Review

Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables

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Abstract

This review describes the biochemical bases for color and firmness changes in fruit and vegetable tissues, since appearance and texture are two of the most fundamental factors affecting the quality of fresh-cut products. The intent is to provide a level of understanding that can be used to underpin future research directions in order to resolve existing issues that limit fresh-cut quality and shelf life. The biochemical mechanisms for enzymatic browning mediated by polyphenol oxidase and phenol peroxidase are described, and the importance of limiting cellular damage during the processing of fresh-cut fruit and vegetable products is emphasized. Also described are two mechanisms of chlorophyll degradation involved in discoloration events in green tissues, and examples of coloring processes specific to particular crops (white blush in carrots, discoloration of Allium spp., secondary browning in apples). The loss of desirable texture in fresh-cut products is a major problem. In fruit this is largely due to a continuation of cell wall disassembly events that are a normal component of ripening, and which result in declining cell wall strength and reduced intercellular adhesion. In some species the process is exacerbated by wound-response ethylene. However, wounding, water loss and ripening-related turgor changes are also important contributors to textural deterioration. In fresh-cut vegetables, water loss and damage-induced lignification are common problems. The effects of factors such as maturity at harvest, processing conditions and various treatments to mitigate quality decline are discussed.

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Keywords: Color; Texture; Pigments; Cell walls; Biochemical changes; Fruit; Vegetables

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Abbreviations: ACSO, S-alk(en)ylcysteine sulfoxide; HGA, homogalacturonan; POD, phenol peroxidase; PG, endo-polygalacturonase; PME, pectin methylesterase; PPO, polyphenol oxidase; RG, rhamnogalacturonan.
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1. Introduction

Appearance and texture changes are two fundamental characters determining the acceptability of fresh-cut fruit and vegetables. The understanding of the processes leading to these changes is essential in developing better approaches to minimizing them and, hence, improving quality and shelf life for the consumer. One common issue is that much of the biochemistry of appearance and textural changes has been, by-and-large, studied in whole plant or tissue systems. The consequences of the cutting operation and post-cutting processes in fresh-cut products have not been extensively studied. However, the value of exploring what is known within fresh-cut products, and melding that knowledge with the current understanding in whole tissue systems, is that it will shed some light on the major issues. Also it is hoped that it may stimulate new research activity to improve the understanding of changes in appearance and texture occurring in fresh-cut fruit and vegetables.

In essence, appearance and texture changes are very tightly linked to tissue deterioration, and as such can and are used as measures of freshness and quality decline in fresh-cut research and industry (Cantwell and Suslow, 2002). Hence, these two characters are probably the most interesting quality attributes with which fresh-cut processors are currently concerned in relation to maximizing shelf life. This review should provide some new insights which will enable researchers to focus experimental hypotheses, and industry to better understand the limits of product shelf life and freshness.

2. Appearance

The appearance of a fresh-cut fruit or vegetable is the attribute most immediately obvious to the consumer, and strongly affects the decision to buy. Many unrelated factors influence appearance, from wound-related effects to drying to microbial colonization. These factors have different causes, and different effects, but all result in an unattractive product. This section will discuss factors common to fresh-cuts of many crops (such as browning in fruit and chlorophyll loss in green produce), plus some examples of problems specific to particular crops (such as white blush in carrots and the development of off-colors in Alliums).

2.1. Cut-edge browning

This is a particular problem in fruit with a white flesh such as apples and pears, but is also a factor in many other fresh-cut fruit and vegetable products. Prior reviews regarding the mechanism and control of enzymatic browning have been confined to the description of the biochemistry of the polyphenol oxidase (PPO) interaction with polyphenols and oxygen (Sapers, 1993; Martinez and Whitaker, 1995). While the browning reactions are central to understanding the biochemical mechanisms, it also is important to evaluate the context of the sub-cellular localization. Fig. 1 provides some detail in regards to localization of phenolic compounds and the enzymes which interact with them to cause browning. Phenolic compound synthesis is associated with the endoplasmic reticulum (Hrazdina and Wagner, 1985). The proteins involved with their synthesis are either incorporated into the endoplasmic reticulum membrane or are loosely associated with it (Hrazdina and Wagner, 1985). Once formed, these compounds are glycosylated and then are extruded within transport vesicles formed from the endoplasmic reticulum membrane. These vesicles are the vehicle by which the phenolic compounds are transported to the vacuole or into the apoplast/cell wall compartment (Hrazdina and Wagner, 1985). There are smaller quantities of phenolic compounds which may be found in chromoplasts, cytoplasm and the mitochondria, but these are normally minute amounts and are associated with specialized metabolic functions (Hrazdina and Wagner, 1985). From this discussion, it follows that the initial event in the oxidative browning process must be the breakdown of membranes within cells of plant tissues and this has been reviewed elsewhere (Toivonen, 2004). Once a physical stress or deteriorative process (e.g. wounding response or senescence) is initiated, the compartmentalization of the cell begins to fail (Marangoni et al., 1996). The consequence of this is the mixing of polyphenol substrates (e.g. catechin, polyphenols) with polyphenol oxidase and/or phenol peroxidases (Degl’Innocenti et al., 2005). This hypothesis is supported by work with fresh-cut potatoes that clearly shows that browning is not rate limited by either the enzymes associated with browning or polyphenol substrate concentration (Cantos et al., 2002). The authors suggest that membrane stability is potentially a major factor controlling the rate of browning.

Most strategies to control cut-edge browning have focused on theoretical approaches to modulate PPO enzyme activities (Martinez and Whitaker, 1995). The most widely used commercial anti-browning formulation available today uses calcium salts and ascorbate (Rupasinghe et al., 2005). Ascorbate is hypothesized to control PPO activity through its ability to reduce quinones to the native diphenols (Nicolas et al., 1994). However, ascorbate has many other possible activities in tissues that might explain its usefulness in inhibiting browning since it is a “univer-
sal” antioxidant (Noctor and Foyer, 1998) and can even quench lipid alkoxyl and peroxyl radicals involved in membrane deterioration (Espín et al., 2000b). It is difficult to ascribe a role to calcium in modulating PPO activity; however, a role in maintaining cell and membrane integrity has been well established (Poovaiah, 1986). It is likely that a formulation containing calcium and ascorbate acts partly to prevent cell and membrane breakdown and also modulate PPO activity in already damaged cells where loss of compartmentalization has occurred. Conversely, there are formulations of PPO inhibitors which act to control browning (Sapers, 1993; Martinez and Whitaker, 1995) and their activities are wide ranging; however most have not been commercially effective or acceptable in fresh-cut products.

