BRUISING PROFILE OF FRESH APPLES ASSOCIATED WITH TISSUE TYPE AND STRUCTURE

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ABSTRACT: Bruising of apples results in millions of dollars in loss annually. To reduce economic loss, a greater understanding of the mechanisms involved is necessary. Our objective was to visualize damage due to bruising, using different methods of microscopy, as an aid to substantiating which mechanisms were most viable. ‘Golden Delicious’ apples at different maturity levels were similarly bruised with an artificial silicon finger attached to an Instron machine. Bruising was induced on freshly harvested fruit and examined after 48 h at room temperature. We used fluorescence microscopy with Calcofluor (to identify cell walls) and CDFA (to identify intact cell membranes) in the bruised and discolored tissue. Together with scanning electron microscopy (SEM), different breakage mechanisms were observed in the bruised volume. These techniques revealed that bruised tissue was comprised of both live cells, and dead cells that appeared burst, crushed or without apparent damage. The greater the amount of intercellular space present in the tissue, the more tissue damage from bruising occurred. Because airspaces weakened the tissue, damage initiated close to these sites. As apples matured, there was an increase in damaged cells surrounding larger intercellular spaces.

Keywords. Apples, Bruising, Harvesting date.

Understanding the mechanisms of bruising at a cellular level in apples is important. If the mechanisms are well-known and understood, damage to apples during harvest could be reduced, benefiting this multi-billion dollar global industry. This requires an understanding of apple tissue morphology, mechanical damage, biochemical reactions in cell fluids released to intercellular spaces, and the biology of bruise healing mechanisms.

APPLE MORPHOLOGY

Apple epidermis is comprised of epidermal cells (the outermost layer of cells), followed by subjacent collenchyma tissue known as the hypodermis. Beneath this are the parenchyma cells (Esau, 1965). The hypodermis may contain up to six layers of collenchyma cells, which contribute to the strength of the epidermis. The outer cells transmit force to the inner parenchyma cells (Knee and Miller, 2002).

The characteristics of each cell type are key to developing an understanding of bruising in apples. Collenchyma tissue consists of cells which are smaller with thick cell walls and more extensive middle lamella structure. Thick walls and close packing make collenchyma tissue strong and resistant to compression deformation. Collenchyma tissue is able to absorb a large amount of mechanical energy because of the combination of high tensile strength along with flexibility and plasticity to resist deformation-caused damage due to external forces. Generally, it is interpreted to function as structurally specialized supporting tissue (Esau, 1965).

Collenchyma cells have specialized, irregularly thickened, pectin-rich, primary cell walls that function in support of growing parts (Taiz and Zeiger, 2002). In contrast, parenchyma tissue is metabolically active tissue, comprised of thin-walled cells with air-filled spaces at the cell corners (Taiz and Zeiger, 2002). These intercellular airspaces may be relatively large, which reduces the amount of cell-to-cell contact (Hulbary, 1944, in Esau, 1965), thereby weakening the tissue (Alvarez et al., 2000a) and resulting in areas vulnerable to damage by external force.

Previous research (Toivonen et al., 2007) showed there were differences in bruise sensitivity by measuring the damage imposed by an external load at different maturity stages. Observing what occurs at a cellular level at different stages with the use of different microscopy techniques could help explain why such differences occur.

APPLE TISSUE MECHANICAL PROPERTIES

Apple cortex cells are anisotropic, (i.e., not homogeneous in all directions) with a radially oriented network of airspaces (Khan and Vincent, 1990). Airspaces are thought to be the weakest areas in apple fruit tissue (Vincent et al., 1991). Alvarez et al. (2000b) emphasize the basic principle of fracture mechanics in all solids is inhomogeneity. Irregularities in tissue structure essentially weaken the cellular matrix and make it more vulnerable to damage. The strength of a material is, therefore, directly related to the magnitude and distribution of these irregularities. Fractures begin at the site...
of such irregularities and grow while traversing the solid, thus creating a new fracture surface.

