that a mixture of blue, green and brown pigments is formed in this pH range (Adams, 1994). A maximum amount of pigment was produced at approximately pH 9, and similar behaviour was found using a chlorogenic acid-glutamine model system. This suggested that the cause of the green discoloration was the reaction between chlorogenic acid and amino acids in the potato. Susceptible potatoes initially turned yellow on cooking, as did chlorogenic acid in water in the pH range 8–9, indicating that the greening was due to the presence of abnormally high levels of chlorogenic acid. Excessive nitrogen fertiliser application can lead to enhanced levels of both chlorogenic acid and amino acids (Leszczynski and Lisinska, 1988) and fertiliser application may therefore be a significant contributory factor leading to green discoloration of cooked new potatoes.

Model system studies have shown that esters of caffeic acid produced greening whilst free caffeic acid did not (Namiki et al., 2001). The green color showed a hue that depended on the structure of the amino acid and on the ester of caffeic acid. Of the amino acids, serine, threonine, proline, cysteine, and cyclic amino acids were found to be negative for greening, although the reason for this is unknown. Evidence was presented that the green pigments were formed as a result of condensation of two molecules of chlorogenic acid with one molecule of amino acid, feasibly by a free radical mechanism. The pigments formed were novel benzacridine derivatives (Figure 5). These compounds were shown to exist in blue, green and yellow forms depending on their oxidation state. The blue pigment was reduced by ascorbic acid to give the green and yellow pigment forms. Thus,
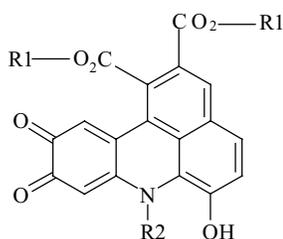
Figure 5 Proposed structure of the green pigment that forms when chlorogenic acid reacts with amino acids in foods (R1 = quinic acid: R2 = amino acid side-chain) (Namiki et al., 2001).

in susceptible new potatoes, the presence of ascorbic acid may influence the green discoloration formed on cooking.

CONCLUSIONS

The chemical and biochemical reactions involved in many raw and processed fruit and vegetable discolorations are broadly understood and the effects of external factors can often be interpreted using this knowledge. In raw materials, only a limited understanding has so far been achieved of internal black and brown discolorations and further information is desirable in the following areas:- biochemical signalling pathways that trigger discolorations; enzymes, reactive oxygen species, and phenolic compounds involved in discoloration; structure of isolated discoloring pigments. In processed materials, further knowledge is required of the role of ascorbic acid in the browning of fruit products and in the "pinking" of Brassicaceous vegetables, and of the structure of the discoloring pigments in many products. For all discolorations, an extension of current knowledge is desirable of the naturally occurring constituents that are associated with acceleration or inhibition of the discoloration reactions, and how processing and storage can lead to variability in these constituents. A greater knowledge of these areas would assist in the development of more efficient methods of controlling raw and processed fruit and vegetable discolorations.

REFERENCES

Adams, J.B. 1973. Thermal degradation of anthocyanins with particular reference to the 3-glycosides of cyanidin I. In acidified aqueous solution at 100°C. *J. Sci. Food Agric.*, **24**:747–762.

Adams, J.B. 1991a. Review: Enzyme inactivation during heat processing of food-stuffs. *Int. J. Food Sci. Tech.*, **26**:1–20.

Adams, J.B. 1991b. Enzymes in individual vegetables. In: *Food Enzymology*. P.F. Fox, Ed., Elsevier Applied Science, London and New York. Vol. 1. pp. 499–543.

Adams, J.B. 1994. Green color development in potato cooking water. *Food Chem.*, **49**:295–298.

Akissoé, N., Hounhouigan, J., Mestres, C., and Nago, M. 2003. How blanching and drying affect the color and functional characteristics of yam (*Dioscorea cayenensis-rotundata*) flour. *Food Chem.* **82**:257–264.

Akissoé, N., Mestres, C., Hounhouigan, J., and Nago, M. 2005. Biochemical origin of browning during the processing of fresh yam (*Dioscorea spp.*) into dried product. *J. Agric. Food Chem.*, **53**:2552–2557.

Ameziane, J., Aplincourt, M., Dupont, L., Heirman, F., and Pierrard, J.-C. 1996. Thermodynamic stability of copper (II), manganese (II), zinc (II) and iron (III) complexes with chlorogenic acid. *Bull. Soc. Chim. Fr.*, **133**:243–249.

Amiot, M.J., Tacchini, M., Aubert, S.Y., and Oleszek, W. 1995. Influence of cultivar, maturity stage, and storage conditions on phenolic composition and enzymatic browning of pear fruits. *J. Agric. Food Chem.*, **43**:1132–1137.

Andjelkovic, M., van Camp, J., de Meulenaer, B., Depaemelaere, G., Socaciu, C., Verloo, M., and Verhe, R. 2006. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chem.*, **98**:23–31.