2.2. Browning reactions

Browning reactions have generally been assumed to be a direct consequence of PPO action on polyphenols (Martinez and Whitaker, 1995), although some have attributed at least a partial role to the action of phenol peroxidase (POD) on polyphenols (Underhill and Critchley, 1995; Richard-Forget and Gaulllard, 1997; Degl’Innocenti et al., 2005). It is difficult to ascribe a significant role to POD when one of its substrates, hydrogen peroxide (H$_2$O$_2$), is generally at very low concentrations in plant cells (Veljovic-Jovanovic et al., 2002). This is for very good reasons, as plant cells regulate hydrogen peroxide levels very tightly due to its implications for oxidative injury (Mittler, 2002). Recent evidence indicates that POD could enhance browning reactions in the presence of ongoing PPO-mediated browning reactions (Richard-Forget and Gaulllard, 1997). While the mechanism of this PPO-coupled browning is not clearly understood, it is possible that the PPO-mediated generation of quinones can lead to H$_2$O$_2$ accumulation, providing a higher concentration of this free-radical species, thus enabling significant levels of POD-mediated polyphenol browning (Jiang and Miles, 1993). The profile of polyphenol substrates in a specific tissue will also impact on the potential for POD-mediated browning since some phenolic substrates (e.g. catechin) can potentially yield more H$_2$O$_2$ than others (e.g. chlorogenic acid) (Cantos et al., 2002). The exact mechanism of this PPO-mediated H$_2$O$_2$ generation has not been elucidated and the existence of this reaction has only been shown in vitro (Jiang and Miles, 1993). Peroxidase-associated browning can be distinguished from PPO-associated browning by the addition of a hydrogen peroxide quenching agent such as catalase, which will prevent browning caused by peroxidase reactions (Underhill and Critchley, 1995; Richard-Forget and Gaulllard, 1997). Further work should be conducted with PPO inhibitors such as tropolone to determine whether POD-mediated browning can occur outside the existence of PPO-associated browning. It would be premature to argue that POD-mediated polyphenol browning is a consistently significant component in browning of fresh-cut fruit and vegetables, although there are sufficient questions raised by the current literature to encourage further work in this area.

The mechanism for browning involves the interaction of polyphenolic substrates with PPO in the presence of oxygen (Fig. 2). PPO catalyzes two reactions: (1) hydroxylation of monophenols to diphenols and (2) oxidation of diphenols to quinones. The hydroxylation reaction is relatively slow and results in colorless products, while the oxidation reaction is relatively rapid and the resultant quinones are colored. Subsequent reactions of the quinones lead to melanin accumulation, which is the brown or black pigment associated with “browning” in plant tissues. The specific reaction sequence which results in brown or black-colored products depends on the specific structure of the polyphenolic substrate.

2.3. Discoloration involving chlorophyll degradation

Yellowing, or loss of green color, is normally considered the major consequence of chlorophyll degradation (Brown et al., 1991; Heaton and Marangoni, 1996; Matile et al., 1999). However, chlorophyll degradation can also lead to color changes akin to browning in fresh-cut products treated with salad dressing (Heaton and Marangoni, 1996; Heaton et al., 1996). Both types of color change are associated with the same pathway for chlorophyll breakdown. However, the browning discoloration is a consequence of incomplete metabolism of the chlorophyll molecule. Fig. 3 shows a generalized scheme for the initial stages of chlorophyll breakdown that eventually lead to colorless products. It is clear that one of two processes precedes the other to yield a common product, pheophorbide, which is the immediate precursor of colorless breakdown products (Matile et al., 1999). In the first of two possible sequences, magnesium dechelatase can act directly on the chlorophyll molecule to produce pheophytin, which is olive-brown in color and this can be converted to pheophorbide via chlorophyllase activity. This can then be converted to colorless products that are eventually transported to the vacuole for storage (Matile et al., 1999). In the alternative sequence, chlorophyllase acts directly on chlorophyll and reduces it to chlorophyllide, which is then converted to pheophorbide by magnesium dechelatase (Heaton and Marangoni, 1996). When a low pH dressing is applied, such as in coleslaw, the low pH mediates the loss of the magnesium from the chlorophyll molecule in the absence of dechelatase.
Fig. 3. Schematic representation of Type I chlorophyll breakdown pathways in green plant tissues. The information to generate the schematic was obtained from Matile et al. (1999).

