

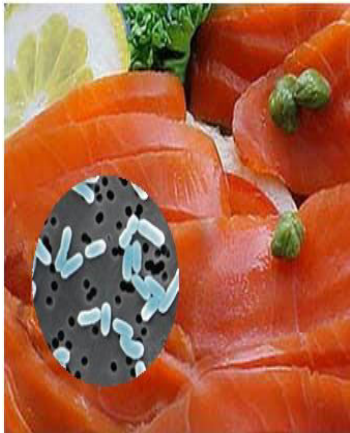
Development of Whey Protein Based Edible Films with pH-controlled Release for Active Packaging

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Food Safety and Packaging



- Microorganisms
- Chemicals
- O₂
- Moisture
- Light

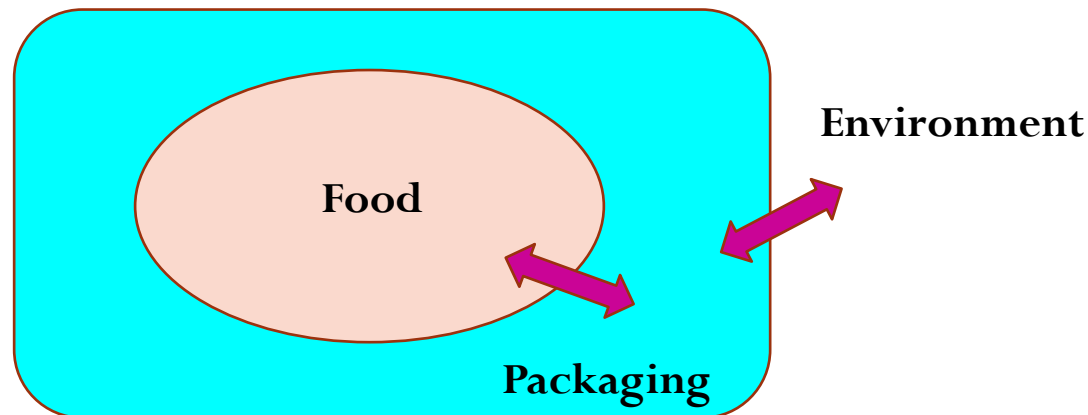
Functional Properties
of Packaging Materials



**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**.

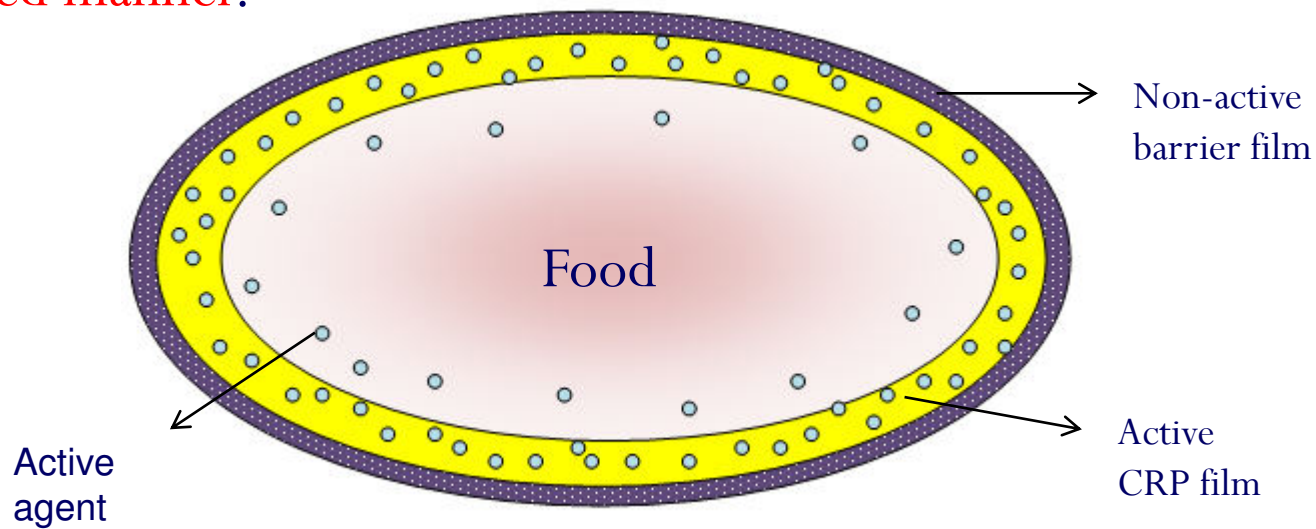
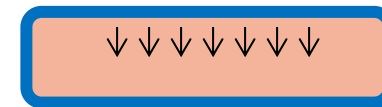
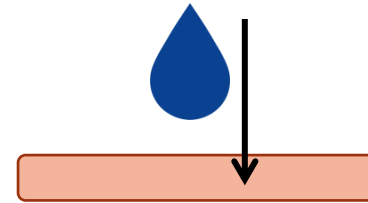