Alvarez et al. (2000a) tested tissues such as carrots, celery, cucumber, and apples under the same external load and found that tissues with smaller cells sustained less damage. Carrots had the toughest tissue due to their small parenchyma cells and lack of intercellular spaces. Cells in direct contact with neighboring cells have greater adhesion, resulting in fewer flaws or inhomogeneities. Such flaws can compound as stress concentrates and promote premature failure. In cucumber and apples, and less in celery, the cellular structure contains more intercellular spaces where cells are not in intimate contact. Moreover, the shape of the cells sometimes prevents adjacent cell walls from touching. Later in fruit development, depolymerisation of the pectic polysaccharides occurs as a result of ripening, further reducing cell adhesion.

Harker et al. (2006) observed that firmer fruit showed fractured and ruptured cells, while softer fruit showed cellular debonding when the tissue was externally damaged. In stored fruit, the failure pattern depended on the firmness of the fruit. Fruit that softened during storage showed both a fracture surface and intact cells, due to cell-to-cell debonding; however, stored, firm apples had the same characteristic as firm apples examined after only one day of storage.

Harker et al. (1997b) showed that cell separation without rupture may be common in ripening soft fruits such as peach, kiwi, and strawberry. That is, whereas in pears, where cell injury was due to cell wall failure and cell fracture, in ripened soft fruit, tissue failure was due to intercellular debonding (De Belie et al., 2000). The main component of intercellular adhesion is the extent of intercellular contact, which is determined by cell shape and packing, water loss, and size or absence of intercellular spaces. These factors change as fruit ripens, leading to larger air spaces and reduced intercellular contact (Glenn and Poovaiah, 1990; Hallett et al., 1992; Harker and Sutherland, 1993), allowing increased tissue deformation under stress. Pierzynowska-Korniak et al. (2002) showed that different apple cultivars have different cells shapes which contribute to their unique mechanical properties.

**Biochemistry**

A third factor to consider in apple bruising is oxidative browning, a result of the release of enzymes during breakdown of cell membranes (Toivonen and Stan, 2004). This occurs when physical stress or deteriorative processes, such as a wound response or senescence, are initiated and compartmentalization of cells begins to fail (Marangoni et al., 1996). The result is the mixing of polyphenol substrates like catechin or polyphenols with polyphenol oxidase and/or phenol peroxidase (Degl’Innocenti et al., 2005). Toivonen and Brummell (2008) point out that membrane stability is a major factor controlling the rate of browning. Ascorbate in the tissues can work to inhibit browning because it is a universal antioxidant (Noctor and Foyer, 1998) and can quench lipid alkoxyl and peroxy radicals involved in membrane deterioration (Espin et al., 2000)

**Observations Regarding Bruising**

More bruise damage has been observed in riper fruit. Brusewitz et al. (1991) found an increase in bruise volume with ripeness. Kvaaale et al. (1968) separated ‘Golden Delicious’ apples into two groups based on skin color (green vs. yellow) which represented different stages of maturity for the fresh market. Saltveit (1984) and Klein (1987) also reported that delayed harvest enhanced fruit sensitivity to bruising.

Apple bruise research has been done mainly with stored fruit, which are physiologically older. Because of degradative changes that occur in the tissue, older fruit contain more air space (Kays, 1997). Moreover, the dissolution of large pectin molecules found in the middle lamella makes the tissue less brittle, which may impart a different bruise mechanism than fresh apples that have no middle lamella dissolution (Kays, 1997). According to Pitt (1982, Pitt and Chen (1983) Holt and Schoorl (1982, 1983, 1984), Van Woensel and De Baerdemaeker (1983), Lin and Pitt (1986), Vincent (1990), Gao and Pitt (1991) Roudot et al. (1991), Khan and Vincent (1990, 1991, 1993a,b) Abbott and Lu (1996), Loodts et al. (2006) bruising is a consequence of breakage of intercellular bonds, propagation of cell wall ruptures and/or cell deflation as a result of loss and diffusion of cell fluid. These conclusions resulted from analyses at the cellular level, and from modeling cell response to external loads.

Holt and Schoorl (1977) proposed a failure mechanism for apple bruising at the cellular level. Their model shows burst cells in the affected area and distorted cells under the burst cells, followed by a layer of unaffected cells some distance away from the compressed surface. Their model was based on the assumption that all apple cells are parenchymatic. Holt and Schoorl (1982) suggested bruising is a mode of failure associated with damaged tissue due to cell bursting. When cells burst, they retain little mechanical strength. Diehl et al. (1979) observed that cells change shape when an external load is applied, and the cell walls stretch because cellular content is relatively incompressible. Therefore, cell breakage occurs when the cell walls fracture. Bruising may also be considered a distortion or shear phenomenon according to Holt and Schoorl (1982). Shearing, unlike cracking, results from slippage. Peleg et al. (1976) showed that slip failure occurred in unripe mango and papaya, while bruising occurred in ripened fruit.