Aquino-Bolaños, E.N., and Mercado-Silva, E. 2004. Effects of polyphenol oxidase and peroxidase activity, phenolics and lignin content on the browning of cut jicama. *Postharv. Biol. Technol.*, **33**:275–283.

Bachem, C.W., Speckmann, G.J., Van der Linde, P.C.G., Verheggen, F.T.M., Hunt, M.D., Steffens, J.C., and Zabeau, M. 1994. Antisense expression of polyphenol oxidase genes inhibits enzymatic browning in potato tubers. *Bio-Technol.*, **12**: 1101–1105.

Bae, R.N., and Lee, S.K. 1990. Factors affecting greening and its control methods in chopped garlic. *J. Kor. Soc. Hort. Sci.*, **31**:358–362.

Bai, B., Chen, F., Wang, Z., Liao, X., Zhao, G., and Hu, X. 2005. Mechanism of the greening colour formation of "Laba" garlic, a traditional homemade Chinese food product. *J. Agric. Food Chem.*, **53**:7103–7107.

Bai, B., Li, L., Hu, X., Wang, Z., and Zhao, G. 2006. Increase in the permeability of tonoplast of garlic (*Allium sativum*) by monocarboxylic acids. *J. Agric. Food Chem.*, **54**:8103–8107.

Bamberg, J.B., and Palta, J.P. 2003. Calcium-rich potatoes: It's in their genes. *USDA Agric. Res. Mag.*, **51**:18–19.

Baritelle, A.L., and Hyde, G.M. 2003. Specific gravity and cultivar effects on potato impact sensitivity. *Postharv. Biol. Technol.*, **29**:279–286.

Barden, C.L., and Bramlage, W.J. 1994. Relationships of antioxidants in apple peel to changes in α-farnesene and conjugated trienes during storage, and to superficial scald development after storage. *Postharv. Biol. Technol.*, **4**:23–33.

Bauchot, A.D., Reid, S.J., Ross, G.S., and Burmeister, D.M. 1999. Induction of apple scald by anaerobiosis has similar characteristics to naturally occurring superficial scald in 'Granny Smith' apple fruit. *Postharv. Biol. Technol.*, **16**:9–14.

Bertazzo, A., Costa, C.V.L., Allegri, G., Schiavolin, M., Favretto, D., and Traldi, P. 1999. Enzymatic oligomerisation of tyrosine by tyrosinase and peroxidase studied by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid Comm. Mass Spectrom.*, **13**:542–547.

Blundstone, H.A.W., Woodman, J.S., and Adams, J.B. 1971. Canned Citrus Products. In: *The Biochemistry of Fruits and their Products*, (A.C. Hulme, Ed.), Academic Press, London and New York. Vol. 2, pp. 543–572.

Burmeister, D.M., and Dilley, D.R. 1995. A 'scald-like' controlled atmosphere storage disorder of Empire apples - a chilling injury induced by CO_2. *Postharv. Biol. Technol.*, **6**:1–7.

Campos-Vargas, R., and Saltveit, M.E. 2002. Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. *Physiol. Plant.*, **114**:73–84.

Cantos, E., Tudela, J.A., Gil, M.I., and Espin, J.C. 2002. Phenolic compounds and related enzymes are not rate-limiting in browning development of fresh-cut potatoes. *J. Agric.Food Chem.*, **50**:3015–3023.

Cantwell, M.I., Peiser, G., and Mercado-Silva, E. 2002. Induction of chilling injury in jicama (*Pachyrhizus erosus*) roots: changes in texture, color and phenolics. *Postharv. Biol. Technol.*, **25**:311–320.

Chandler, B.V., and Clegg, K.M. 1970. Pink discoloration in canned pears. *J. Sci. Food Agric.*, **21**:315–328.

Cheng, G.W., and Crisosto, C.H. 1997. Iron-polyphenol complex formation and skin discoloration in peaches and nectarines. *J. Am. Soc. Hort. Sci.*, **122**:95–99.

Cheynier, V., Fulcrand, H., Guyot, S., Oszmianski, J., and Moutounet, M. 1995. Reactions of enzymically generated quinones in relation to browning in grape musts and wines. *ACS Symp. Ser.*, No. 600:130–143.

Choi, S.W., and Sapers, G.M. 1994. Purpling reaction of sinapic acid model systems containing L-DOPA and mushroom tyrosinase *J. Agric. Food Chem.*, **42**:1183–1189.

Choi, Y.-J., Tomás-Barberán, F. A., and Saltveit, M. E. 2005. Wound-induced phenolic accumulation and browning in lettuce (*Lactuca sativa* L.) leaf tissue is reduced by exposure to n-alcohols. *Postharv. Biol. Technol.*, **37**:47–55.

Chu, G.C.L., Leung, E.S.K., and Kawaji, M. 2004. Reversibility of lychee pericarp red color in relation to pericarp pH, activity of polyphenol oxidase, and particle size of brown pigment. *Fruits*, **59**:17–23.

Clegg, K.M. and Morton, A.D. 1965. Carbonyl compounds and the non-enzymic browning of lemon juice. *J. Sci.Food Agric.*, **16**:191–198.

