Fresh-Cut Product Biology

Mikal E. Saltveit
Mann Laboratory, Department of Plant Sciences, University of California, Davis, CA 95616-8631
mesaltveit@ucdavis.edu

CONCLUSION
1. Fresh-cut fruits and vegetables usually respond differently than whole fruits and vegetables.
2. Knowledge of basic biology of whole and fresh-cut commodities helps devise strategies to optimize quality retention during handling and marketing.
3. Unexpected things will happen when fresh-cut fruits and vegetables are produced and marketed.

The Opportunities for Wounding are Many and Varied

- **Harvesting**: Sufficient injury to separate from parent plant
- **Transporting**: Vibration, compression, temperature abuse, etc.
- **Handling**: Unloading, sorting, cleaning, packaging, etc.
- **Processing**: Trimming, abrading, segmenting, etc.

Fresh-cut = Wounding

- **Fresh-cut requires wounding** (e.g., lightly processing, minimally processed) the plant tissue.
- **Types of wounds include**: abrasion, chopping, cutting, dicing, grating, mincing, peeling, scraping, slicing, shredding, tearing, etc.
- **The response depends** on the type of wound and other previous and/or subsequent stresses.
- **Plants have evolved a limited number** of genetically preset physiological responses to wounding.

The Responses to Wounding are also Many and Varied

- **Physical response**: Mechanical changes to existing tissue
- **Biochemical response**: Reactions with existing cellular components (e.g., substrates and enzymes)
- **Physiological response**: Induction of the synthesis or activation of enzymes and accumulation of their products

Cellular Membranes

Plants are composed of millions of cells.

Each cell is divided into individual compartments by membranes.

This division protects the cell from unwanted reactions between enzymes and substrates that may cause damage.
Generalized Plant Cell

Cell wall  Amyloplast  Endoplasmic reticulum  Middle lamella  Nucleus  Mitochondria

Vacuole (storage)

Cell membrane  Plasmodesmata  Golgi complex  Chloroplast  Cytoplasm  Air space

Cell Membranes

Maintaining membrane integrity is crucial to maintaining cell vitality and product quality.

- The outer cell membrane isolates and protects the cell from the environment.
- The vacuole membrane isolates stored organic acids, sugars, and pigments.
- The mitochondria membrane isolates reactive energy-producing reactions.
- The lysosome membrane isolates degrading enzymes from the rest of the cell.

Cellular Turgor

Maintaining turgor is crucial to maintaining texture and product appeal.

Solvent (water) movement across the cell's semi-permeable membranes is driven by osmosis.

The solution inside a cell is more concentrated than outside, so water preferentially moves into a cell.

Water uptake by the cell would increase its volume.

The cell wall constrains the cell expansion and maintains a constant volume, so the incoming water increases the pressure within the cell; i.e., the turgor.

Loss of Turgor

Turgor can be lost because of:

- Water loss: reduced water content, reduced volume, wilting.
- Cell wall softening: reduced resistance to pressure, cells expand, pressure (turgor) decreases.
- Loss of membrane semi-permeability: solutes diffuse through the membrane, gradient reduced, pressure decreased, loss of turgor.

Loss of Turgor

A commodity is made up of cells grouped into different tissue systems.

- The cuticle is a layer of waxes on the outside and is a barrier to water loss and pathogen entry.
- The epidermis is the first layer of living cells. It produces the cuticle and also acts as a barrier.
- The inner tissues comprises most of what we eat and is called the periderm and/or mesoderm.
**Product Structure**

A commodity is made up of different tissue systems

- **Structural tissue** supports the cells and gives the commodity its shape and texture.
- **Vascular tissue** is interspersed in the commodity and provides the plumbing to move material around.
- **Air spaces** are between the cells and are pathways for gas diffusion (O₂, CO₂, H₂O, C₂H₄).