Figure 1. CRP System

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 !

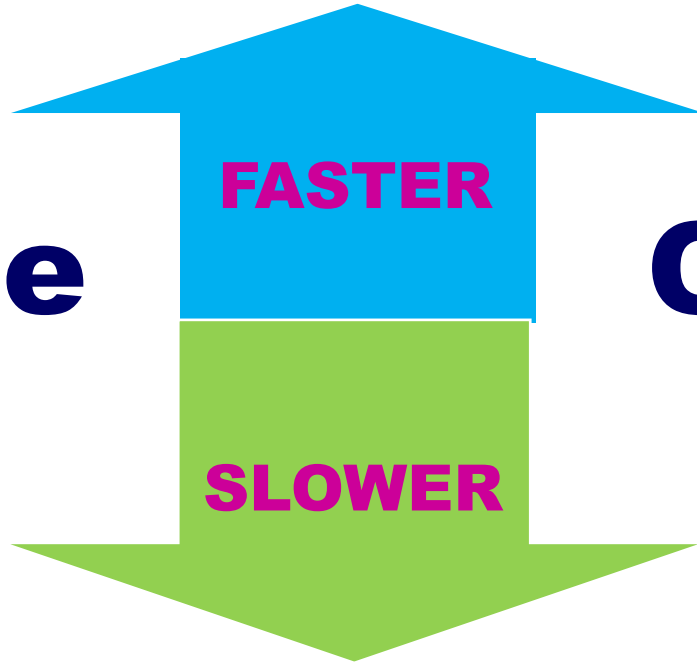
**Release
Rate**

FASTER

**Growth
Rate**

SLOWER

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

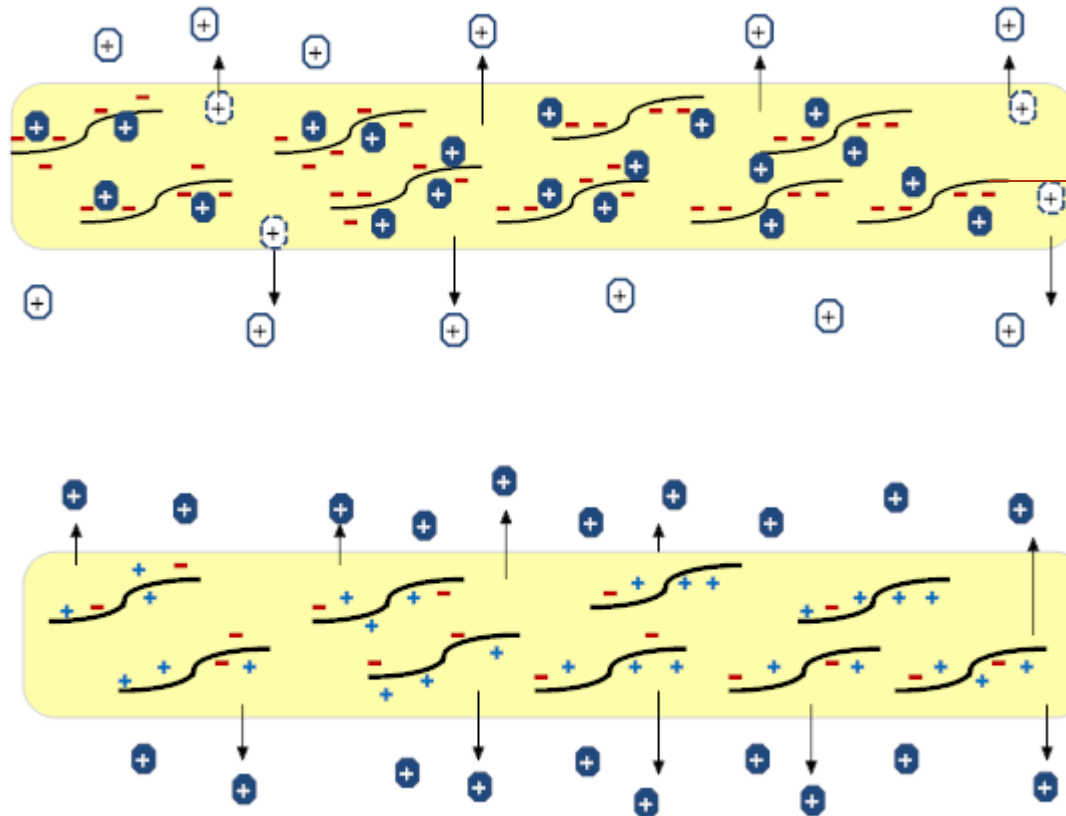
- 20% of milk proteins
- pI \approx 5.4
- One of the most commonly used biodegradable film material
- Forms transparent brittle films
- Good O₂, CO₂, lipid and aroma barrier properties