According to Harker et al. (1997a), the actual mechanism of breakage is characterized by three modes of failure: cell fracture, rupture, and cell-to-cell debonding. Fracturing is characterized by cells cleaving across the equator whereas rupture is characterized by cell bursting and collapse. Cell-to-cell debonding on the other hand, is characterized by the separation of cells with no damage, or only minor deflation or distortion, while other cells remain intact. It may be that the weakened middle lamella in the tissue is less rigid with polymers that are able to move relative to each other, or that the weakened middle lamella results in tissue that is still rigid, but not as strong. Extrapolating from cell-to-cell bonds to tissue-level mechanics, the second alternative is more likely to result in brittle tissue behavior.

Bruising is an important problem for the fruit and vegetable industry in general, and for the fresh market apple industry in particular. In the U.S. in 2001, apple bruising costs an estimated $113 million in loss (Varith, 2001). Reduction of bruising in the fruit and vegetable industry could provide an annual payback of millions of dollars (Barietelle and Hyde, 2001). Because bruising is a complicated process many theories have been suggested but few verified because
of the inability to observe and measure what is happening to the cells. Our objectives in using different microscopy techniques were to determine: 1) the location of damaged cells within the imposed bruised tissue, and 2) the percentage of living cells versus dead cells in the bruised tissue volume.

**MATERIALS AND METHODS**

**EXPERIMENTAL DESIGN AND FRUIT SELECTION**

Apples used in this study (*Malus domestica* Borkh cv. ‘Golden Delicious’) came from a commercial organic orchard located in Orondo, Washington, in both 2006 and 2007. Tree vigor and cropping were uniform and irrigation, fertilizer, and spraying practices were the same across all treatments. Fruit were harvested at different maturity levels based on skin color. Green apples were harvested 143 days after full bloom (dafb) in 2007 and 132 dafb in 2008; creamy colored apples 150 dafb in 2007 and 139 dafb in 2008; and yellow apples 158 dafb in 2007 and 146 dafb in 2008. Typically, ‘Golden Delicious’ are harvested based on peel ground color change as described by Mitcham et al. (2008). In this work we added the intermediate creamy/white color as a treatment.

Six fruit from three different trees were harvested at the three color intervals during the commercial harvest season, as detailed above, for a total of three treatments based on maturity index/skin color. Harvested fruit, free of apparent damage, were transported in tri-layered European apple boxes, nested in cylindrical holes cut in low-density foam with an additional foam sheet on the bottom of the box to protect fruit from damage.

**BRUISE INDUCTION**

An Instron Model 1350 (Instron Industrial Products, Grove City, Pa.) was used to apply an external 10-mm deformation on the apples using a silicon cylinder similar with the shape and rigidity to a human finger called ‘creepy finger 13-0053’ (Loftus International, Salt Lake City, Utah). Its dimensions are: length: 10 cm and diameter 3 cm. The imposed force was applied at harvest to the same general location on each fruit (fig. 1). Deformation rate of the artificial finger was 0.425 mm/s rate (25.5 mm/min or 1 in./min), and a 10 mm deflection into the surface of the fruit. Bruising was evaluated and tissue samples prepared for observation using confocal and scanning electron microscopy (SEM) 48 hours after treatment.

**PREPARATION OF TISSUE FOR SEM**

Tissue from bruised apples was cut into approximately 1-cm³ thick sections and observed using a Quanta 200F SEM (FEI Company, Hillsboro, Oreg.), under low vacuum mode. Depending on the bruise size, the whole discolored area was cut in half and observed under the microscope (fig. 1). Since the experimental methods for studying *in situ* deformation are limited and that the tissue is not studied in its natural state due to the procedures used to preserve the tissue, this method was selected to analyze the tissue under its natural state. With this technique, we expected to see the natural state of the damaged tissue within the bruised volume without being altered by the fixing or dehydration procedures of the other techniques.