**Parts of a Whole Commodity**

- Epidermis (below the cuticle)
- Structural tissue (support, protection)
- Air spaces (voids between cells)
- Cuticle (outside waxy covering)
- Periderm or Mesoderm (rest of the internal tissue)
- Vascular tissue (the plumbing)

**Examples of Fresh-cut Responses to Wounding**

**Carrot - ‘Baby’ Carrots**
- Surface whitening
- Edible coatings (make coatings from commodity)

**Celery - Petiole Segments**
- Pithiness, vascular elongation, flaring of ends

**Tomato - Ripening Mature-green Slices**
- Altered water activity and gaseous atmosphere
- Unexpected sprouting of seeds (i.e. vivipary)

**Lettuce - Browning Disorders**
- Russet spotting
- Wound-induced browning – cause & wound signal

**Immediate and Subsequent Physical Effects of Wounding**

- **Mechanical shock to tissue**
  - Bruises, cracks, fractures in tissue
  - Hydraulic shocks propagate through tissue

- **Removal of cuticle and epidermis**
  - Alter gas diffusion
  - Increase or decrease of Carbon dioxide levels fall
  - Oxygen levels rise
  - Accelerated water loss
  - Entry of contaminants
  - Loss of structural integrity

- **Surface debris changes appearance**
  - White blush formation

- **Accelerated or uneven water loss**
  - Changes in turgor
  - Changes in texture
  - Activates senescence changes

- **Liquid on cut surface blocks pores**
  - Reduced gas diffusion
  - Elevated CO₂, Reduced O₂
  - Accelerates water loss
  - Provides substrate for microbes

- **Exposure to contaminants**
  - Microbes, pesticides, dust, etc.

**White-blush development on fresh-cut carrots**

Is it a physical change (e.g., surface debris) OR a physiological change? (e.g., wound-induced lignin synthesis)

**Fresh-cut carrots are produced from 3 inch long segments cut from longer carrots**

- Discarded

  Abruading the surface of the carrot pieces produces rounded edges but also leaves cellular debris on the surface.
Development of white-blush on fresh-cut ‘baby’ carrots

Water loss

Notice surface debris

Abrading the carrot’s surface leaves cellular debris that forms the white-blush when adhering moisture is lost.

Re-wetting the carrot’s surface reverses white-blush the development on fresh-cut carrot pieces

Moisture on the carrot’s surface reduces white-blush formation during storage and marketing

Notice water droplets

How could white-blush develop in a closed bag?

White-blush can develop during storage

Movement of water from respiring carrots to the inner surface of a closed bag can remove enough water to produce white-blush.
Water loss from cut celery petiole segments

- Water loss occurs at the cut ends
- Soft tissue collapses at cut ends
- Vascular tissue is ridged
- Vascular strands protrude from cut ends

Ripening Slices of Mature-green Tomato Fruit

Mature-green tomato  Red-ripe tomato

Changing Internal Gas Concentrations can Produce Unexpected Effects

Altering water activity and internal gas composition can stimulate responses that were eliminated during domestication and breeding of commercial cultivars

Viviparous – germinating while still in the fruit

Seeds germinated during fruit ripening

Some responses to wounding produce unexpected and unwanted effects

Gas Exchange in Whole Commodities

- Openings in the epidermis
  Stomata, hairs, trichomes, lenticels
- Openings at the stem or blossom ends
  Stem scars, pedicel, open calyx
- Injuries provide additional openings
  Unrestricted gas movement
**Gas Exchange**

Stem scar

80-90% Gas exchange

Blossom end  Lenticels  Impermeable skin

**Sites of Gas Exchange are sites of Water Loss**

Fine hairs were broken off during harvest

Cuts and abrasions  Stem scar

Water Loss after 3 days  Fresh cut

**Wounding Greatly Alters Rates of Gas Exchange**

Removal of the epidermal layer and blocking the surface of the tissue with rinse water has

**TWO EFFECTS**

*First:* it inhibits gas diffusion

(gas moves through water 10,000 times slower than through air)

*Second:* it enhances gas diffusion

(the unprotected surface is exposed as the liquid is re-absorbed or evaporates)

**Harvested Crops are Living Organisms**

Metabolism of Stored:

- Carbohydrates
- Organic acids
- Sugars
- Fats
- Oxygen
- Carbon dioxide
- Water vapor
- Heat

**Subsequent Biochemical and Physiological Changes**

- **Wound signal (nature, speed)**
  - Hormone (trauma), Wall fragment
  - Hydraulic change, Bioelectrical wave

- **Membrane depolarization**
  - Increased permeability
  - Mixing of cellular compounds
  - Calcium & signal transduction
  - Vacuole contents