The low pH can also inactivate chlorophyll degrading enzymes which have a pH optimum around 7.0 (McFeeters et al., 1971; Suzuki et al., 2002; Arkus et al., 2005) and hence the result is an accumulation of pheophytin and the transformation of green tissue to an olive-brown color (Heaton and Marangoni, 1996). The above description involves very controlled, relatively well-described transformations. Such chlorophyll breakdown is classified as Type I by Brown et al. (1991). Type II breakdown is far less controlled and is mediated by oxygen radicals (Brown et al., 1991). Another feature of Type II breakdown is that the membrane systems within the cell are normally disrupted prior to their initiation (Matile et al., 1999). Several possible mechanisms have been reported for Type II breakdown of chlorophyll, although this list is probably only limited by the restricted number of experiments conducted to date. One of the difficulties of examining Type II degradation is isolating stable intermediates or identifiable end-products (Brown et al., 1991). The first potential mechanism for Type II breakdown involves fatty acids and either chlorophyll oxidase or lipoxygenase (Martinoia et al., 1982; Lüthy et al., 1984; Thomas, 1986). These two paths share many commonalities, such as being dependent on the presence of free fatty acids and being strictly dependent on the availability of oxygen (Lüthy et al., 1984). However, they differ in that the chlorophyll oxidase does not generate linolenate hydroperoxide and it can act on a wider range of fatty acid substrates including oleic and stearic acids (Lüthy et al., 1984). The purported mechanism of chlorophyll bleaching involves the reaction of the peroxo radicals formed when these two enzymes oxidize their lipid substrates (Thomas, 1986). The peroxo radicals can directly attack chlorophyll molecules, causing the loss of structure and color. The second mechanism relates to peroxidase-associated reactions (Martinoia et al., 1982; Shibata et al., 1995). There are a number of peroxidase reactions that can lead to oxidation of chlorophylls, including reactions using as substrate phenolic compounds (Martinoia et al., 1982) and nitrite (Shibata et al., 1995). The peroxidase mechanism appears not to be a direct oxidation by hydrogen peroxide mediated by peroxidase, rather it is purported to be due to the direct oxidation of the chlorophyll molecule by phenoxy radicals that are generated by peroxidase reaction with the p-hydroxyl group of certain phenolic compounds (Fig. 4; Yamauchi et al., 2004). The interaction of peroxidase with these phenolic compounds also results in the concomitant production of superoxide anions (Fig. 4; Yamauchi et al., 2004) which can directly oxidize the chlorophyll molecule (Brown et al., 1991; Yamauchi et al., 2004).

From the discussion in the previous paragraph, it becomes clear that free-radical events can be correlated with chlorophyll loss, suggesting that events which lead to uncontrolled production of radicals are a prerequisite for Type II chlorophyll breakdown. Previous workers have suggested that intact cells

Fig. 4. Schematic for three possible Type II chlorophyll breakdown pathways in damaged cells of green plant tissues. Schematic was constructed from information obtained from Martinoia et al. (1982), Lüthy et al. (1984), Thomas (1986), Brown et al. (1991), Shibata et al. (1995) and Yamauchi et al. (2004). CHLOX: chlorophyll oxidase; CHL-POD: chlorophyll peroxidase; LOX: lipoxygenase.
show a controlled senescence process closely described by Type I breakdown events (Brown et al., 1991; Matile et al., 1999; Hörtensteiner and Feller, 2002). However later in the senescence or stress response timeline membrane breakdown occurs and this leads to loss of sub-cellular compartmentalization, intermixing of many enzyme systems and their potential substrates thus leading to multiple possible oxygen radical production scenarios and oxidation of chlorophyll (Martinioa et al., 1982; Matile et al., 1999; Hörtensteiner and Feller, 2002).

Studies on chlorophyll loss in fresh-cut product have predominately produced evidence for Type II chlorophyll breakdown, i.e. breakdown induced by oxygen radical oxidation of the chlorophyll molecule (Brown et al., 1991). Chlorophyll breakdown in parsley leaves has in one case been shown to involve pheophytin accumulation (Yamauchi and Watada, 1991), and in another case to involve two breakdown intermediates, pheophytin and an unknown believed to be 13'-hydroxychlorophyll (Amir-Shapira et al., 1987). In broccoli, there are numerous reports strongly supporting the hypothesis that peroxidase- and/or chlorophyll oxidase- and/or lipooxygenase-mediated chlorophyll breakdown are important in whole product storage and packaged fresh-cut product (Zhang et al., 1994; Funamoto et al., 2002, 2003; Costa et al., 2005, 2006). Spinach leaf chlorophyll loss has been associated with peroxidase-mediated breakdown as well (Yamauchi and Watada, 1991). In a final example, excised cabbage discs show chlorophyll losses strongly associated with lipooxygenase activity and concomitant fatty acid degradation (Chéour et al., 1992).

The implications of whether Type I or II breakdown mechanisms are involved in the loss of chlorophyll in a particular fresh-cut product provide some new insight as to how to approach reduction in the rate of chlorophyll losses. For example, there has been much understanding developed in pre-harvest chlorophyll loss in crops, showing the importance of Type I break down pathways in the orderly regulation of chlorophyll content in response to water and heat stresses, insect feeding and aging (Majumdar et al., 1991; Fang et al., 1998; Ni et al., 2001; Hörtensteiner, 2006). However, in the case of fresh-cut product, the issue appears to be Type II reactions. Hence, those working to improve quality losses in fresh-cut product through breeding will have to be asking different questions than those working to breed improved crops in the field. The question relating to fresh-cut chlorophyll loss should not be focused specifically on chlorophyll catabolism; rather on approaches to maintain cell membrane integrity and control fatty acid oxidation reactions (Chéour et al., 1992).

2.4. White blush in carrots

Minimally processed, cut-and-peel carrots suffer from a very specific surface discoloration named “white blush” which occurs as consequence of exposure of damaged cell wall materials on processed or cut edges to drying conditions (Tatsumi et al., 1991, 1993; Avena-Bustillos et al., 1994; Cisneros-Zevallos et al., 1995). While the drying is a physical process, post-processing accumulation of lignified material can occur as a wound response, intensifying the incidence and severity of this “white blush” (Bolin and Huxsoll, 1991; Bolin, 1992; Howard and Griffin, 1993; Howard et al., 1994a; Cisneros-Zevallos et al., 1995). Despite the fact it is a wound-associated process, ethylene does not appear to be a part of the mechanism which initiates this particular lignification process (Howard and Griffin, 1993). Lignification is a process which involves a series of many enzyme conversions beginning with phenylalanine ammonia lyase, which is the initial reaction in the phenylpropanoid pathway producing soluble phenolics (Hennion et al., 1992) all the way through to syringaldazine oxidase, a cell wall-associated isoform of peroxidase that converts syringaldazine to lignin (Goldberg et al., 1985). The definitive demonstration that lignification is important to “white blush” formation was provided by Howard et al. (1994a). They showed that steam treatment inhibited phenylalanine ammonia lyase and syringaldazine oxidase activities in minimally processed carrots and these declines in activity could be correlated with reduced accumulation of soluble phenolic compounds and lignin in treated carrots. Steam-treated carrots were also shown to have significantly reduced levels of “white blush” (Howard et al., 1994a). Since the physical appearance and lignification are intensified with increased roughness of the processing, the use of fine abrasives to polish the carrot surface results in less “white blush” (Bolin and Huxsoll, 1991). Similarly, use of sharp cutting implements will also reduce the wound response and lignin accumulation (Tatsumi et al., 1991; Bolin and Huxsoll, 1991). In addition, “white blush” can be controlled with treatments that alter tissue pH and hence enzyme activity (Bolin and Huxsoll, 1991; Bolin, 1992).

