Use of Anti-browning Agents and Calcium

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Fresh-Cut Shelf Life Limitations

• Microbial spoilage
• Color *
  – Enzymatic (oxidative) browning
  – Loss of color, bleaching
• Texture *
  – Dessication – loss of moisture
  – Loss of cell integrity/translucency
• Flavor
  – Off-flavor or off-odor development
• Nutrients
  – Oxidation or change in biological activity

* Presentation will focus on these attributes.

Off-flavor and odor production

Many off-flavor reactions catalyzed by lipoxygenase. Keep cool and reduce O2.

Fresh – Cut Fruit & Vegetable Color

Enzymatic* browning in cut apples

*Polyphenol oxidase catalyzed phenolic oxidation.

Enzymatic* browning in lettuce

*Polyphenol oxidase catalyzed phenolic oxidation.
Loss of green color - bleaching

May be catalyzed by acid, and/or lipoxygenase and chlorophyllase.

Plant Tissue/Cells

Location of Quality Components

- **Water vs. Lipid Soluble Components**
  - **Water Soluble** (70-90% of plant tissue)
    - Vacuole, Cytoplasm, Cell Wall
    - Apoplast between cells
    - Most of Cell Contents
  - **Lipid Soluble** (10-30% of plant tissue)
    - Membranes (plasma, tonoplast and surrounding all organelles)
    - Plastids and Lipid Bodies

Fruit & Vegetable Color

- **Water soluble**
  - Red to purple: anthocyanins
  - Brown, grey, black, pink: phenolics
- **Fat soluble**
  - Yellow, orange, red: carotenoids (ex: lycopene, beta-carotene)
  - Green: chlorophyll

Types of Browning

- **Enzymatic** (most important in fresh-cut)
  - Polyphenol oxidase catalyzed
- **Non-Enzymatic**
  - Maillard sugar-amine reaction, concentrated solutions
  - Carmelization sugars, high temperatures
  - Ascorbic acid oxidation of ascorbic acid
  - Lipid browning
**Enzymes**

- **Proteins** – that catalyze reactions by lowering activation energy
- Found naturally in plants, animals and microorganisms
- Responsible for metabolic processes, many reactions which result in quality loss
- Sensitive to temperature, pH, oxygen, light and substrate concentration

**Polyphenol oxidase (PPO)**

- Two enzyme types - catalyze oxidation of
  - mono-phenolics: o-diphenoloxidases
  - di-phenolics: laccases
to form brown compound
- Requires oxygen for reaction
- In plants, active pH range 6 to 7
- Contains copper as prosthetic group
- Somewhat unstable to heat
- Enzyme localized in plastids, while substrates (phenolics) are in vacuole
- Genes have been cloned

**Polyphenol oxidase (PPO)**

- Because enzyme & substrate are physically separated, browning doesn’t occur until -
  - Cutting, bruising, senescence
  - In presence of oxygen

**Factors Affecting Enzyme Activity**

- Cultivar
- Maturity
- Tissue (fruit, flower, tuber, stem etc.)
- Phenolics (substrates) present
- pH, oxygen, temperature, light
- Mechanical damage

**Phenylalanine Ammonia Lyase**

- Key enzyme in phenolic biosynthesis
- Mechanical injury (wounding) and ethylene can stimulate phenolic metabolism
- Phenolics are substrates for PPO; increased concentration stimulates browning
**Apricot cultivars - Lightness differences**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>DL*</th>
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<tbody>
<tr>
<td>Henderson</td>
<td>26.3</td>
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<tr>
<td>Moniqui</td>
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<tr>
<td>Rouge de Roussillon</td>
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<td>Rouge de Fournes</td>
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<td>Polonais</td>
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<td>Bebeco</td>
<td>5.3</td>
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<tr>
<td>Precoce de Tyrinthe</td>
<td>3.7</td>
</tr>
</tbody>
</table>

DL* = Difference in lightness from cutting to later time period. Higher number indicates more browning in same time.

After Radler, 1997

**Relative PPO activity**

**Different tissues in apple cultivars**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Relative PPO activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peel</td>
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<tr>
<td>Red Delicious</td>
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<tr>
<td>Golden Delicious</td>
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<td>McIntosh</td>
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<td>Fuji</td>
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<td>Gala</td>
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<tr>
<td>Granny Smith</td>
<td>43</td>
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<tr>
<td>Jonagold</td>
<td>43</td>
</tr>
<tr>
<td>Elstar</td>
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</tbody>
</table>

**Prevention - Enzymatic Browning**

- **Physical**
  - Reduction of temperature and/or oxygen, use of:
    - Refrigeration
    - Controlled atmospheres
    - Modified atmosphere packaging
    - Edible coatings
  - Treatment with heat, gamma-irradiation or high pressure

- **Preservatives**
  - Means utilize compounds which act to:
    - Inhibit the enzyme
    - Remove its substrates (oxygen and phenolics)
    - Function as preferred substrate

**Chemical Anti-browning Agents**

- Acidulants
- Reducing Agents
- Chelating Agents
- Complexing Agents
- Enzyme Inhibitors