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

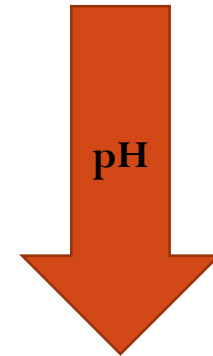
WPI films

- **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

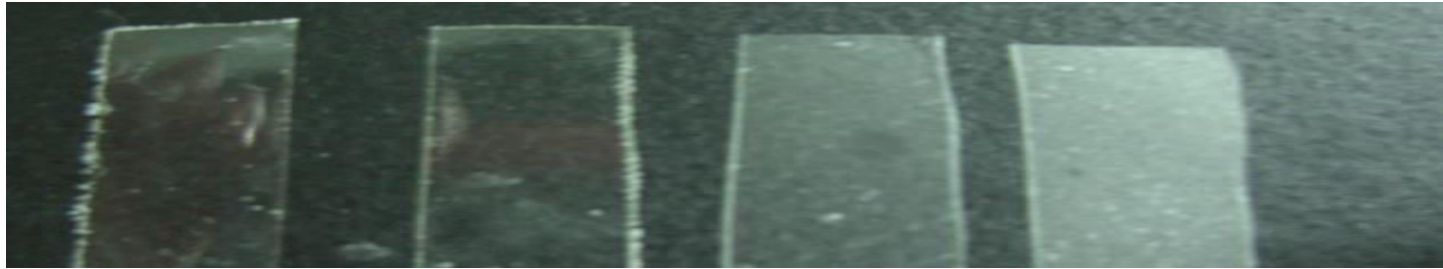
- Activation of Lyz based antilisterial activity
- *Listeria monocytogenes* - Smoked fish processing (Smoked salmon)
 - MAP → lost after opening the pack
 - Vacuum packaging has no absolute effect on *L. Monocytogenes*

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



Control

LYS

LYS +

Oleic acid (9%)