Clegg, K.M., 1966. Citric acid and the browning of solutions containing ascorbic acid. *J. Sci. Food Agric.*, **17**:546–549.

Corsini, D., Stark, J., and Thornton, M. 1999. Factors contributing to the blackspot bruise potential of Idaho potato fields. *Am. J. Pot. Res.*, **76**:221–226.

Corsini, D. 2001. Components of blackspot bruising. Presented at the University of Idaho Potato Conference, January 18th, 2001.

Costa, M.L., Civello, P.M., Chaves, A.R., and Martínez, G. A. 2005. Effect of ethephon and 6-benzylaminopurine on chlorophyll degrading enzymes and a peroxidase-linked chlorophyll bleaching during post-harvest senescence of broccoli (*Brassica oleracea* L.) at 20°C. *Postharv. Biol. Technol.*, **35**:191-199.

Curry, E.A. 2000. Farnesene and squalene reduce scald in apples and pears. *Acta. Hort. (ISHS)*, **518**:137–144.

Dat, J., Vandenabeele, S., Vranova, E., Van Montagu, M., Inze, D., and Van Breuseegem, F. 2000. Dual action of the active oxygen species during plant stress responses *Cell Mol Life Sci.*, **57**:779–795.

Debicki-Pospišil, J., Lovric, T., Trinajstic, N., and Sabljic, A. 1983. Anthocyanin degradation in the presence of furfural and 5-hydroxymethylfurfural. *J. Food Sci.*, **48**:411–416.

Fernandez-Trujillo, J.P., Nock, J.F., Kupferman, E.M., Brown, S.K., and Watkins, C.B. 2003. Peroxidase activity and superficial scald development in apple fruit. *J. Agric. Food Chem.*, **51**:7182–7186.

Florissen, P., Ekman, J.S., Blumenthal, C., McGlasson, W.B., Condroy, J., and Holford, P. 1996. The effects of short heat-treatments on the induction of chilling injury in avocado fruit (*Persea americana* Mill). *Postharv. Biol. Technol.*, **8**:129–41.

Franck, C., Baetens, M., Lammertyn, J., Scheerlinck, N., Davey, M.W., and Nicolaï, B.M. 2003. Ascorbic acid mapping to study core breakdown development in 'Conference' pears. *Postharv. Biol. Technol.*, **30**:133–142.

Franck, C., Lammertyn, J., Ho, Q. T., Verboven, P., Verlinden, B., and Nicolai, B. M. 2007. Browning disorders in pear fruit. *Postharv. Biol. Technol.*, **43**:1–13.

Friedman, M. 1997. Chemistry, biochemistry, and dietary role of potato polyphenols. A review. *J. Agric. Food Chem.*, **45**:1523–1540.

Fry, S.C., Dumville, J.C., and Miller, J.G. 2001. Fingerprinting of polysaccharides attacked by hydroxyl radicals *in vitro* and in the cell walls of ripening pear fruit. *Biochem. J.*, **357**:729–737.

Fukuda, M., Kunisada, Y., Noda, H., Tagaya, S., Yamamoto, Y., and Kida, Y. 1995. Effect of storage time of potatoes after harvest on increase in the ascorbic acid content by wounding. *J. Jap. Soc. Food Sci. Technol.*, **42**:1031–1034.

Fukumoto, L.R., Toivonen, P.M.A., and Delaquis, P.J. 2002. Effect of wash water temperature and chlorination on phenolic metabolism and browning of stored iceberg lettuce in photosynthetic and vascular tissues. *J. Agric. Food Chem.* **50**:4503–4511.

Funamoto, Y., Yamauchi, N., and Shigyo, M. 2003. Involvement of peroxidase in chlorophyll degradation in stored broccoli (*Brassica oleracea* L.) and inhibition of the activity by heat treatment. *Postharv. Biol. Technol.*, **28**:39–46.

Gallardo-Guerrero, L., Gandul-Rojas, B., and Mínguez-Mosquera, M. I. 1999. Chlorophyll pigment composition in table olives (cv. Gordal) with green staining alteration *J. Food Prot.*, **62**:1167–1117.

Gallardo-Guerrero, L., Hornero-Mendez, D., and Mínguez-Mosquera, M.I. 2002. Pectins as a possible source of the copper involved in the green staining alteration of cv. Gordal table olives. *J. Agric. Food Chem.,* **50**:6746–6751.

Gallardo-Guerrero, L., Milicua, J.C.G., Salvador, A.M., Jaren-Galan, M., and Mínguez-Mosquera, M. I. 2003. Pigment-lipoprotein complexes in table olives (cv. Gordal) with green staining alteration. *J. Agric. Food Chem.,* **51**:1724–1727.

Gandul-Rojas, B., Gallardo-Guerrero, L., and Mínguez-Mosquera, M. I. 1999. Identification of oxidized chlorophylls and metallochlorophyllic complexes

of copper in table olives (cv. Gordal) with green staining alteration. *J. Food Prot.*, **62**:1172–1177.

