Effect of Edible Coatings on Quality and Shelf Life of Fresh Mushrooms (*Agaricus bisporus*)

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Mushroom Production

- Over 800 million lb of white button mushrooms were produced in U.S. during 2008-09
- 85% sold as fresh mushrooms
- Fresh mushrooms ranked 13th among 20 most consumed raw vegetables in the United States

Gormley, 1975; USDA, 2009; CFR, 2009
Mushroom Quality Characteristics

- Color
- Cap Opening (Maturity Index)
- Moisture Loss (Shriveling)
- Microbial Contamination (*Pseudomonas, Flavobacterium*)
- Stem Elongation
## Respiration Rates at 5°C

<table>
<thead>
<tr>
<th>Class</th>
<th>mg CO₂kg⁻¹h⁻¹</th>
<th>Commodities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Low</td>
<td>&lt; 5</td>
<td>Nuts, dates</td>
</tr>
<tr>
<td>Low</td>
<td>5 – 10</td>
<td>Apple, citrus, grape, kiwifruit, onion, potato</td>
</tr>
<tr>
<td>Moderate</td>
<td>10 – 20</td>
<td>Apricot, banana, cherry, peach, nectarine, pear, plum, fig, cabbage, carrot, lettuce, pepper, tomato</td>
</tr>
<tr>
<td>High</td>
<td>20 – 40</td>
<td>Strawberry, blackberry, raspberry, cauliflower, lima bean, avocado</td>
</tr>
<tr>
<td>Very High</td>
<td>40 – 60</td>
<td>Artichoke, snap bean, Brussels sprouts, cut flowers</td>
</tr>
<tr>
<td>Extremely High</td>
<td>&gt; 60</td>
<td>Asparagus, broccoli, <strong>mushroom</strong>, pea, spinach, sweet corn</td>
</tr>
</tbody>
</table>

Saltveit, 2004
Microbial Contamination

- Bacterial populations increase from 7.3 to 8.4 log CFU/g stored for 6 days at 4°C
  - 54% Pseudomonas fluorescens
  - 10% Flavobacterium spp.

- Rate of fresh mushroom deterioration directly related to initial microbial load

Chikthimmah and Beelman 2006; Beelman and Guthrie 1989
Moisture Loss

- Unlike fruits and vegetables, mushroom lack epidermal structure
  - High transpiration rate from fruiting body
  - Water loss comparable to that of free water surface

- Weight loss caused by transpiration
  - Toughening and shriveling of cap
  - Money loss when sold by weight
  - Loss of consumer acceptability

San Antonio and Flegg 1964; Gormley 1975
Mushroom Shelf Life Extension

- Increasing demand for fresh mushrooms by consumers
- Need for technologies that will maintain quality and safety of fresh mushrooms
  - Respiration Rate Control
  - Bacterial Contamination
  - Browning
  - Weight Loss
Mushroom Shelf Life Extension

- Technologies proposed to extend shelf life of fresh mushrooms
  - Antimicrobial wash
  - Modified atmosphere packaging (susceptible to temperature abuse)
  - Modified humidity packaging (reduced humidity reduces weight)
  - Irradiation (not accepted by consumers)
Edible Coatings/Films

- Material used for enrobing foods to extend their shelf life

  - Provide replacement and/or fortification of natural layers to prevent moisture losses
  - Selectively allow controlled exchange of $\text{O}_2$, $\text{CO}_2$ and ethylene
  - Prevent loss of important components (vitamins, minerals)
  - Transporting functional ingredients (controlled release)
Antimicrobials in Edible Coatings

- Edible coatings can incorporate food additives, such as anti-browning agents, antimicrobials, antioxidants, flavors, colorants, spices, nutraceuticals and probiotics

- Antimicrobial edible coatings inhibit spoilage and pathogenic bacteria by controlled release: maintaining effective concentrations of active compounds on food surfaces

- There are several categories of antimicrobials that can be incorporated into edible films and coatings:
  - Organic acids
  - Polypeptides (nisin)
  - Plant essential oils
  - Nitrites and sulfites

Martín-Belloso, et al. 2009; Gennadios and Kurth 1997; Franssen and Krochta 2003
Fresh Cut Apples

- Coated with carrageenan and WPC, or WPI
- Extended shelf life to 3 weeks
- Storage 4°C
Avocado

- Coated with methyl cellulose
- Extended shelf life from 6 to 10 days
Peeled Carrots

- Coated with xanthan gum or alginate
- Added Calcium, Vitamin E
- Extended shelf life from 4 to 8 days

Mei et al. 2002; Amanatidou et al. 2000
Cut Mangoes

- Coated with chitosan
- Storage 6°C
- Extended shelf life from 3 to 7 days

Chien et al. 2005
Lettuce

- Coated with alginate
- Added CaCl$_2$
- Increased crispness
Peeled Litchi

- Coated with chitosan
- Extended shelf life from 3 to 6 days
- Storage -1°C
Mushrooms

• Coated with Calcium Alginate
  • Higher L-values
  • Weight loss reduction

• Coated with Chitosan
  • Decrease in phenolic enzyme activity
  • Less bacterial counts (cfu/g)
  • Lower ΔL* values

Nussinovitch and Kampf 1993, Hesham A.A 2007
Research Goals

- To screen biopolymer based coating materials for their compatibility with the mushroom surface.
- To determine the effect of the coating along with antimicrobials and chelating agents on the quality and shelf-life of fresh mushrooms:
  - Color change
  - Weight loss of fresh mushrooms
  - Microbial count of fresh mushrooms
- To determine the effect of edible coating on $O_2$, $CO_2$, and water vapor transmission rates.
Nisin and EDTA