- **Loss of protoplasmic streaming**
- **Membrane disorganized**
  - Lipids oxidized
  - Free fatty acids produced

- **Elevated respiration**
  - CO₂, heat production, O₂ consumption

- **Oxidative reactions**
  - Non-respiratory O₂ consumption, phenolic oxidation and tissue browning

- **Compositional changes**
  - Polysaccharides, organic acids, aroma, sugar to acid balance, loss of flavor, toughening

- **Induction of wound healing**
- **Induction of enzymes**
  - Altered protein synthesis
  - Altered phenolic metabolism
  - Phytoalexins, browning substrates

- **Elevated ethylene production**

**PHYSICAL WOUND**

Physiological signal(s)

- Wound healing
- Cell division & differentiation
- Periderm formation
- Altered texture & appearance

- Heat
- Poor flavor
- Reduced growth
- Tissue browning
- Suberin
- PPO
- Phenolic compounds
- Limited expansion
- Oxygen
- Reduced substrate
- Phenolic metabolism
- PPQ
- Limited carbohydrate, organic acids, vitamins
Flow-through system to measure carbon dioxide production

Air under pressure
Restriction maintains a constant flow
Data recorder and analyzer

- Test fruit
- Moisture trap
- Infrared CO2 analyzer

Maintains a constant pressure

Respiration of a sliced mature-green tomato fruit

The flow-through system took about 12 minutes to reach equilibrium for a whole 123 g tomato fruit producing 12 mL CO2/kg h at 21 °C (70 °F).

Respiration of a sliced mature-green tomato fruit

Efflux of CO2 from fruit cut into eights at time zero (0.0 hours).

The initial efflux of CO2 is from CO2 dissolved in water within the fruit (wavy lines from 0.0 to 0.4 h). It is followed by efflux of CO2 produced from wound-induced respiration (red dots from 0.1 to 1.0). These wound-induced changes overlap and add to the basal rate of normal, non-wounded respiration.

Respiration of Intact and Shredded Lettuce

- Shredding greatly increases respiration rates
- Temperature control is extremely important

Respiration of Intact and Segmented Cauliflower

In contrast to lettuce, cauliflower heads and florets (typical commercial size, 40g) have very similar respiration rates & quality changes during storage.
**Respiration of Wounded Tissue in MAP**

Fresh-cut lettuce in MAP at 10°C

**Oxygen or Carbon dioxide levels (%)**

- Oxygen (4%)
- Equilibrium carbon dioxide (5%)
- Excess' carbon dioxide
- Deficient' oxygen
- Equilibrium oxygen (4%)

**Time (h)**

0 10 20 30 40 50

**Reducing Wound-induced Increases in Metabolic Changes**

- Minimize the amount of wounding (best method to minimize wound-induced changes)
- Increases are usually short lived (can adversely affect MA and MAP)
- Wounding may trigger other changes (promote ripening and phenolic metabolism, senescence)
- Can be reduced by cold temperatures (possibility of chilling injury in some commodities)
- Chilling injury is less important in fresh-cut (rapid use, fully ripe; so chilling sensitive tissue can be stored near 0°C for a short time)

**Anaerobic respiration**

What is the difference between yogurt and beer? Both are products of fermentation (i.e., anaerobic respiration)

In yogurt, pyruvic acid is converted to lactic acid without CO₂ production

In beer, pyruvic acid is converted to ethanol with CO₂ production

**Sugar**

Pyruvic acid

\[\text{CH}_3\text{CHOHCOOH}\]

Lactic acid

\[\text{CH}_3\text{CHOHCOOH}\]

TCA Cycle (Aerobic)

Ethanol

\[\text{CH}_3\text{CHOHCH}_2\text{OH}\]

LDH

ADH

PDC

**O₂ + C (sugar) → CO₂**

Equal volumes of oxygen are consumed and equal volumes of carbon dioxide are produced

However, the solubility of oxygen in water (31 mL/L) is far less (3.5%) than carbon dioxide (878 mL/L), so the volume of gas decreases as respiration converts O₂ into CO₂ and into the CO₂ is dissolved within the tissue

**Anaerobic respiration**

**O₂ + C (sugar) → CO₂**

Equal volumes of oxygen are consumed and equal volumes of carbon dioxide are produced