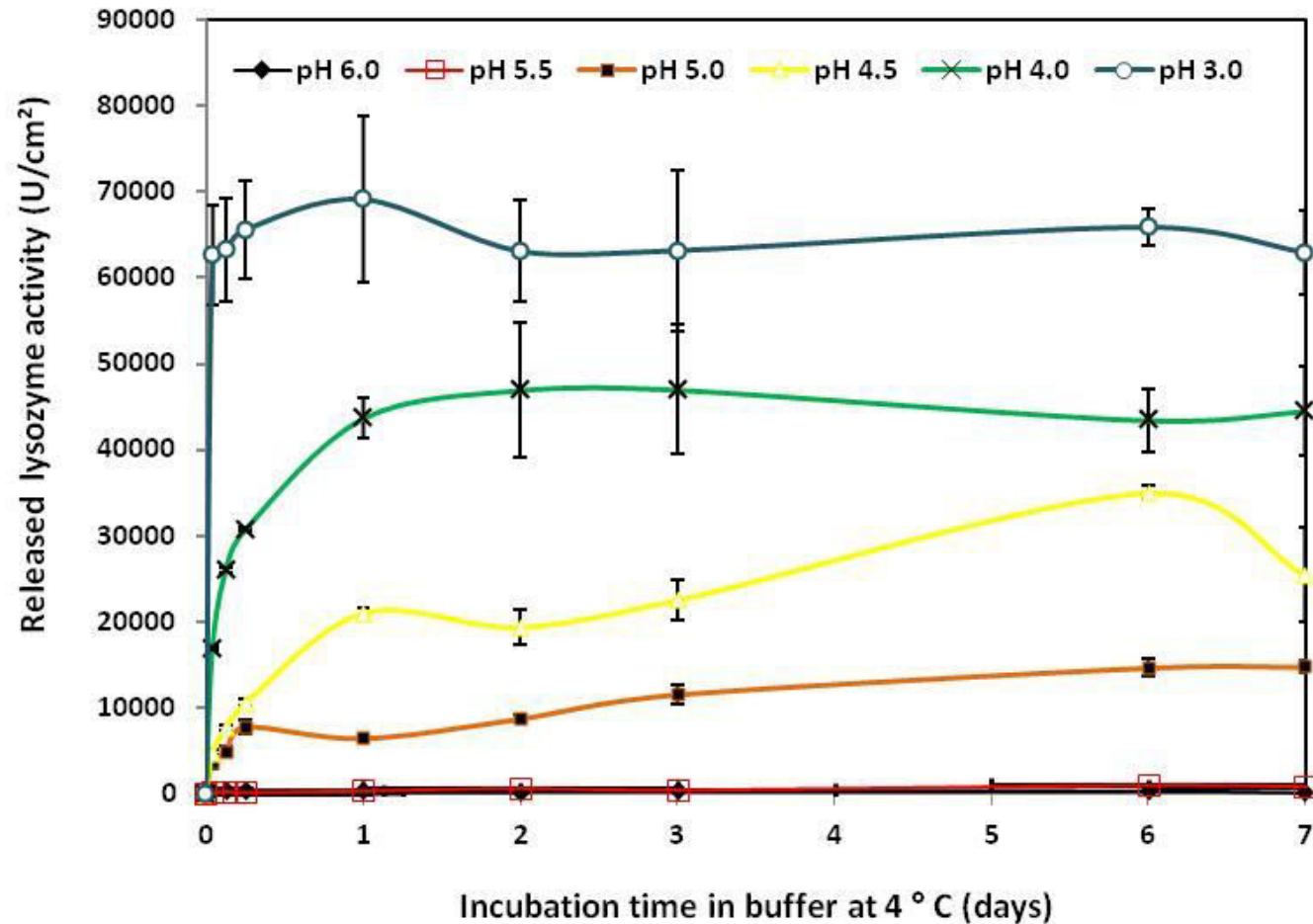
LYS +

Beeswax (30%)

