Development of Whey Protein Based Edible Films with pH-controlled Release for Active Packaging

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Food Safety and Packaging

- Microorganisms
- Chemicals
- O₂
- Moisture
- Light

Functional Properties of Packaging Materials

Better shelf-life and quality
Active and Antimicrobial Packaging

A packaging system releases active/antimicrobial compounds on the food surface to delay microbial growth.

- Inhibit pathogenic bacteria
- Control spoilage
- Use minimum amounts of active compounds

✔ Extend shelf life
✔ Safe and High quality foods
Controlled Release Packaging (CRP) System

Innovative form of active packaging where antimicrobial and antioxidant compounds incorporated into a polymer and release in a controlled manner.

Figure 1. CRP System
Controlled Release

- Direct addition of antimicrobials
  - Excessive use!
  - Neutralization!
- Antimicrobial sprays or dips
  - Diffusion into center of food!

Controlled release antimicrobial packaging
- Prevent too high / too low concentration of active substances at food surface
- Prevent sensorial or toxicological problems (use lower amounts of active agent)
Diffusion into Food!

Release Rate

Growth Rate

SLOWER

FASTER

Microbial Spoilage!
pH-Controlled Release in WPI films

- pH-triggering mechanism for controlled release of lysozyme from WPI films

- Can be triggered by adjusting the pH of food when antimicrobial activity is needed
  - Before cold storage
  - After freezing-thawing
  - Before transportation and market display
Edible Films and Antimicrobials

**Whey Protein Isolate**
- 20% of milk proteins
- $pI \approx 5.4$
- One of the most commonly used biodegradable film material
- Forms transparent brittle films
- Good $O_2$, $CO_2$, lipid and aroma barrier properties

**Lysozyme**
- Hydrolases
- $pI \approx 9.2$
- Molecular weight $\approx 14.7$ kDa
- Found in tears, mucus, egg white
- One of the most frequently used biopreservative
- Antimicrobial activity mainly on gram-positive bacteria
WPI films

- Whey protein film (WPI)
- WPI – Oleic acid blend film (9% oleic acid)
- WPI – Beeswax composite film (30% beeswax)

: Free lysozyme
: Bound lysozyme

Negatively charged WPI

Neutral pH

Below pI (pH 5.4)
Potential Application

- Activation of Lyz based antilisterial activity
- *Listeria monocytogenes* - Smoked fish processing (Smoked salmon)
  - MAP → lost after opening the pack
  - Vacuum packaging has no absolute effect on *L. Monocytogenes*

pH-triggered release system can be employed as a hurdle to reduce the risk, especially during the storage of remaining food after the first consumption.

Cold smoked salmon
Produced WPI Films

- Released LYS activity into buffer
- Released LYS activity on smoked salmon
- In-vitro antimicrobial activity of films
- Antimicrobial activity of the films coated on smoked salmon
- Morphological properties

<table>
<thead>
<tr>
<th>Control</th>
<th>LYS</th>
<th>LYS + Oleic acid (9%)</th>
<th>LYS + Beeswax (30%)</th>
</tr>
</thead>
</table>

Produced WPI Films with:
- LYS
- Oleic acid (9%)
- Beeswax (30%)
LYS Activity of the Films

- LYS release at different pHs
LYS Activity of the Composite and Blend Films

A. pH 5.5, pH 5.0, pH 4.5, pH 4.0

B. pH 4.5

C. pH 5.0

D. pH 5.5
Film Morphology

Control WPI film

WPI + LYS

WPI + Oleic acid

WPI + bees wax
Release on Smoked Salmon

OLE & BW

![Graph showing release of enzyme activity on Smoked Salmon with different incubation times and conditions.](image)
## Antimicrobial Activity against L. innocua

<table>
<thead>
<tr>
<th>Film composition</th>
<th>Average zone area (mm²) at 4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>LYZ (mg/cm²)</td>
<td>OLE (%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>- - - -</td>
<td>-</td>
</tr>
<tr>
<td>- - - +</td>
<td>-</td>
</tr>
<tr>
<td>0.7 - - -</td>
<td>51.7 ± 8.5&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.7 - - +</td>
<td>101.1 ± 15.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.7 9 -</td>
<td>121.5 ± 14.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.7 9 - +</td>
<td>141.6 ± 26.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.7 - 30 -</td>
<td>54.5 ± 7.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.7 - 30 +</td>
<td>58.5 ± 21.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
## Antimicrobial Activity against *L. innocua*

**L. innocua counts during storage at 4°C (log CFU/g)**

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uncoated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.98 ± 0.08^{a,A}</td>
<td>4.82 ± 0.10^{ab,B}</td>
<td>4.82 ± 0.10^{a,B}</td>
<td>4.80 ± 0.11^{a,B}</td>
<td>4.83 ± 0.13^{a,B}</td>
</tr>
<tr>
<td><strong>WPI/OLE (control)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>4.92 ± 0.10^{a,A}</td>
<td>4.72 ± 0.10^{bc,C}</td>
<td>4.73 ± 0.08^{b,C}</td>
<td>4.78 ± 0.09^{a,BC}</td>
<td>4.84 ± 0.11^{a,B}</td>
</tr>
<tr>
<td><strong>WPI/OLE+LYS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.92 ± 0.08^{a,A}</td>
<td>4.68 ± 0.07^{c,B}</td>
<td>4.69 ± 0.15^{b,B}</td>
<td>4.74 ± 0.12^{a,B}</td>
<td>4.49 ± 0.12^{b,C}</td>
</tr>
<tr>
<td><strong>WPI/OLE (acidified)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.97 ± 0.04^{a,A}</td>
<td>4.84 ± 0.11^{a,B}</td>
<td>4.77 ± 0.13^{ab,B}</td>
<td>4.80 ± 0.12^{a,B}</td>
<td>4.57 ± 0.09^{b,C}</td>
</tr>
<tr>
<td><strong>WPI/OLE+LYS (acidified)</strong></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>4.97 ± 0.07^{a,A}</td>
<td>4.49 ± 0.13^{d,B}</td>
<td>4.45 ± 0.13^{c,BC}</td>
<td>4.27 ± 0.19^{b,BC}</td>
<td>4.24 ± 0.15^{c,C}</td>
</tr>
</tbody>
</table>
Conclusion

- *LYS release mechanism based on acidification of the film was tested*

- *The films were successfully applied on smoked salmon slices*

- *Activation of edible films by consumer before consumption or after for the remaining part of the food is possible*

- *To optimize the concentration of LYS and increase the efficiency of film activity, further food applications are needed.*
thank you!