**Acidulants**

- **pH optimum** for PPO is 6.0-6.5
- Little activity is detected below pH 4.5 (Whitaker 1994)
- Irreversible inactivation may occur at pH < 3.0
- Usually used in common with other agents
- Common acidulants:
  - Citric acid
  - Malic acid

**Citric acid**

\[
\text{Citric acid} = \text{CH}_2\text{C(OH)}\text{C(OH)}\text{COOH}
\]

Inhibits PPO by reducing pH and chelating copper prosthetic group.
- Also inhibits oxidation by chelating other metal ions
- Synergistic with ascorbic acid
- Very commonly used on fresh-cut
Reducing Agents

- Cause reduction of colorless o-quinones resulting from PPO action back to o-diphenols
- Reducing agent is irreversibly oxidized; therefore consumed
- Common reducing agents:
  - ascorbic acid
  - cysteine
  - other thiols

Ascorbic acid

Reduces quinones to phenolic compounds
- acid and salt forms used
- salt (neutral pH) form may be more active
- water soluble
- often used in combination with citric acid

Erythorbic Acid

Reduces quinones to phenolic compounds
- Isomer of ascorbic acid
- Acid and sodium salt used
- Sodium salt may be more effective
- Cheaper (1/5 cost) than ascorbic acid

Chelating Agents

- Agents complex copper in the active site of PPO, therefore inhibit the enzyme
- Common chelating agents*:
  - EDTA – chelates many metals
  - Sporix – polyphosphate that chelates Fe, Ca, Mg, Al
- *All GRAS

Complexing Agents

- Agents capable of entrapping or forming complexes with PPO substrates or reaction products
- Results vary with specific cyclodextrin and more complex mixtures of phenolics
- Common complexing agents:
  - Cyclodextrins – sugar molecules in a ring formation
  - cyclic non-reducing oligosaccharides
Enzyme Inhibitors

- Sulfites inhibit PPO, but banned on use in fresh fruits and vegetables.
- One of inhibitors with the most potential is 4-hexyl resorcinol
- FDA GRAS and EU approval status for crustaceans and shrimp only
  - Additional approval requires testing on commodity of interest
  - Used in combination with ascorbic acid

Sulfites

\[
\begin{align*}
\text{Na}_2\text{SO}_3, \\
\text{NaHSO}_3, \\
\text{SO}_2
\end{align*}
\]
Inhibit polyphenol oxidase
React with PPO intermediates to form colorless products
- no longer GRAS for fruits & vegetables served raw, sold raw or presented to customer as raw
- foods containing detectable level of sulfiting agent (10 ppm) must label contents

Substrate Analogs

- These agents inhibit PPO by mimicking phenolic substrates
- Over prolonged storage (>24 hr), Sapers et al. (1998) found severe browning developed.
- Suggested that cinnamates and benzoates undergo slow but gradual conversion to PPO substrates

Cinnamic Acid & Benzoic Acid

- Inhibit o-diphenol oxidase by acting as substrate analogues
- GRAS - approved for food use

Methyl jasmonate

Inhibits browning
- natural plant product
- very slightly soluble in water (soluble in alcohol)
- can be applied as gas
Physical methods - Browning prevention

1. Exclusion of oxygen (CA, MAP, edible coatings, sugar, salt)
2. Temperature reduction
3. pH adjustment
4. Heat
5. ‘Advanced methods’
   1. Gamma irradiation (heat)
   2. High pressure (pressure & heat)
   3. Pulsed electric fields (heat)
   4. Microwave (heat)

Fresh – Cut
Fruit & Vegetable Texture

Dessication in cut carrots

Loss of integrity/translucency

Dessication - loss of integrity

Textural Integrity Preservation

- Genetic background
- Maturity
- Morphology, cell wall and middle lamella structure
- Cell turgor
- Water content
- Biochemical factors, enzyme activity
Texture – Relation to Structure

TEXTURAL PROPERTIES
(firmness: viscoelasticity, turgor)

STRUCTURAL COMPONENTS
(cell wall, middle lamella, plasma membrane)

Effect of Calcium
- Calcium forms ionic bonds between pectin molecules with negative charge – Firmness!
- Pectinesterase cleaves methyl esters to form more free carboxylic acid groups allowing more Ca$^{2+}$ to bind, creating stronger walls.
- Calcium ions also stabilize cell membranes, which may have an effect on turgor pressure, membrane permeability and integrity.

\[
\text{COO}^- \quad \text{Ca}^{2+} \quad \text{OOCC}
\]

\[
\text{COOCH}_3 \quad \text{CH}_2\text{OOCC}
\]

Fresh-cut Melon Firmness with Calcium

Turgor pressure is a function of the osmotic pressure in the tissue

Egg – Box model
- Calcium interaction with free carboxyl groups on adjacent pectin chains
- “Egg-box” model
Use of Calcium in Fresh-cut

- Concentrations typically used ~ 0.5-1.0%
- Must be labeled
- May be used in combination with low temperature blanching for PME activation and additional firming
- Either CaCl₂, calcium lactate or calcium ascorbate may be used