Gapper, N.E., Bai, J., and Whitaker, B.D. 2006. Inhibition of ethylene-induced α-farnesene synthase gene PcAFS1 expression in 'd'Anjou' pears with 1-MCP reduces synthesis and oxidation of α-farnesene and delays development of superficial scald. *Postharv. Biol. Technol.*, **41**:225–233.

García-Viguera, C., and Bridle, P. 1999. Influence of structure on color stability of anthocyanins and flavylium salts with ascorbic acid. *Food Chem.*, **64**:21–26.

Gong, Y., and Mattheis, J.P. 2003a. Physiological responses of 'Braeburn' and 'Gala' apples to high CO_2. *Hortsci.*, **38**:859.

Gong, Y., and Mattheis, J.P. 2003b. Effects of low oxygen on active oxygen metabolism and internal browning in 'Braeburn' apple fruit. *ISHS Acta Hort.,* **628**:533–539.

Green, M.A., and Fry, S.C. 2005. Vitamin C degradation in plant cells via enzymatic hydrolysis of 4-O-oxalyl-L-threonate. *Nature,* **433**:83–87.

Griffiths, D.W., Bain, H., and Dale, M.F.B. 1992. Development of a rapid colorimetric method for the determination of chlorogenic acid in freeze-dried potato tubers. *J. Sci. Food Agric.,* **58**:41–48.

Griffiths, D.W., and Bain, H. 1997. Photo-induced changes in the concentrations of individual chlorogenic acid isomers in potato (*Solanum tuberosum*) tubers and their complexation with ferric ions. *Pot. Res.*, **40**:307–315.

Haines, M.M., Mattinson, D.D., and Fellman, J.K. 2005. Farnesylation of components of the ethylene signalling pathway may be necessary to the development of superficial scald in apples. *ISHS Acta Hort.*, **682**:313–320.

Hayashi, T., Hoshii, Y., and Namiki, M. 1983. On the yellow product and browning of the reaction of dehydroascorbic acid with amino acids. *Agric. Biol. Chem.*, **47**:1003–1009.

Hershkovitz, V., Saguy, S.I., and Pesis, E. 2005. Postharvest application of 1-MCP to improve the quality of various avocado cultivars. *Postharv. Biol. Technol.*, **37**:252–264.

Hewajulige, I.G.N., Wijeratnam, R.S.W., Wijesundera, R.L.C., and Abeysekere, M. 2003. Fruit calcium concentration and chilling injury during low temperature storage of pineapple. *J. Sci.Food Agric.*, **83**:1451–1454.

Huang, X.-M., Wang, H.-C., Yuan, W.-Q., Lu, J.-M., Yin, J.-H., Luo, S., and Huang, H.-B. 2005. A study of rapid senescence of detached litchi: roles of water loss and calcium. *Postharv. Biol. Technol.*, **36**:177–189.

Hughes, J.C., and Evans, J.L. 1969. Studies on after-cooking blackening. V. Changes in after-cooking blackening and the chemistry of Majestic and Ulster Beacon tubers during the growing season. *Eur. Pot. J.*, **12**:26–40.

Hughes, J.C., and Swain, T. 1962. After-cooking blackening in potatoes. III. Examination of the interaction of factors by *in-vitro* experiments. *J. Sci. Food Agric.*, **13**:358–363.

Imai, S., Akita, K., Tomotake, M., and Sawada, H. 2006. Identification of two novel pigment precursors and a reddish-purple pigment involved in the blue-green discoloration of onion and garlic. *J. Agric. Food Chem.*, **54**:843–847.

Jankowski, K.M., Parkin, K.L., and von Elbe, J.H. 1997. Nonuniform browning or 'mottling' in French fry products associated with a heterogeneous distribution of reducing sugars. *J. Food Proc. Pres.*, **21**:33–54.

Jiang Y., Duan X., Joyce, D., Zhang, Z., and Li, J. 2004. Advances in understanding of enzymatic browning in harvested litchi fruit. *Food Chem.*, **88**:443–446.

Johnson, S.M., Doherty, S.J., and Croy, R.R.D. 2003. Biphasic superoxide generation in potato tubers. A self-amplifying response to stress. *Plant Physiol.*, **131**:1440–1449.

Kahn, V. 1977. Some biochemical properties of polyphenol oxidase from two avocado varieties differing in their browning rates. *J. Food Sci.*, **42**:38–43.

Ke, D., and Saltveit, M.E. Jr. 1989. Developmental control of russet spotting, phenolic enzymes, and IAA oxidase in cultivars of iceberg lettuce. *J. Am. Soc. Hort. Sci.*, **114**:472–477.

Kochhar, S., Conklin, P.L., Brown, S.K., and Watkins, C.B.A. 2003. A quantitative and qualitative analysis of antioxidant enzymes in relation to susceptibility of apple fruit to superficial scald. *J. Am. Soc. Hort. Sci.*, **128**:910–916.

Krifi, B., Chouteau, F., Boudrant, J., and Metche, M. 2000. Degradation of anthocyanins from blood orange juices. *Int. J. of Food Sci. Tech.*, **35**(3):275–283.