- Nisin is an antimicrobial protein or bacteriocin produced by some strains of *Lactococcus lactis* subsp. *lactis*
  - Antimicrobial activity against gram+ bacteria
  - Limited antimicrobial activity against gram- bacteria, yeasts and molds
- EDTA is a chelating agent with high affinity to divalent metallic ions
  - When divalent ions are removed from outer membrane of gram- bacteria, they become vulnerable to antimicrobial agents

Delves-Broughton et al. 1996; Delves-Broughton 1993
Gram + & Gram -
Mode of Action of Nisin & EDTA in Gram- Bacteria

Outer Wall

Cytoplasmic Membrane

Lipopolysacharides

Peptidoglycan

Phospholipids

EDTA

Ca^{2+}
Mode of Action of Nisin & EDTA in Gram- Bacteria

Nisin

EDTA

Ca^{2+}

Outer Wall

Lipopolysaccharides

Peptidoglycan

Cytoplasmic Membrane

Phospholipids

Nisin
Controlled Release of Nisin & EDTA
Controlled Release of Nisin & EDTA
Controlled Release of Nisin & EDTA
Nisin in Calcium Alginate

- By immobilizing nisin in calcium alginate gels, a reduction of gram+ bacteria in ground beef was achieved for up to seven days.

- EDTA was shown to enhance Nisin activity against gram- bacteria in buffer solutions.

Cutter and Siragusa 1997; Delves-Broughton 1993
Nisin+EDTA in Calcium Alginate

- **Nisin + EDTA** in a calcium alginate immobilized system was shown to inhibit *E. coli* O157:H7 in ground beef.

- **Nisin + EDTA** in a calcium alginate system was shown to reduce mesophillic and psycrophillic bacteria in northern snakehead fillets:
  - Lower water loss
  - Higher score in sensory analysis using 9-point hedonic scale
Significance & Innovation

- Increase in sales of fresh mushrooms calls for technologies to extend their shelf life.

- The application of an **edible coating** containing an **antimicrobial agent** prior to packaging will provide a low cost, **controlled-release** technology that will increase fresh mushroom shelf life.
Materials

- Mushrooms: obtained from the MTDF of Penn State University
- Carbohydrate based Bipolymers
- CaCl$_2$
- Antimicrobial: Nisin
- Chelators: Disodium EDTA, Sodium Citrate
Methodology
Biopolymer Selection

- Water soluble
- Liquid at coating temperature (4°C), form a water insoluble matrix
- Permeable to O₂ and CO₂
- Allow release of functional ingredients (i.e. Nisin, EDTA)
Mushrooms are randomly selected on day 0 and L, a, b values are recorded with a Minolta Chromameter (Model CR-400)

One tray is selected randomly on each day, and the L, a, b values in two random points of the cap are recorded for all mushrooms

The mean L values, $\Delta L$ values and $\Delta E$ values will be calculated

$$\Delta L = L_F - L_0$$

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$
The maturity index is assigned on day 0 and after days 1 – 10 using the extent of cap opening based on the following 7-point scale.

<table>
<thead>
<tr>
<th>Stage</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description of Sporophore</td>
<td>Veil intact (tight)</td>
<td>Veil intact (stretched)</td>
<td>Veil partially broken (less than half)</td>
<td>Veil partially broken (greater than half)</td>
<td>Veil completely broken</td>
<td>Cap open, gills well exposed</td>
<td>Cap open, gill surface flat</td>
</tr>
</tbody>
</table>

Guthrie, 1984; Modified from Hammond 1976
Weight Loss

- Mushrooms are weighed (±0.1g) prior to placing into tray on day 0
- One tray will be selected randomly from 1 – 10 days, and the weight of the mushrooms will be recorded
Standard Plate Count

- One package is evaluated for microbial count on day 0, and during storage.
- All mushrooms in each package are blended with equal weight of 0.1% peptone water in a sterilized blender for 2 minutes.
- The homogenate is serially diluted and spread plated on agar plates.
- Petri dishes are incubated at 37°C for 48 hours.
- The mean of duplicate plates is a single measurement/package, and reported as CFU/g.
Preliminary Experiments
Effect of Coating on Quality

<table>
<thead>
<tr>
<th></th>
<th>Control Uncoated</th>
<th>Treatment 1 Ca-Alginate + Nisin</th>
<th>Treatment 2 Ca-Alginate + EDTA</th>
<th>Treatment 3 Ca-Alginate + Nisin + EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 0</strong></td>
<td><img src="image" alt="Control Uncoated" /> <img src="image" alt="Treatment 1" /> <img src="image" alt="Treatment 2" /> <img src="image" alt="Treatment 3" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Control Uncoated" /> <img src="image" alt="Treatment 1" /> <img src="image" alt="Treatment 2" /> <img src="image" alt="Treatment 3" /></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Day 7</strong></td>
<td><img src="image" alt="Control Uncoated" /> <img src="image" alt="Treatment 1" /> <img src="image" alt="Treatment 2" /> <img src="image" alt="Treatment 3" /></td>
<td></td>
<td></td>
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Appearance of Coated Mushrooms
Appearance of Coated Mushrooms after cooking
Ongoing Studies

- Screen biopolymer based coating materials for their compatibility with mushroom surface
- Determine the effect of the coating along with antimicrobials and chelating agents on the quality and shelf-life of fresh mushrooms:
  - Color change
  - Weight loss
  - Microbial count
- Determine the effect of edible coating on $O_2$, $CO_2$, and water vapor transmission rates
- Cost Calculation (approx. $0.10/lb of fresh mushrooms)
Questions?