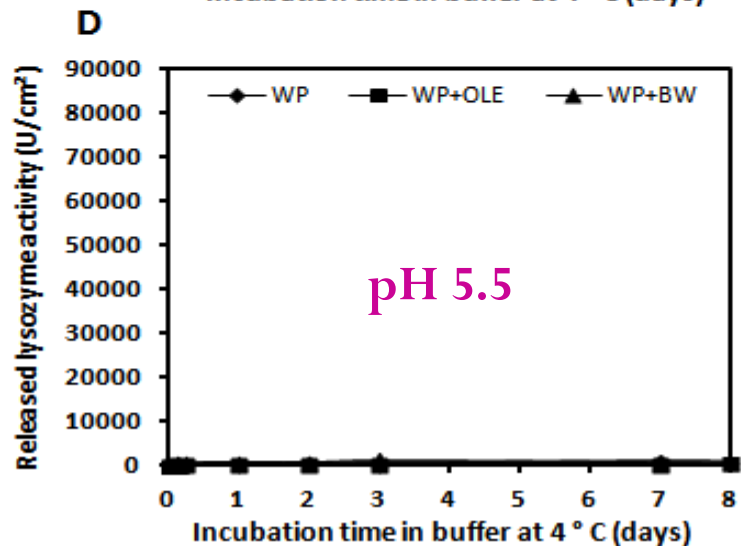
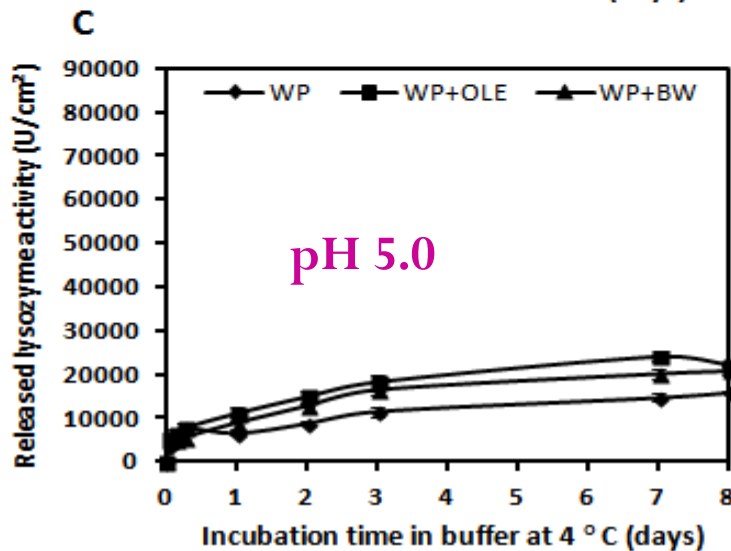
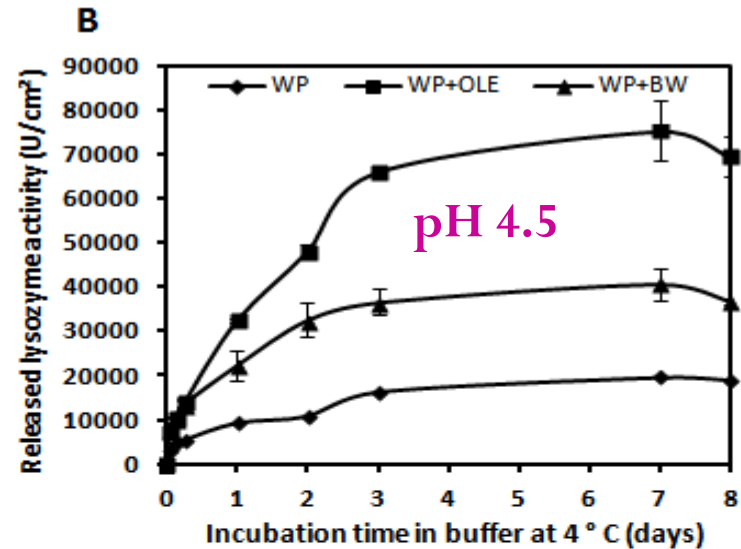
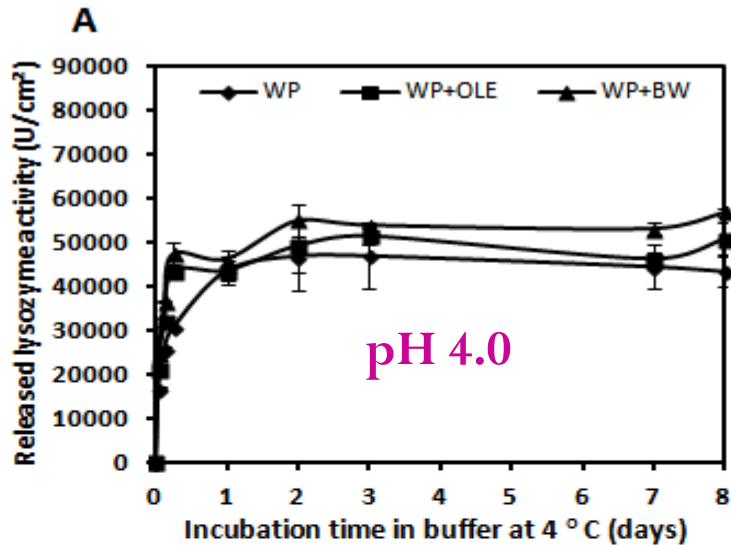
- Released LYS activity into buffer
- Released LYS activity on smoked salmon
- In-vitro antimicrobial activity of films
- Antimicrobial activity of the films coated on smoked salmon
- Morphological properties