Kubec, R., Hrbačová, M., Musah, R.A., and Velíšek, J. 2004. Allium discoloration: precursors involved in onion "pinking" and garlic greening. *J. Agric. Food Chem.*, **52**:5089–5094.

Kurata, T., Fujimaki, M., and Sakurai, Y. 1973. Red pigment produced by the reaction of dehydro-L-ascorbic acid with alpha-amino acid. *Agric. Biol. Chem.*, **37**:1471–1477.

Lamb, C., and Dixon, R.A. 1997. The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **48**:251–275.

Lærke, P.E., Brierley, E.R., and Cobb, A.H. 2000. Impact-induced blackspots and membrane deterioration in potato (*Solanum tuberosum* L.) tubers. *J. Sci. Food Agric.*, **80**:1332–1338.

Lærke, P.E., Christiansen, J., and Veierskov, B. 2002. Color of blackspot bruises in potato tubers during growth and storage compared to their discoloration potential. *Postharv. Biol. Technol.*, **26**:99–111.

Larrigaudiere, C., Lentheric, I., Pintó, E., and Vendrell, M. 2001. Short-term effects of air and controlled atmosphere storage on antioxidant metabolism in Conference pears. *J. Plant Physiol.* **158**:1015–1022.

Larrigaudiere, C., Lentheric, I., Puy, J., and Pinto, E. 2004. Biochemical characterisation of core browning and brown heart disorders in pear by multivariate analysis. *Postharv. Biol. Technol.*, **31**:29–39.

Lattanzio, V., Linsalata, V., Palmieri, S., and van Sumore, C.F. 1989. The beneficial effect of citric and ascorbic acid on the phenolic browning reaction in stored artichoke (*Cynara scolymus* L.) heads. *Food Chem.*, **33**:93–106.

Lattanzio, V., Cardinali, A., Di Venere, D., Linalata, V., and Palmieri, S. 1994. Browning phenomena in stored artichoke (*Cynara scolymus* L.) heads: enzymic or chemical reactions? *Food Chem.*, **50**:1–7.

Lee, C-H., and Parkin, K.L. 1998. Relationship between thiosulfinates and pink discoloration in onion extracts as influenced by pH. *Food Chem.*, **61**:345–350.

Lee, E.-J., Cho, J.-E., Kim, J.-H., and Lee, S.-K. 2007. Green pigment in crushed garlic (*Allium sativum* L.) cloves: Purification and partial characterization. *Food Chem.*, **101**:1709–1718.

Leszczynski, W., and Lisinska, G. 1988. Influence of nitrogen fertilisation on chemical composition of potato tubers. *Food Chem.*, **28**:45–52.

Lindquist, F.E., Dietrich, W.C., Masure, M.P., and Boggs, M.M. 1951. Effect of enzyme inactivation on quality retention in frozen Brussels sprouts. *Food Technol.*, **5**:198–199.

Macheix, J-J., Sapis, J-C., and Fleuriet, A. 1991. Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines. *Crit. Rev. Food Sci. Nutr.*, **30**:441–486.

Maeda, Y., Ozawa, Y., and Uda, Y. 1982. Studies on the yellowing mechanism of salted radish pickles. I. Precursor of yellow substance in fresh radish root and some factors affecting the yellowing thereof. *J.Jap.Soc.Food Sci.Tech.*, **29**:522–528.

MacNabnay, M., Dean, B.B., Bajema, R.W., and Hyde, G.M. 1999. The effect of potassium deficiency on chemical, biochemical and physical factors commonly associated with blackspot development in potato tubers. *Am. J. Pot. Res.*, **76**:53–60.

Mahadeviah, M., Ranganna, S., Sastry, L.V.L., Bhatnagar, H.C., Siddappa, G.S., Sathyavathi, V.K., Shah, G,R., Mookerjee, D.K., and Prabhakar, J. V. 1965. Some practical measures to prevent pink discoloration in canned cabbage. *J. Food Sci. Technol. (India)*, **2**:10–16.

Markakis, P. 1974. Anthocyanins and their stability in foods. *Crit. Rev. Food Technol.*, **4**:437–456 .

Matheis, G., and Belitz, H.D. 1978. Studies on enzymic browning of potatoes (*Solanum tuberosum*). IV. Relationship between tyrosine turnover and rate of browning. *Zeits. Lebensm. U. Forsch.*, **167**:97–100.

Matich, A.J., Banks, N.H., and Rowan, D.D. 1998. Modification of α-farnesene levels in cool-stored 'Granny Smith' apples by ventilation. *Postharv. Biol. Technol.*, **14**:159–170.

Matsuoka, H., Takahashi, A., Ozawa, Y., Yamada, Y., Uda, Y., and Kawakishi, S. 2002. 2-[3-(2-Thioxopyrrolidin-3-ylidene)methyl]-tryptophan, a novel yellow pigment in salted radish roots. *Biosci. Biotechnol. Biochem.*, **66**:1450–1454.