**FLUORESCENCE MICROSCOPY**

The LSM 510 meta laser scanning microscope (Carl Zeiss MicroImaging Inc., Thornwood, N.Y.) was used to observed live and dead tissue using the fluorescent dyes Calcofluor white (1 drop mixed with 1 drop of distilled water) and 5(6)-CFDA, SE; DFSE (5-(and-) 6-carboxyfluorescein succinimidyl ester, mixed isomers (CDFA, C1157) from Invitrogen (Life Technologies, Carlsbad, Calif.). Four mL of DMSO was mixed with 25 mg of CFDA and 1 µL of this solution mixed with 1 mL of distilled water. The bruise was cut and 0.5-mm thick slices of tissue were mounted onto white snow coat micro slides (1 in. x 3 in. x 1.00 mm) and dyed with CDFA for 10 min. A drop of the calcofluor solution was added and covered with a cover slip.
RESULTS

BRUISE FORMATION

All apples in the study formed a bruise under the point of 10-mm deflection nominal force applied in the 10-mm deflection was 50N. Bruise volume varied by apple maturity, ranging from 583.0 mm³ in green to 1024.2 mm³ in white to 3565.4 mm³ in yellow stages.

The formation of the bruise was in a conical shape, larger on the top layers and narrower at the lower layers. The tissue that was underneath the area where the external damage occurred turned into a brownish color (fig. 2).

Figure 2. Discolored (bruised) area of compressed ‘Golden Delicious’ apple.

SEM

Using SEM, it was observed that bruise damage starts under the collenchyma cell layer, approximately six layers under the epidermis. Where larger intercellular spaces were located, there was a large amount of dead, burst, or crushed cells. More cells were damaged as harvest dates progressed, and the damage was observed to be adjacent to the larger airspaces and transmitted to the neighboring cells (fig. 3). In all treatments the hypodermis was intact. In some cases, some compactness of the hypodermis layer was observed (fig. 4); however, there was no apparent damage (fig. 5 - images of tissue from green stage maturity). At an angle of 45° relative to the direction of the applied load (fig. 6), we observed a crack (large intercellular space), in addition to a crack in a parallel plane to the applied load within the fruit tissue. The crack parallel to the load was not surrounded by discolored tissue.

Figure 3. SEM images of control (a) and bruised (b) apple tissue.
In our study, cell bursting and detachment were observed under SEM (fig. 3) but this was not observed in detailed under fluorescence microscopy (data not shown).

**Fluorescence Microscopy**

Use of CFDA and Calcofluor dyes indicated in the bruised area cells were not all dead (damaged cell membrane), but rather a mixture of dead and live cells. Cells adjacent to larger airspaces were dead and the amount of dead cells varied depending on the maturity of the apple. The more mature the fruit was, the greater the number of dead cells surrounding the airspaces. The hypodermis layer of cells was generally intact, beneath which areas of dead cells were visible (fig. 5).

It appeared that the collenchyma cells transmitted the applied force to the underlying parenchyma cells, sustaining no apparent damage themselves (fig. 7). We also observed greater damage with increasing apple maturity due to larger airspaces and cell detachment, causing a larger area of discolored, damaged tissue (figs. 8 and 9).

**Discussion**

One of our primary assumptions in this work was that the imposed deflection of 10 mm using the silicon finger is representative of that imposed by a picker’s finger. We used this deflection distance because it consistently gave us a reproducible bruise. Keeping the deflection constant was probably unrealistic; however, we elected to keep this variable constant rather than increasing variation due to reducing the applied force or deflection distance. In this regard, it is important to note, first, that the deflection of 10 mm represents both the compression of the finger pad touching the apple and the distance it was pushed below the plane of the fruit surface. On a green, firm apple this force of roughly 50 N likely represents a “worst case scenario” since 1) not all pickers grasp the fruit identically, 2) not all fingers exert the same amount of force when removing the fruit, and 3) not every fruit is bruised because the fruit removal force and, therefore, the force required to “pull” the fruit off the tree varies due to varying degrees of pedicel abscission layer formation. Second, as the apple increases in maturity on the tree, both flesh firmness and fruit removal force decrease. These are somewhat opposing in their effects on bruising since softer fruit could make bruising easier, but decreasing fruit removal force allows the fruit to be removed from the tree more easily. Indeed, sometimes, just a slight movement of the fruit is all that is required to break the well-formed pedicel abscission layer. Other cultural and physiological issues may also be involved and we refer the reader to Toivonen et al. (2007) who addressed numerous factors affecting bruising and bruise recovery.