However, the solubility of oxygen in water (31 mL/L) is far less (3.5%) than carbon dioxide (878 mL/L), so the volume of gas decreases as respiration converts O₂ into CO₂ and into the CO₂ is dissolved within the tissue

As O₂ declines the cells become anaerobic and produce large amounts of CO₂
**Anaerobic respiration**

\[ C \text{ (sugar)} \rightarrow \text{CO}_2 \]

As O\(_2\) declines the cells become anaerobic and produce large amounts of CO\(_2\). When the tissue becomes saturated with CO\(_2\), it starts to accumulate in the bag and increases its volume.

**Ethylene Should be Minimized to Maintain Quality**

Wounding can stimulate ethylene production and accumulation in enclosed spaces.

Low levels of ethylene promote the ripening of climacteric fruit and vegetables.

Even small amounts of ethylene (e.g., 0.02 µl·l\(^{-1}\)) can adversely affect the storage life of non-climacteric vegetables.

**Effect of Wounding on the Respiratory Climacteric**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Non-climacteric fruits, Immature fruits, Vegetative tissue</th>
<th>Non-wounded</th>
<th>Wounded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide</td>
<td>Non-wounded</td>
<td>Wounded</td>
<td></td>
</tr>
<tr>
<td>Ethylene</td>
<td>Non-wounded</td>
<td>Wounded</td>
<td></td>
</tr>
</tbody>
</table>

Wounding can cause an early onset of the climacteric.

**There is a Positive and a Negative Feedback of Ethylene on Ethylene production**

- Vegetable tissue and immature fruit have **negative feedback**, where ethylene **inhibits** its production.

- Mature and ripening fruit have **positive feedback**, where ethylene **promotes** its production.

**Negative feedback**

\[ \text{Immature} \quad \text{C}_2\text{H}_4 \quad + \quad -1 \text{ MCP} \]

\[ \text{Time} \]

**Positive feedback**

\[ \text{Mature} \quad \text{C}_2\text{H}_4 \quad + \quad 1 \text{ MCP} \]

\[ \text{Time} \]

**Negative feedback**

\[ \text{Immature} \quad \text{C}_2\text{H}_4 \quad + \quad -1 \text{ MCP} \]

\[ \text{Time} \]

**Positive feedback**

\[ \text{Mature} \quad \text{C}_2\text{H}_4 \quad + \quad 1 \text{ MCP} \]

\[ \text{Time} \]

\[ -1 \text{ MCP} \]
 Controlling the Effects of Ethylene

Should ethylene be removed from the atmosphere, or should the tissue be made less sensitive to ethylene?

- Ventilation may be impractical in CA or MA
- One of the beneficial effects of traditional CA is that low oxygen reduces ethylene synthesis, and low oxygen and elevated carbon dioxide reduces sensitivity to ethylene
- Pre-storage treatments such as heat-shock and 1-MCP can render the commodity less sensitive to ethylene

Ethylene is not involved in all wound responses

- Negatively regulated in non-climacteric tissues
- Wound-induced increase is usually transitory
- PAL increased before inductive C2H4 rise
- Response even when C2H4 action blocked

Changes after cutting

Dynamic changes that occur in respiration over time are often accompanied by other changes in metabolism

- Induced synthesis of phenolic compounds
- Browning of injured and adjacent tissue
- Textural changes; softening, toughening
- Changes in aroma profile

Color changes after cutting

- Freshly cut
- 1 Hour
- 4 Hours
- 24 Hours

Not all tissues respond similarly to wound-induced browning

<table>
<thead>
<tr>
<th>Tissues that brown rapidly have:</th>
<th>Tissues that brown slowly have:</th>
</tr>
</thead>
<tbody>
<tr>
<td>High levels of phenolic compounds</td>
<td>Low levels of phenolic compounds</td>
</tr>
<tr>
<td>Low levels of antioxidants</td>
<td>High levels of antioxidants</td>
</tr>
<tr>
<td>Adequate PPO, POD activity</td>
<td>Low levels of PPO, POD activity</td>
</tr>
<tr>
<td>‘Leaky’ membranes</td>
<td>Intact membranes</td>
</tr>
</tbody>
</table>