2.5. Discoloration in Alliums

Onions, garlic and leeks can develop pink, red, green, blue-green or blue discolorations as a consequence of cell disruption (Joslyn and Peterson, 1960; Körner and Berk, 1967; Lukes, 1986). It is not common in fresh-cut product, but in instances where cutting operations or handling lead to significant tissue damage, this discoloration can develop (Howard et al., 1994b). The chemistry of this discoloration is peculiar to Allium species and both isoalliin and alliinase are prerequisites for the discoloration to occur (Kubec et al., 2004). In brief, isoalliin and other S-alk(en)ylcysteine sulfoxides (ACSOs) are enzymatically cleaved by the enzyme alliinase to yield 1-propenyl-containing thiosulfanates. These thiosulfanates (termed “color developers”) interact with free amino acids to produce pigmented compounds, the color of which is dependent on the identity and proportion of the various thiosulfate species that are generated by the alliinase (Kubec et al., 2004). In intact Allium tissues, the ACSOs are generally localized in the cytoplasm, while the alliinase is localized in the vacuole (Randle and Lancaster, 2002). In addition, alliinase is highly concentrated in the bundle sheath cells of the vasculature of bulb tissue, with very little found in leaf tissue (Randle and Lancaster, 2002). In summary, as with browning and chlorophyll degradation, loss of cellular and tissue integrity needs to occur before the alliinase action on ACSOs can occur, and the resultant compounds must then interact with free amino acids in the damaged milieu of tissue and cell juices.
2.6. Secondary browning in apples

Secondary browning has recently been identified as a quality limitation in fresh-cut apples (Toivonen, 2006). It can be discriminated from cut-edge browning in two ways: (1) secondary browning is localized in nature as opposed to the diffuse nature of cut-edge browning and (2) the timing of occurrence. Cut-edge browning occurs within hours of cutting, whereas secondary browning begins to appear in fresh-cut fruit at any time between 1 and 3 weeks in storage, the exact time of incidence being governed by the temperature handling history of the product (Toivonen, 2006). Application of anti-browning solution at cutting does not control it to any great extent (Toivonen and Delaquis, 2006). Observations in non-browning apples that have been produced using insertion of an antisense PPO transgene demonstrate that the browning is not a consequence of PPO activity within the apple slice tissue, rather it can be attributed to the action of tyrosinase exuded from the fungal spores germinating on the cut surfaces of the slices (Toivonen, 2004; and unpublished data). This phenomenon points out a challenge in the study of fresh-cut fruit and vegetables, and that is the understanding of the true biochemical reality of cut products. The reality is that the biochemistry of surface-associated microorganisms may be partially responsible for biochemically mediated changes in quality as well as the constitutive biochemistry within the product tissue.

3. Texture

Texture comes in many guises (crispness, hardness, mealliness, flouriness, grittiness, etc. (Harker et al., 1997a)), and the consumer has an expectation that cutting and storing a product will not interfere with the anticipated sensory properties. Many aspects of texture can be quantified objectively, particularly those related to mechanical properties. To the consumer, there are two factors that most influence the mouth feel of a fruit or vegetable: firmness and juiciness. Firmness is determined largely by the physical anatomy of the tissue, particularly cell size, shape and packing, cell wall thickness and strength, and the extent of cell-to-cell adhesion, together with turgor status. Many of these factors are inter-related, for example tissues with small cells tend to have a greater content of cell walls, a lower relative amount of cytoplasm and vacuole (cell sap), a greater area of cell-to-cell contact, and low amounts of intercellular air spaces, making the tissue firmer and apparently less juicy.

Although cell wall thickness and strength are major contributors to firmness, these are characteristic of a species and a tissue and are determined largely by genetic factors. Unlike vegetables (stems, roots/tubers, leaves), the cells of ripening fruit flesh are generally relatively weak. Also unlike vegetables, the cell walls of fruit undergo a natural degradation during fruit ripening, reducing cell wall firmness and intercellular adhesion. This leads firstly to the attainment of a desirable eating texture and then, as senescence begins, to a loss of this desirable texture. In addition to cell wall strength and properties, firmness is also related to the turgor properties of a tissue or organ, which is affected by factors such as the accumulation of photosynthate and the water status of the cells. Turgor can be affected by environmental conditions, such as sunlight received during growth, and is substantially reduced by water loss from the tissue. During fruit ripening, there is a decline in turgor which contributes to textural changes (Shackel et al., 1991; Harker and Sutherland, 1993), probably due partly to an accumulation of osmotic solutes in the cell wall space (Almeida and Huber, 1999), and partly to postharvest water loss from the ripening fruit (Saladie et al., 2007). Factors affecting texture may change substantially either pre- or post-harvest, due to changes in cell size, intercellular adhesion, starch/sugar conversion, water loss, cell wall composition and cell wall strength.