Apple parenchyma tissue was observed to suffer varying mechanical failures depending on the force applied. Contrary to what Upchurch (1985) found, the presence of larger intercellular spaces in undamaged tissue, our findings show that tissue with larger airspaces is more vulnerable to bruising. As Tu et al. (1996) observed, larger intercellular spaces are due to degradation of the middle lamella and a reduction of cell adhesion or part of the apple’s tissue properties. Zamorskyi (2007) observed that ‘Golden Delicious’ apples have denser intercellular spaces and uneven cell size and structure which also confirms what Alvarez et al. (2000b) reported regarding inhomogeneities in structure that weakens tissue and makes it more vulnerable to damage. Our finding that collenchyma cells transmit the applied force to the underlying parenchyma cells agreed with those of Knee and Miller (2002). Similarly, we observed that collenchyma cells were not damaged, while the parenchyma cells absorbed all the energy and therefore burst or were crushed, resulting in cell death. All our observations are consistent with those of other investigators. Mohsenin (1970) showed that in a bruised area, layers of cells remain unbroken, and even among the damaged layers, groups of cells survived. Holt and Schoorl (1983) estimated that only 0.03% of cells are fractured in bruised areas. Using TEM, we did not observe cell membrane damage (data not shown), however, such damage has been reported by Rodriguez et al. (1990).
Figure 5. Confocal imaging of bruised vs. undamaged apple tissue (blue dye showing cell walls of dead cells and green dye showing intact live cell membranes). (a) Damaged cells (parenchyma cells) under undamaged hypodermis cells (collenchymas cells), (b) healthy undamaged cells.

As reported by Alvarez et al. (2000b) and Vincent et al. (1991) we observed also that generally, irregularities (air-space) in tissue structure weakens the tissue and makes it more vulnerable to damage. Where tissue was cracking parallel to the applied load but not discolored, we suggest this may be due to minor damage in the tissue such as Holt and Schoorl (1983) observed in potato tissue. As Donald et al. (2003) explained, experimental methodologies for studying in situ deformation are limited and it is uncertain whether the procedures used to preserve the tissue, such as vacuum for dehydration and coating in SEM, fixing and embedding for light microscopy alter tissue structure.

Numerous and varied definitions and symptoms of bruising can be found in the literature. A bruise can be due to cell injury that results in flesh browning (Mohsenin et al., 1962); a discoloration or fracture of the tissue (Chen et al., 1987); cell bursting (Holt and Schoorl, 1977); cell rupture (Diehl et al., 1979; Pitt, 1982) and flesh browning (Robitaille and Janick 1973); an injured area which is flat, soft and brown with a high respiration rate (Ericsson and Tahir, 1996); a dark spot (Blahovec, 1999); or just an indentation of the surface and a slight discoloration (Crisosto et al., 1993) where tissue is compressed or impacted. Based on our observations, we would define a bruise as an area of discolored tissue comprising an array of undamaged and burst and crushed cells (fig. 8), along with cells that have not been physically damaged. The discoloration is only an indication of where the damage can be found. This can occur inside the cell if the cell membrane is damaged or outside if the cell contents are released to the intercellular spaces when the cell ruptures.

CONCLUSIONS

Based mainly on tissue properties, our major findings regarding the mechanism of bruising of freshly harvested ‘Golden Delicious’ apples are: 1) cell wall rupture or damage is not required for bruise induction; 2) parenchyma cells are involved in the discoloration of bruised tissue in ‘Golden Delicious’ apples because they are more fragile than the collenchyma cells; 3) cracking as well as rupture appear to be present in the damaged tissue; 4) damaged tissue is made up of a mixture of dead and live cells; 5) cell rupture appears to
be facilitated by intercellular spaces which are the weakest point in the tissue; and 6) cells adjacent to intercellular spaces are more vulnerable to damage presumably due to the lack of cell-to-cell contact, thereby allowing them to expand and rupture more easily than cells that are tightly packed. Although there is much yet to do, these data add to our understanding of the mechanisms underlying bruising of apple flesh at harvest.

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