Use of Anti-browning Agents and Calcium

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Outline of Presentation
- Causes & Types of Browning
- Location of Browning Agents in Plant Cells
- Enzymes – Polyphenol Oxidase and Phenylalanine Ammonia Lyase
- Varietal & Tissue Differences in Browning
- Prevention of Browning
- Texture and Loss of Cellular Integrity
- Prevention of Loss of Integrity

Causes & Types of Browning

Causes of Browning
- Wounding / Mechanical damage / Cutting
- Senescence and Cell / Tissue breakdown
- Temperature abuse
- Chilling injury
- Disease
- CO₂ injury
  (see apples)

Types of Browning
- **Enzymatic** (most important in fresh-cut)
  - Polyphenol oxidase
  - Phenylalanine ammonia lyase
- **Non-Enzymatic** (not important in fresh-cut)
  - Maillard - sugar-amine reaction, concentrated solutions
  - Carmelization - sugars, high temperatures
  - Ascorbic acid oxidation
  - Lipid browning

Slide courtesy of Dr. Panita Ngamchuachit
Location of Browning Agents in Plant Cells

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

Plant Tissues/Cells
Location of Browning Components
- Water vs. Lipid Soluble Components
  - Water Soluble (70-90% of plant tissue)
    - Most of Cell Contents
    - Vacuole, Cytoplasm, Cell Wall
    - Phenolic substrates in vacuole
  - Lipid Soluble (10-30% of plant tissue)
    - Membranes (plasma, tonoplast and surrounding all organelles)
    - Plastids and Lipid Bodies
    - Polyphenol oxidase in chloroplast

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
Enzymes

**Polyphenol Oxidase**
- Catalyzes oxidation of mono-phenolics to di-phenolics which form brown compounds
- 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

**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

**Factors Affecting Enzyme Activity**
- Variety or Cultivar
- Maturity
- Tissue (fruit, flower, tuber, stem etc.)
- Phenolics (substrates) present
- pH, oxygen, temperature, light
- Mechanical damage

**Varietal & Tissue Differences in Browning**
Clingstone Peach Varieties
Initial color after cutting and color after 24 hours at RT

Enzymatic Browning in Varieties of Cut Apples
Photo courtesy of Adel Kader.

Apricot cultivars - Lightness differences
(oxidized - unoxidized) After Radler, 1997

Relative PPO activity
Different tissues in apple cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Relative PPO activity</th>
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<tbody>
<tr>
<td></td>
<td>Peel</td>
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<td>Red Delicious</td>
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<td>Golden Delicious</td>
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<td>Granny Smith</td>
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<tr>
<td>Jonagold</td>
<td>43</td>
</tr>
<tr>
<td>Elstar</td>
<td>10</td>
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</tbody>
</table>

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

Prevention of Browning

Chemical Anti-browning Agents
1. Acidulants
2. Reducing Agents (most used on fresh-cut)
3. Chelating Agents
4. Complexing Agents
5. 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

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 or calcium ascorbate
- Thiols: cysteine or glutathione
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

PPO in Treated Fresh-cut Eggplant
PPO activity inhibited in 0.4% solutions of either calcium citrate or ascorbate. Barbagallo et al., 2012 PB&T

Fresh-Cut Artichokes + Cysteine
Addition of 0.5% cysteine resulted in less browning. Amodio et al., 2011 PB&T

Cut Apples + Citric & Ascorbic
0.5% citric + 0.5% ascorbic – least browning

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

- 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

4-Hexylresorcinol

- Inhibits polyphenol oxidase
- Approved for use on shrimp to control browning
- Not approved for fresh-cut

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 shock and refrigeration

**Apple Slices – Antibrowning Agents and Whey Protein Concentrate**

Using combination of chemical anti-browning agent with WPC (physical) is best.

*Perez-Gago et al., 2006* PB&T

**Texture and Loss of Cellular Integrity**

**Loss of Integrity - Translucency**

*Photo courtesy of Adel Kader.*

**Loss of Integrity - Dessication**

*Photo courtesy of Adel Kader.*
**General Plant Cell Structure**

- Cell wall (contains pectin)
- Middle lamella (pectin)
- Plasma membrane

**Cell wall strengthening with Calcium**

- Calcium interaction binds free carboxyl groups on adjacent pectin chains
- “Egg-box” formation – firmer texture

- Polygalacturonic acid chains (pectins)
- Calcium ions

**Factors Affecting Textural Integrity**

- Genetic/varietal background
- Maturity
- Morphology, cell wall and middle lamella structure
- Cell turgor pressure
- Water content
- Biochemical factors, enzyme activity (pectin methylesterase, polygalacturonase)

**Use of Calcium in Fresh-cut**

- Concentrations typically used ~ 0.5-2.5%
- Either CaCl₂, calcium lactate or calcium ascorbate may be used (ascorbate will also assist with prevention of browning)
- Must be labeled
- May be used in combination with low temperature blanching for PME activation, more demethylation of pectins and additional firming

**Turgor Pressure is a Function of the Osmotic Pressure in the Tissue**
Calcium Dips on Fresh-cut Mangoes

To determine the effects of calcium treatments on instrumental quality and consumer acceptance

Varieties
- 'Kent', 'Tommy Atkins'

Calcium sources
- CaCl₂, Ca-lactate

Concentrations
- 0, 0.068, 0.136, or 0.204M

Dip times
- 0, 1, 2.5, or 5min

In both varieties
- ↑Ca Conc. + ↑dip time = ↑firmness
- Ca treated samples are firmer than the water dips, and the untreated controls
- Firmness retention was higher in cubes treated with CaCl₂

Consumer test

Consumer liking strongly corresponded to mango variety

Cluster 1
- n = 130
- 'Kent'
- lower Ca conc.
- CaCl₂ (at 0.136 M)

Cluster 2
- n = 53
- 'Tommy Atkins'
- (for all treatments except 0.204M)

Calcium studies- Conclusions

- **CaCl₂**: suitable for fresh-cut processing based on
  - better tissue firming
  - consumer preference
- **Optimal calcium treatment**
  - 'Tommy Atkins' 2.5 min dip in 0.136 M CaCl₂
  - 'Kent' 1.0 min dip in 0.136 M CaCl₂
- **'Kent'**: suitable for fresh-cut processing in terms of
  - consumer preference

Fresh-Cut Eggplant - Firmness

Optimal firmness with 0.4% calcium ascorbate dips (also beat anti-browning).
Barbagallo et al. 2012. PB&T

Fresh-cut Melon Firmness with Calcium

Optimal firmness with 2.5% calcium lactate at either 25 or 60°C.
Dipping berries in chitosan with or without calcium gluconate reduces texture loss.
Hernandez-Munoz et al. 2006 PB&T