Perceived juiciness is also affected by the cellular makeup of a tissue, large cells having a greater relative content of cell sap and tending to split open more easily. The nature of tissue failure with biting and chewing determines juiciness, whether cell walls split open releasing juice, or whether tissue splits by cell separation along the middle lamellae, with little cell rupture. Tensile tests examining failure under stress found that in crisp fruit such as apple and watermelon the tissue broke apart abruptly, due to cells splitting open across the primary cell walls (Glenn and Pooovaiah, 1990; Harker et al., 1997b). Carrot showed some elasticity and bending followed by failure, but the fracture surface also showed that cells had split open. However, muskmelon showed a mixture of cell rupture and cell separation along the middle lamellae at the fracture surface, while banana showed almost exclusively cell separation without cell rupture (Harker et al., 1997b). Cell separation without rupture may be typical of fruit which are very soft when ripe, such as peach, kiwifruit and strawberry. In pears, tissue failure when unripe was due to cell wall failure and cell fracture, whereas with increasing softening failure became partly then exclusively due to intercellular debonding (De Belie et al., 2000). However, although ruptured cells at the fracture surface may not be evident in very soft fruit, a layer of juice typically overlays the broken surface (Harker and Sutherland, 1993; Harker and Hallett, 1994), possibly accounting for the juicy texture. Whether this juice is a naturally occurring extra-cellular fluid or is an intracellular fluid released by membrane damage during the application of tension has not been determined (Harker et al., 1997a). Fruit with a juicy texture in the mouth presumably contain cells with plenty of juice which is easily released during chewing, owing to the weakening of cell walls during ripening. Firmness can change rapidly during fruit ripening or in fresh-cut fruit and vegetables, and numerous handling and storage treatments are applied to slow down textural change.

3.1. Loss of textural quality in ripening whole fruit

Many of the changes occurring in fresh-cut fruit are a continuation of the normal ripening events that lead to softening, combined with or influenced by the effects of tissue cutting and wounding. Thus an understanding of normal ripening-related softening is important before considering the special case of fresh-cut fruit.

Fruit can be divided into groups based upon their softening behavior, or upon the regulation of their ripening process.
Bourne (1979) divided fruit into:

1. Those that soften greatly as they ripen, such as apricot, strawberry, peach, plum, kiwifruit, European pear and most berries. These fruit have a soft, melting texture.

2. Those that soften moderately as they ripen, such as apple, quince, cranberry, Asian pear, bell pepper and watermelon. These fruit tend to have a crisp, fracturable texture.

Fruit can also be divided into two groups based on the role of ethylene in their ripening process (Lelièvre et al., 1997):

1. Climacteric fruit that produce large amounts of ethylene, and in which ripening is promoted by ethylene, such as tomato, peach, apple, banana and kiwifruit.

2. Non-climacteric fruit that produce only low basal amounts of ethylene throughout ripening and in which ripening is insensitive to exogenous ethylene, such as grape, strawberry, watermelon, pineapple and citrus.

Some climacteric fruit are melting (peach, avocado) and some are crisp (apple, quince), whereas non-climacteric fruit can also be melting (strawberry, blackberry) or crisp (watermelon, cranberry), indicating that there is no relationship between climacteric or non-climacteric status and fruit texture. Rather, fruit firmness and texture are most closely associated with cell wall structure and composition, and particularly with the cell wall changes that occur during ripening.

The cell wall provides rigidity and strength, and it is against the resistance of the wall that the osmotic pressure of the protoplast exerts force and provides turgor. Nevertheless, primary cell walls are extensible, somewhat elastic and are capable of being loosened and allowing growth. In contrast, secondary cell walls are inextensible, heavily thickened and often lignified. Fruit parenchyma cells mostly have primary cell walls, usually relatively thin and weakened. Vegetable tissues generally have a much higher proportion of cells with thickened and lignified cell walls, and usually (in their raw state) are much harder than ripe fruit.

Primary cell walls are composed of rigid cellulose microfibrils held together by networks of matrix glycans (hemicelluloses) and pectins, together with smaller amounts of structural proteins and phenolics (Carpita and Gibeaut, 1993). In most species, the predominant matrix glycan is xyloglucan, with the remainder being largely substituted or unsubstituted xylans and some glucomannan. Pectins are characterized by their large content of galacturonic acid, and are of four types: homogalacturonan (HGA, a large linear unsubstituted polymer of galacturonic acid), xylogalacturonan (HGA possessing occasional substitution with single xylose residues), and two types of branched rhamnogalacturonans. Rhamnogalacturonan I (RG-I) has a backbone of alternating residues of galacturonic acid and rhamnose, to which are attached large side chains of galactan and arabinan. Rhamnogalacturonan II (RG-II) has a backbone like HGA, with a complex side-chain structure containing many different sugars including several rare ones (Carpita and Gibeaut, 1993).

All of the above polysaccharides are found throughout the primary cell wall. However, the middle lamella is pectin rich and is composed almost entirely of HGA. The middle lamella acts as the glue holding neighboring cells together, and as such is the primary determinant of intercellular adhesion. During plant development, HGA in the cell wall becomes increasingly negatively charged, due to the removal of methylester groups by pectin methylesterase (PME), leaving carboxylic acid groups. In the presence of calcium, domains of negatively charged galacturonic acid residues in different HGA molecules associate together through ionic Ca\(^{2+}\) bonds, forming a calcium-pectate gel that adds to wall strength and provides most of the intercellular bonding in ripe fruit.

The other main component of intercellular adhesion is the extent of intercellular contact, which is determined by cell shape and packing, water loss and the size or absence of intercellular air spaces. These are factors which may change as a fruit ripens, generally leading to larger air spaces and reduced intercellular contact (Glenn and Poovaiah, 1990; Hallett et al., 1992; Harker and Sutherland, 1993), thus allowing increased tissue deformability under stress.

During fruit ripening, cell wall polysaccharides are extensively modified by a variety of ripening-related enzymes secreted from the symplast into the cell wall space. These changes affect the structure and strength of the wall, and ultimately bring about fruit softening. Both pectins and matrix glycans are degraded, although the nature of the changes occurring is species-specific and even cultivar-specific (Brummell, 2006). Fig. 5 shows the modification of major cell wall components occurring during softening in a melting-flesh peach variety. A series of changes is initiated sequentially, so that, at different times during ripening, softening and textural alterations are the result of different sets of cell wall modifications. However, no fruit species is typical, and such schemes (and indeed the mechanism resulting in softening) may differ significantly in other species.