McGarry, A., Hole, C.C., Drew, R.L.K., and Parsons, N. 1996. Internal damage in potato tubers: a critical review. *Postharv. Biol. Technol.*, **8**:239–258.

Mondy, N.I., and Chandra, S. 1979. Quality of potato tubers as affected by freezing: I. Texture, ascorbic acid and enzymatic discoloration. *Am. Pot. J.*, **56**:119–124.

Mondy, N.I., and Munshi, C.B. 1993. Effect of maturity and storage on ascorbic acid and tyrosine concentrations and enzymatic discoloration of potatoes. *J. Agric. Food Chem.*, **41**:1868–1871.

Muneta, P., and Kaisaki, F. 1985. Ascorbic acid-ferrous iron complexes and after cooking darkening of potatoes. *Am. Pot. J.*, **62**:531–536.

Munshi, C.B. 1994. Effect of mineral nutrition, sprout inhibitors, and naturally-occurring toxicants on potato quality. *Diss. Abs. Int.*-B, **54**:4979.

Murata, M., Shinoda, Y., and Homma, S. 2002. Browning of model orange juice solution and changes in the components. *Int. Cong. Ser.*, **1245**:459–460.

Naim, M., Schutz, O., Zehavi, U., Rouseff, R.L., and Haleva-Toledo, E. 1997. Effects of orange juice fortification with thiols on *p*-vinylguaiacol formation, ascorbic acid degradation, browning, and acceptance during pasteurisation and storage under moderate conditions. *J. Agric. Food Chem.*, **45**:1861–1867.

Namiki, M., Yabuta, G., and Koizumi, Y. 2001. Green pigment formed by the reaction of chlorogenic acid (or caffeic acid esters) with a primary amino compound during food processing. In: *Chemistry and Physiology of Selected Food Colorants*, ACS Symposium Series 775. (J.M. Ames, and T.F. Hofmann, Eds.), American Chemical Society, Washington, pp. 113–131.

Nicolas, J.J., Richard-Forget, F.C., Goupy, P.M., Amiot, M.J., and Aubert, S.Y. 1994. Enzymatic browning reactions in apple and apple products. *Crit. Rev. Food Sci. Nut.*, **34**:109–157.

Okunev, A.S., and Pokrovskaya, M.V. 1987. Complex formation of chlorogenic acid with iron (III). *Izv. Vyssh. Uchebn. Zaved., Pishch. Tekhnol.*, **5**:101–102.

Omidiji, O., and Okpuzor, J. 1996. Time course of PPO-related browning of yams. *J. Sci. Food Agric.*, **70**:190–196.

Onayemi, O. 1986. Some chemical factors affecting the quality of processed yam. *J. Food Sci.*, **51**:161–164.

Orozco-Cárdenas, M.L., and Ryan, C.A. 1999. Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. *Proc Natl Acad Sci USA.,* **96**:6553–6557.

Ozawa, Y., Uda, Y., and Kawakishi, S. 1993. Effects of pH, metal ions and ascorbic acid on formation of yellow pigments from tetrahydro-beta-carboline derivatives . *J. Jap. Soc. Food Sci. Tech.*, **40**:528–531.

Partington, J.C., Smith, C., and Bolwell, G.P. 1999. Changes in the location of polyphenol oxidase in potato (*Solanum tuberosum* L.) tuber during cell death in response to impact injury: comparison with wound tissue. *Planta*, **207**:449–460.

Paull, R.E., and Rohrbach, K.G. 1985. Symptom development of chilling injury in pineapple fruit. *J. Am. Soc. Hort. Sci.,* **110**:100–105.

Pechous, S.W., Watkins, C.B., and Whitaker, B.D. 2005. Expression of α-farnesene synthase gene AFS 1 in relation to levels of α-farnesene and conjugated trienols in peel tissue of scald-susceptible 'Law Rome' and scald-resistant 'Idared' apple fruit. *Postharv. Biol. Technol.*, **35**:125–132.

Peiser, G., Lopez-Galvez, G., Cantwell, M. and Saltveit, M.E. 1998. Phenylalanine ammonia lyase inhibitors do not prevent russet spotting lesion development in lettuce midribs. *J. Amer. Soc. Hort. Sci.*, **123**:687–691.

Piretti, M.V., Gallerani, G., and Brodnik, U. 1996. Polyphenol polymerization involvement in apple superficial scald. *Postharv. Biol. Technol.*, **8**:11–18.

Poei-Langston, M.S., and Wrolstad, R.E. 1981. Color degradation in an ascorbic acid-anthocyanin-flavanol model system. *J. of Food Sci.*, **46**:1218–1222, 1236.

Ranganna, S., and Parpia, H.A.B. 1974. Pink discoloration in canned banana (*Musa paradisiaca*) and field bean (*Dolichos lablab* var. *lignosus Prain*) II Chemical changes in leucoanthocyanidins during processing. *Lebensm. Wiss. Technol.*, **7**:111–119.

Ranganna, S., and Setty, L. 1968. Nonenzymatic discoloration in dried cabbage. Ascorbic acid – amino acid interactions. *J. Agric. Food Chem.*, **16**:529–533.