Different procedures are used to prevent browning in the two tissue types

- Reduce levels of phenolic compounds (genetic selection, cultural practices)
- Supply antioxidants, exclude oxygen
- Reduce PPO, POD activity
- Increase membrane stability
- Prevent phenolic accumulation (reduce wound signal, and enzyme and phenolic synthesis)
- Increase membrane stability
- Later, exclude oxygen

Enzymatic Browning of Injured Tissues

Artichokes are naturally high in phenolic compounds, and will rapidly brown when injured tissue (e.g., at the edge of a cut) is exposed to oxygen in the air. This browning is an oxidation of existing phenolic compounds that is facilitated by enzymes (PPO, POD) already in the cells. No wound-induced physiological response is necessary for this browning to occur.
Wounds Induce Phenolic Metabolism that Leads to Tissue Browning

Lettuce leaves usually have low levels of phenolic compounds (depends on cultural practices and previous stresses). It takes a few days after wounding to synthesize the enzymes of phenolic metabolism, and for them to synthesize phenolic compounds that accumulate and subsequently cause tissue browning.

Wound Responses of Lettuce

Wounding and ethylene both promote PAL activity and the accumulation of phenolic compounds and tissue browning.

However, the wound response does not involve ethylene, but is initiated by a de novo synthesized wound signal that diffuses into adjacent tissue from the wound site and stimulates phenolic metabolism.

Wounding Produces a Wound Signal

- Signal produced at site of injury
- Diffuses or propagates into adjacent tissue
- Diminishes with time and distance from wound
- Induces wound responses in non-injured tissue
- Induces enzyme activity and phenolic synthesis
- Phenolic compounds accumulate
- Tissue browning occurs

Wounding greatly increases the severity of russet spotting

Russet spotting on whole or wounded lettuce mid-rib segments induced by exposure to 1.0 ppm ethylene in air at 5 °C.
Controlling Browning in Lightly Processed Lettuce & Celery

- Minimize stress before & after harvest
- Use sharp knives during processing
- Market the product rapidly
- Store and ship at 0 °C
- Use packaging that excludes oxygen
- Increase carbon dioxide in package
- Interfere with the wound signal

Mechanical injury induces browning

Through evolution plants acquired an impressive, but limited ability to respond to stresses.

Some responses take precedence over others.

The metabolic response induced by one stress (e.g., heat-shock) can be used to redirected the response to another stress (e.g., wounding).

Are there other methods to control browning?

There are Many Opportunities for Wounding

Controlling Browning in Lightly Processed Lettuce & Celery

- Minimize stress before & after harvest
- Use sharp knives during processing
- Market the product rapidly
- Store and ship at 0 °C
- Use packaging that excludes oxygen
- Increase carbon dioxide in package

Controlled or modified atmospheres

Some responses take precedence over others.

The metabolic response induced by one stress (e.g., heat-shock) can be used to redirected the response to another stress (e.g., wounding).
**PHOSPHOLIPIDS IN MEMBRANE**

- Wound, Pest, Pathogen, Elicitor
- Phosphatidic acid (PA)
- Inositol C3 to C7 (proposed to hydrolyze)
- Phosphatidic alcohols
- Diacylglycerol (DAG)
- 13-Hydroxylinolenic acid (13-HPOT)
- Allene oxide synthase (AOS)
- Allene oxide cyclase (AOC)
- Reductase and 3ß-oxidations
- Lipoxygenase (LOX)
- Lipolytic acyl hydrolase (LAH)
- Diacylglycerol (DAG)
- Phosphatidate phosphatase

Knowing the metabolic pathways involved in wound responses gives us the opportunity to target specific treatments to enhance the beneficial effects and diminish the deleterious effects of wounding.

Otherwise we are just using a ‘spray and pray’ approach to solving problems.

---

**CONCLUSION**

1. Fresh-cut fruits and vegetables respond differently than whole fruits and vegetables.
2. Knowledge of basic biology of whole and fresh-cut commodities helps devise handling and marketing strategies to optimize quality retention.
3. Unexpected things will happen when fresh-cut fruits and vegetables are produced and marketed.
4. Genetic modifications may prevent deleterious wound-induced changes, but they may also make the plant more susceptible to stresses during growth.