![Fig. 5. Schematic representation of softening and changes to cell wall components occurring during maturation and ripening of melting-flesh peach. Note that the chronological order and extent of most of these events varies between fruit types, with some processes being reduced or absent in other species. Figure modified from Brummell (2006), based on data in Brummell et al. (2004). RG-I, rhamnogalacturonan-I.](image-url)
Pectins generally undergo the earliest modification, with the loss of RG-I side chain galactan beginning before ripening is initiated. In tomato, almost half of the cell wall galactose content is lost during green fruit maturation, with the rate of loss increasing substantially after ripening begins (Kim et al., 1991). A substantial loss of polymeric cell wall galactose is a feature of ripening in most, but not all, fruit (Gross and Sams, 1984; Redgwell et al., 1997a). Transgenic suppression of a tomato ripening-related β-galactosidase enzyme activity showed that reducing the degradation of cell wall galactan early in ripening causes a reduction of firmness later in ripening (Smith et al., 2002). Degradation of RG-I arabinan side chains also occurs, presumably due to the activity of a ripening-related α-arabinosidase (Tateishi et al., 2005). In apple, degradation of a branched arabinan occurs prior to softening (Peña and Carpita, 2004), whereas in melting flesh peach a striking loss of polymeric arabinose occurs during ripening and softening (Brummell et al., 2004). As with the loss of cell wall galactose, loss of cell wall arabinose proceeds during ripening in most species of fruit (Gross and Sams, 1984; Redgwell et al., 1997a). It seems likely that the degradation of the large side chains of RG-I is part of the process that increases the porosity and openness of the wall, and which may allow increasing access of degradative enzymes resident in the cell wall space to polysaccharide substrates.

The demethylesterification of pectic HGA also begins during fruit maturation or early in ripening, accomplished by the activity of PME. PME activity peaks early in ripening, but is reduced during fruit maturation or early in ripening, accomplished by the activity of PME (Phan et al., 2003; Callahan et al., 2004). In tomato, the degree of pectin methylesterification occurs before softening (Barnavon et al., 2001; Brummell et al., 2004). In tomato, the degree of pectin methylesterification declines from 90% in mature green fruit to 35% in pink and red ripe fruit (Koch and Nevins, 1989). Calcium cross-links between demethylsterified HGA molecules in the middle lamella form an important component of the bonding between adjacent cells. Transgenic suppression of PME activity in tomato decreases pectin demethylesterification, and reduces the depolymerization of pectin by endo-polygalacturonase (PG) during ripening (Tieman et al., 1992). It is well known that PG requires a pectin substrate that is at least partially demethylsterified (Wakabayashi et al., 2000). The firmness of transgenic tomatoes with reduced PME activity was reduced during ripening (Phan et al., 2007), but as fruit entered the over-ripe stage the integrity of the tissue became severely compromised, presumably due to the reduced ability to form calcium cross-bridges between pectin molecules (Tieman and Handa, 1994). Pectin demethylsterification also contributes to the changing ionic conditions in the apoplast during ripening, the charged domains playing a role in lowering pH and altering ion balance, which may modify the activity of enzymes and the diffusion of charged proteins within the wall matrix (Almeida and Huber, 1999, 2007).

Depolymerization of matrix glycans is a common feature of fruit ripening (Brummell, 2006), with the possible exception of apple and some soft berries (Percy et al., 1997; Vicente et al., 2007a, 2007c). It has been observed to start coincident with the beginning of ripening, to continue progressively throughout ripening, and in most cases to correlate with softening (Huber, 1984; O’Donoghue and Huber, 1992; Brummell et al., 1999, 2004). Ripening-related glycan hydrolases include endo-1,4-β-glucanases, endo-1,4-β-xylanases, endo-1,4-β-mannanases and xyloglucan endotransglycosylases, although the role of these enzymes and the cause of xyloglucan depolymerization during softening remain unclear (Brummell and Harpster, 2001).

Depolymerization of pectins is much more variable between species, both in when it begins and in the extent of chain cleavage occurring (Brummell, 2006). In some fruit, such as tomato and avocado, pectin depolymerization begins during early to mid-ripening (Huber and O’Donoghue, 1993), whereas in others, such as peach and pepino, it occurs late in ripening (O’Donoghue et al., 1997; Brummell et al., 2004). In strawberry, banana and blueberry little pectin depolymerization can be detected (Huber, 1984; Wade et al., 1992; Vicente et al., 2007b), whereas depolymerization is moderate in tomato and peach and extensive in avocado (Huber and O’Donoghue, 1993; Brummell et al., 2004). Experiments in transgenic non-softening mutant tomato fruit have shown that PG-mediated polyuronide degradation alone may not be sufficient to cause softening (Giovannoni et al., 1989), and in many species pectin depolymerization does not correlate with softening, at least during early ripening. However, pectin depolymerization appears to be the major cause of firmness loss in some soft berries (Vicente et al., 2007a, 2007c). Also, non-melting flesh peach and domesticated pepper cultivars are natural mutants in which softening is greatly reduced (relative to melting flesh peach and wild pepper accessions, respectively), due to genetic lesions that result in deficient PG gene expression (Rao and Paran, 2003; Callahan et al., 2004). In tomato, transgenic suppression of PG results in improved shelf life due to improved middle lamella integrity (Kramer et al., 1992; Langley et al., 1994), and in strawberry suppression of pectate lyase results in firmer fruit (Jiménez-Bermúdez et al., 2002). Depolymerization of middle lamellae during ripening is visible under the electron microscope (Crookes and Grierson, 1983; Hallett et al., 1992), and is a major cause of reduced intercellular adhesion, which affects firmness. Thus pectin depolymerization forms a component of softening, and reducing it would be expected to increase shelf life.

In addition to cell wall weakening, two other results of altered cell wall disassembly during ripening are that pectins become increasingly soluble (extractable), and that the cell wall swells. These two factors are often correlated in extent (Redgwell et al., 1997b), although whether they are related to each other or whether both are consequences of some other event has not been resolved. Overexpression of a ripening-related expansin in tomato results in enhanced fruit softening and a marked increase in matrix glycan depolymerization, whereas its suppression results in firmer fruit and reduced pectin depolymerization (Brummell et al., 1999). Expansins are cell wall-modifying proteins that lack hydrolytic activity, and are thought to act by loosening the bonding between matrix glycans and cellulose microfibrils (Cosgrove, 2000). Presumably, the altered glycan and pectin depolymerization observed in transgenic fruit is due to a modified accessibility of existing hydrolases to
polysaccharide substrates. This suggests that loosening of the glycan-cellulose network by expansin may be one of the causes of cell wall swelling during ripening. Whatever the mechanism, the cell wall becomes a swollen, more porous and hydrated structure during ripening, and this combined with cell wall disassembly and degradation of the middle lamella reduces the strength of the wall and decreases intercellular adhesion.