Rattanathanalerk, M., Chiewchan, N., and Srichumpoung, W. 2005. Effect of thermal processing on the quality loss of pineapple juice. *J. Food Eng.*, **66**:259–265.

Robertson, G.L., and Samaniego, C.M.L. 1986. Effect of initial dissolved oxygen levels on the degradation of ascorbic acid and the browning of lemon juice during storage. *J. Food Sci.*, **51**:184–187.

Sabba, R.P., and Dean, B.B. 1994. Sources of tyrosine in genotypes *of Solanum tuberosum* L. differing in capacity to produce melanin pigments. *J. Am. Soc. Hort. Sci.*, **119**:770–774.

Salisbury, F.B., and Ross, C.W. 1985. Mineral nutrition. In : Plant physiology, 3rd edition, Wadsworth Publishing Company, Belmont, California pp. 96–113.

Salunkhe, D.E., Wu, M.T., and Do,, J.Y. 1978. Effects of long-term storage on quality of processed foods 1. Meal, ready-to-eat, individual ration items packed in flexible retortable pouches. *J. Food Qual.*, **2**: 75–103.

Saltveit, M.E. 2000. Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. *Postharv. Biol. Technol.*, **21**:61–69.

Saltveit, M.E. 2004. Effect of 1-methylcyclopropene on phenylpropanoid metabolism, the accumulation of phenolic compounds, and browning of whole and fresh-cut 'iceberg' lettuce. *Postharv. Biol. Technol.*, **34**:75–80.

Saltveit, M.E., and Loaiza-Velarde, J.G. 2000. Method of preventing wound-induced browning in produce. *US Pat.* 6113958.

Saquet, A.A., Streif, J., and Bangerth, F. 2003. Energy metabolism and membrane alterations in relation to brown heart development in 'Conference' pears during delayed controlled atmosphere storage. *Postharv. Biol. Technol.*, **30**:123–132.

Sarma, A.D., Sreelakshmi, Y., and Sharma, R. 1997. Antioxidant ability of anthocyanins against ascorbic acid oxidation. *Phytochem.*, **45**:671–674.

Saure, M.C. 2005. Calcium translocation to fleshy fruit: its mechanism and endogenous control. *Sci. Hort.*, **105**:65–89.

Schaller, K., and Amberger, A. 1974. The correlation between constituents responsible for blackspots of potatoes. *Qual. Plant.: Plant Foods Hum. Nut.*, **24**:191–198.

Schwimmer, S. 1981. *Source Book of Food Enzymology*, The AVI Publishing Company Inc., Westport. Connecticut, USA.

Selvarajah, S., Bauchot, A.D., and John, P. 2001. Internal browning in cold-stored pineapples is suppressed by a postharvest application of 1-methylcyclopropene. *Postharv. Biol. Technol.*, **23**:167–170.

Shaw, P.E., Tatum, J.H., and Berry, R.E. 1977. In: *Developments in Food Carbohydrate-I*. G.G. Birch, and R.S. Shallenberger, (Eds.), Applied Science Publishers, London pp.91.

Shigenaga, T., Yamauchi, N., Funamoto, Y., and Shigyo, M. 2005. Effects of heat treatment on an ascorbate-glutathione cycle in stored broccoli (*Brassica oleracea* L.) florets. *Postharv. Biol. Technol.*, **38**:152–159.

Sim, S.K., Ohmann, S.M., and Tong, C.B. 1997. Comparison of polyphenol oxidase in tubers of *Solanum tuberosum* and the non-browning tubers of *S. hjertingii*. *Am. Pot. J.*, **74**:1–13.

Stevens, L.H., and Davelaar, E. 1996. Isolation and characterization of blackspot pigments from potato tubers. *Phytochem.*, **42**:941–947.

Stevens, L.H., Davelaar, E., Kolb, R.M., Pennings, E.J.M., and Smits, N.P.M. 1998. Tyrosine and cysteine are substrates for blackspot synthesis in potato. *Phytochem.*, **49**:703–707.

Strachan, G., and Nonnecke, I.L. 1961. Non-enzymatic browning in cauliflower. *Can. J. Plant Sci.*, **41**:377.

Sun, J., Jiang, Y., Wei, X., Shi, J., You, Y., Liu, H., Kakuda, Y., and Zhao, M. 2006. Identification of (–)-epicatechin as the direct substrate for polyphenol oxidase isolated from litchi pericarp. *Food Res. Intnl.*, **39**:864–870.

Takahama, U. 2004. Oxidation of vacuolar and apoplastic phenolic substrates by peroxidase: Physiological significance of the oxidation reactions. *Phytochem. Rev.*, **3**:207–219.

Thornton, M.K., and Workman, M. 1987. Changes in ascorbic acid content of blackspot-resistant and –susceptible potatoes following bruising. *Hort. Sci.*, **22**:455–456.