3.2. Loss of textural quality in fresh-cuts

The texture of a fruit or vegetable tissue is a composite of numerous factors, some of which are genetic, some environmental, and some due to postharvest handling and storage. Different cultivars vary widely in their rate of textural deterioration (Gorny et al., 2000; Abbott et al., 2004; Saftner et al., 2005), and there is not always a relationship between the rate of softening in whole fruit and in fresh-cut pieces (Aguayo et al., 2004). Even for a particular cultivar, the conditions under which the plants are grown can affect the subsequent shelf life of the fresh-cut product (Hong et al., 2000; Bett-Garber et al., 2005). Maturity at the time of processing strongly affects product shelf life (Beaulieu et al., 2004; Soliva-Fortuny et al., 2004; Lana et al., 2005), particularly for fruit since during ripening they become increasingly soft and susceptible to transport and handling damage. For vegetables such as jicama, freshness at the time of processing strongly affects product shelf life (Aquino-Bolaños et al., 2000). Compared with fruit, vegetables generally have a much greater proportion of cells with thickened secondary walls and consequently are much firmer and less susceptible to softening. Also, the natural progression of cell wall modifications that causes softening in fruit does not occur in vegetable tissues, and loss of textural quality is related to ageing processes and senescence, water loss, reduced turgor and wounding effects including the leakage of osmotic solutes. Fresh-cuts of vegetables generally present fewer problems than fresh-cuts of fruit, many species of which soften rapidly owing to natural ripening events. However, in delicate products such as spinach, wilting can be one of the major causes of loss of visual appearance and texture (Piagentini et al., 2002).

Fresh-cut products are wounded tissues, and consequently they deteriorate more rapidly and their physiology differs from that of intact fruit and vegetables. The various processes of peeling, coring, chopping, slicing, dicing or shredding cut through cells and release cell contents at the sites of wounding. Subcellular compartmentalization is disrupted at the cut surfaces, and the mixing of substrates and enzymes which are normally separated can initiate reactions that normally do not occur. Many of the postharvest treatments and storage conditions applied to fresh-cuts are designed to ameliorate the initial effects of wounding and wounding-induced responses. For both fruit and vegetables, wounding and mechanical injury result in increased rates of respiration and production of ethylene, with effects being observed very rapidly, often within minutes to a few hours (Rosen and Kader, 1989; Abe and Watada, 1991; Agar et al., 1999; Escalona et al., 2003).

The severity of wounding caused by the various stages of producing fresh-cuts has a major effect on product shelf life, and can be greater for climacteric fruit for which wound-induced ethylene promotes ripening and softening. In climacteric fruit, wound-induced ethylene would have the same effect as treating tissue with exogenous ethylene, causing a hastening of ripening and softening. Both for fruit and vegetables, ethylene production can promote senescence and its removal or antagonism by 1-methylcyclopene extends product shelf life (Abe and Watada, 1991; Jeong et al., 2004). In vegetables, lignification and the development of hardening can be an undesirable effect of wounding (Everson et al., 1992; Viña and Chaves, 2003). Lignification close to the site of damage is a defense response of plants following injury (Vance et al., 1980). In fresh-cut carrot, tissue damage and lignification were minimized by the use of very sharp blades for processing (Barry-Ryan and O’Beirne, 1998).

Processes of plant senescence increase as soon as a tissue is harvested from the plant, and involve degradative changes in membranes, cell walls, subcellular organelles, proteins and texture. Wounding (fresh-cut processing) activates not only ACC synthase and ethylene production (Yu and Yang, 1980), but also generates a number of hormonal and other signals (hydraulic, electrical) which mediate defense and stress responses (León et al., 2001). These signals induce the activation of suites of defense and stress genes, and result in altered mRNAs and protein expression (Mehta et al., 1991; Karakurt and Huber, 2007). Ripening can be rapidly initiated by wounding in pre-climacteric fruit (Starrett and Latties, 1993), and the rate of softening of fresh-cut fruit pieces is often markedly more rapid than in intact whole fruit (O’Connor-Shaw et al., 1994; Paul and Chen, 1997; Karakurt and Huber, 2003). Tissue softening is frequently the major problem limiting the shelf-life of fresh-cut products (e.g., Agar et al., 1999), which even when refrigerated can become unacceptable in as little as 2 days for tropical fruit such as papaya (O’Connor-Shaw et al., 1994). In fresh-cut papaya, the amount of ethylene produced is related to the extent of damage inflicted, and the firmness of sliced tissue declines rapidly during 48h, relative to the slow decline in firmness of intact fruit (Paul and Chen, 1997). It is well known that PG is an ethylene-regulated enzyme (Brummell and Harpster, 2001), and that its activity is increased by damage such as bruising (Moretti et al., 1998). Accordingly, increased PG activity has been observed in fresh-cut papaya (Karakurt and Huber, 2003). Relative to identically stored intact fruit, pectin from fresh-cut papaya declines in total amount, increases in solubility and exhibits depolymerization, all consistent with the observed increase in PG activity (Karakurt and Huber, 2003). Although PME activity is not affected, large increases in the activity of α- and β-galactosidase have also been observed, suggesting that increased cell wall hydrolase activity is a major contributor to the accelerated cell wall disassembly and increased softening of fresh-cuts. This may be particularly severe at the cut surface, since where tissue integrity is disrupted the extent of pectin depolymerization can greatly exceed that normally occurring in vivo, presumably due to increased accessibility of PG to its substrate or to ionic conditions more favorable for PG activity (Huber and O’Donoghue, 1993; Brummell and Labavitch, 1997; Almeida and Huber, 2007).
The other major factors in the loss of desirable texture are water loss and osmotic changes (Shackel et al., 1991; Saladié et al., 2007). Water loss leads to a loss of turgor and crispness, and is rapid in fresh-cut products due to the absence of a cuticle and sub-epidermal layers and the exposure of internal tissues. However, water loss can be greatly retarded by appropriate packaging. Accelerated senescence in fresh-cuts can lead to membrane deterioration due to the accumulation of increased amounts of lipooxygenase and phospholipase (Karakurt and Huber, 2003). Lack of membrane integrity allows the leakage of cellular osmotic solutes into the apoplastic space, which then results in water movements and turgor loss. Washing of green bell pepper slices after cutting improves firmness retention, probably due to the removal from the cut surfaces of solutes and stress-related signalling compounds such as acetaldehyde and phenolics (Toivonen and Stan, 2004). An additional benefit may be that washing increases the activities of catalase, peroxidase and superoxide dismutase, enzymes which are all involved in scavenging oxygen free radicals that contribute to membrane injury, and which normally decline during senescence (Kanazawa et al., 2000). In fresh-cut pear slices, firmness retention is substantially greater under atmospheres of 100% N₂ rather than in the presence of O₂, since oxidative damage causes a reduction in membrane integrity, cellular leakage and the flooding of intercellular spaces (Soliva-Fortuny et al., 2002a). Studies incubating pear tissue in hypotonic or hypertonic solutions found that for soft tissues turgor has little effect on tensile strength, since tissue failure is almost exclusively due to cell separation (De Belie et al., 2000). However, in firm tissues in which tissue failure is due to rupture of the primary cell wall, turgor strongly affects tensile strength. In melon, similar experiments have shown that cell plasmolysis and loss of turgor reduces firmness by more than 50% (Rojas et al., 2001).