Thybo, A.K., Christiansen, J., Kaack, K., and Petersen, M.A. 2006. Effect of cultivars, wound healing and storage on sensory quality and chemical components in pre-peeled potatoes. *LWT-Food Sci. Tech.*, **39**:166–176.

Thygesen, P.W., Dry, I.B., and Robinson, S.P. 1995. Polyphenol oxidase in potato. A multigene family that exhibits differential expression patterns. *Plant Physiol.*, **109**:525–531.

Toivonen, P.M.A., and Sweeney, M. 1998. Differences in chlorophyll loss at 13°C for two broccoli (*Brassica oleracea* L.) cultivars associated with antioxidant enzyme activities. *J. Agric. Food Chem.*, **46**:20–24.

Uylaser, V., and Sahin, I. 2002. A research on the suitability of cauliflowers for canned-type pickle production. *Gida*, **27**:115–120.

Van Llelyveld, L.J., Gerrish, C., and Dixon, R.A. 1984. Enzyme activities and polyphenols related to mesocarp discoloration of avocado fruit. *Phytochem.*, **23**:1531–1534.

Veltman, R.H., Kho, R.M., van Schaik, A.C.R., Sanders, M.G., and Oosterhaven, J. 2000. Ascorbic acid and tissue browning in pears (*Pyrus communis* L. cvs Rocha and Conference) under controlled atmosphere conditions. *Postharv. Biol. Technol.*, **19**:129–137.

Veltman, R.H., Lenthéric, I., van der Plas, L.H.W., and Peppelenbos, H.W. 2003. Internal browning in pear fruit (*Pyrus communis* L. cv Conference) may be a result of a limited availability of energy and antioxidants. *Postharv. Biol. Technol.*, **28**:295–302.

Wang-Pruski, G., and Nowak, J. 2004. Potato after-cooking darkening. *Am. J. Pot. Res.,* **81**:7–16.

Watkins, C.B., Barden, C.L., and Bramlage, W.J. 1993. Relationships between alpha-farnesene, ethylene production and superficial scald development of apples *ISHS Acta Hort.*, **343**:155–160.

Weaver, M.L., and Hautala, E. 1970. Study of hydrogen peroxide, potato enzymes and blackspot. *Am. Pot. J.*, **47**:457–468.

Whitaker, B.D. 1998. Phenolic fatty-acid esters from the peel of 'Gala' apples and their possible role in resistance to superficial scald. *Postharv. Biol. Technol.*, **13**:1–10.

Whitaker, B.D., Solomos, T., and Harrison, D.J. 1998. Synthesis and oxidation of α-farnesene during high and low O_2 storage of apple cultivars differing in scald susceptibility. *ISHS Acta Hort.*, **464**:165–170.

Wills, R.B.H., and Tirmazi, S.I.H. 1982. Inhibition of ripening of avocados with calcium. *Sci. Hort.*, **16**:323–330.

White, P.J., and Broadley, M.R. 2003. Calcium in Plants. *Ann. Bot.*, **92**:487–511.

Woolf, A.B., and Ferguson, I.B. 2000. Postharvest responses to high temperatures in the field. *Postharv. Biol. Technol.*, **21**:7–20.

Woolf, A.B., Requejo-Tapia, C., Cox, K.A., Jackman, R.C., Gunson, A., Arpaia, M.L., and White, A. 2005. 1-MCP reduces physiological disorders of 'Hass' avocados. *Postharv. Biol. Technol.*, **35**:43–60.

Xuetong-Fan, and Mattheis, J.P. 2000. Yellowing of broccoli in storage is reduced by 1-methylcyclopropene. *HortSci.*, **35**:885–887.

Yamauchi, N., Funamoto, Y., and Shigyo, M. 2004. Peroxidase-mediated chlorophyll degradation in horticultural crops. *Phytochem. Rev.*, **3**:221–228.

Zauberman, G., Fuchs, Y., and Akerman, M. 1985. Peroxidase activity in avocado fruit stored at chilling temperatures. *Sci. Hort.*, **26**:261–265.

Zerbini, P.E., Rizzolo, A., Brambilla, A. Cambiaghi, P., and Grassi, M. 2002. Loss of ascorbic acid during storage of Conference pears in relation to the appearance of brown heart. *J. Sci. Food Agric.*, **82**:1007–1030.

Zerdin, K., Rooney, M.L., and Vermuë, J. 2003. The vitamin C content of orange juice packed in an oxygen scavenger material. *Food Chem.*, **82**:387–395.

Zhang, Z., Pang, X., Xuewu, D., Ji, Z., and Jiang, Y. 2005. Role of peroxidase in anthocyanin degradation in litchi fruit pericarp. *Food Chem.*, **90**:47–52.

Zhou, Y., Dahler, J.M., Underhill, S.J.R., and Wills, R.B.H. 2003. Enzymes associated with blackheart development in pineapple fruit. *Food Chem.*, **80**:565–572.

Zhuang, H., Hildebrand, D.F., and Barth, M.M. 1995. Senescence of broccoli buds is related to changes in lipid peroxidation. *J. Agric. Food Chem.*, **43**:2585–2591.