Calcium, usually either as a solution of calcium chloride or of calcium lactate, is commonly used for the maintenance of firmness in fresh-cuts. Substantial delays in softening have been reported for numerous fruit species (e.g., Rosen and Kader, 1989; Agar et al., 1999; Luna-Guzmán and Barrett, 2000; Gorny et al., 2002; Soliva-Fortuny et al., 2002b). Similar treatments were also effective in fresh-cut vegetables such as carrot and lettuce (Martín-Diana et al., 2006; Rico et al., 2007). Calcium probably acts in two ways. Firstly, calcium ions form ionic bridges between demethylsterified pectin molecules to produce cross-linked polymer networks in the middle lamella. This improves cell-to-cell adhesion and thus mechanical strength, and delays the normal degradation of intercellular connections in ripening fruit. Secondly, calcium acts to retard senescent changes. In shredded carrot, firmness has been retained by a calcium treatment that reduces senescence-associated membrane lipid changes and so helps preserve membrane integrity (Picchioni et al., 1996). Calcium treatment also lowers lipase activity in fresh-cut cantaloupe melon, which would also be expected to defer membrane deterioration (Lamikanra and Watson, 2004). Calcium dips are often combined with chemicals such as ascorbate or cysteine which prevent browning, and the dipping treatment itself acts to rinse enzymes and solutes from injured cells at the cut surfaces.

As with other quality attributes of fresh-cuts (such as flavor and off-flavors, aroma, color and appearance), texture is affected by storage temperature, by controlled or modified atmosphere and by ethylene accumulation. Many of these factors interact, and there may be a trade-off between the mitigation of different aspects of quality deterioration. For example, low temperature inhibits ethylene production (Artés et al., 1999), but can induce chilling injury in some (particularly tropical) fruit species. Modified atmospheres also reduce ethylene production, but can induce anaerobic metabolism and the development of compounds responsible for off-flavors (Gil et al., 1998). Ethanol and heat treatments that are beneficial for prolonging texture and visual shelf life are deleterious to aroma (Bai et al., 2004). Such factors vary between species and cultivar, so handling and storage conditions need to be optimized for each product.

4. Conclusions

The foregoing discussion clearly demonstrates that knowledge regarding the biochemical mechanisms of both color and texture change is extensive, although by no means complete. The application of this knowledge to specific color and texture problems is essential for quality management in fresh-cut fruit and vegetable products. Solving some problems may use relatively simple technology, such as the application of edible coatings which are effective against both browning development and textural deterioration (Olivas and Barbosa-Canovas, 2005). The use of various combinations of treatments may also be advantageous (e.g., Gorny et al., 2002; Aguayo et al., 2006; Martín-Diana et al., 2006; Rico et al., 2007), but these must be optimized for each species and even for each cultivar. As is pointed out in several examples, such as the anti-browning dips used on fresh-cut apples, the results of treatments are not always predictable, and unanticipated effects (both positive and negative) may result.

The best starting point generally lies in identifying commonalities to previous work, such as whether the issues pertain to vegetative mature or immature tissues, whether a fruit is climacteric or non-climacteric, and/or whether a fruit has a melting or non-melting character as it ripens. For example, the use of 1-methylcyclopropene can be efficacious with climacteric fruit, but may be ineffective with non-climacteric fruit or vegetables. However, biological variation and species differences add to the complexity, and make it difficult to provide a one-solution-fits-all scenario. So, despite the in-depth knowledge of mechanisms, work on a case-by-case basis continues to be essential to resolving color and texture change problems in particular fresh-cut fruit or vegetables. In the future, a greater understanding of the biochemical mechanisms involved will lead to the identification of key factors for each species or cultivar, and may help direct breeding efforts towards developing cultivars with reduced susceptibility to browning or textural deterioration. For genetic intervention approaches, differences in the mechanism of ripening-related fruit softening evident between species (Brummell, 2006), and variations between cultivars, will necessitate different genetic targets. These targets must be clearly ascertained before embarking on transgenic manipulations, which can be prolonged in the case of fruit trees. This
review has attempted to classify existing knowledge, highlighting differences and commonalities between commodities, and it is hoped that such a perspective will lead to significant new approaches to improving the quality of fresh-cut fruit and vegetables.

References

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Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana<br>Persea americana