LYS Activity of the Films

- LYS release at different pHs

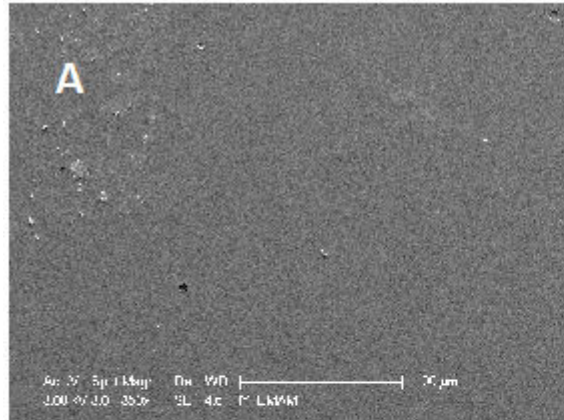


LYS Activity of the Composite and Blend Films

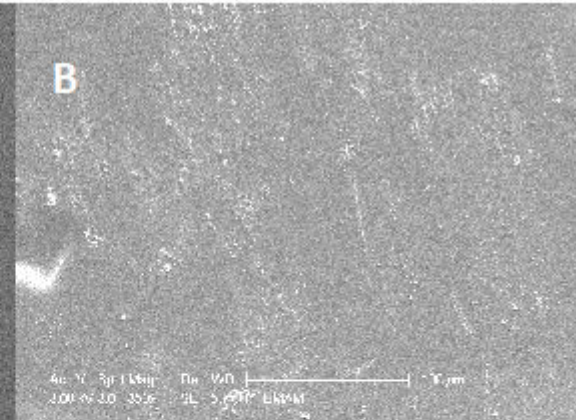


Film Morphology

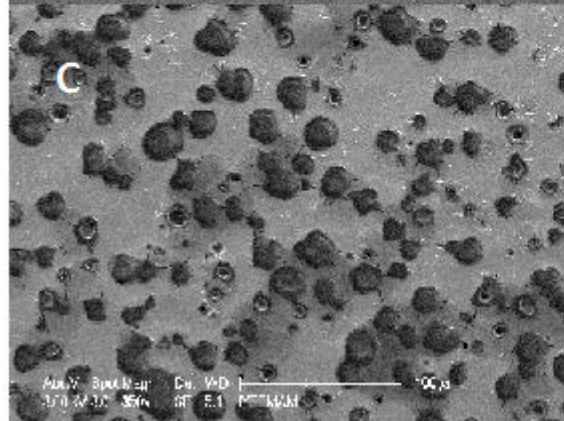
Control WPI film



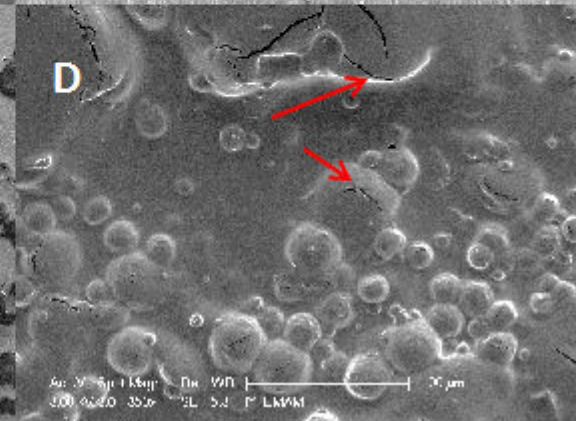
WPI + LYS



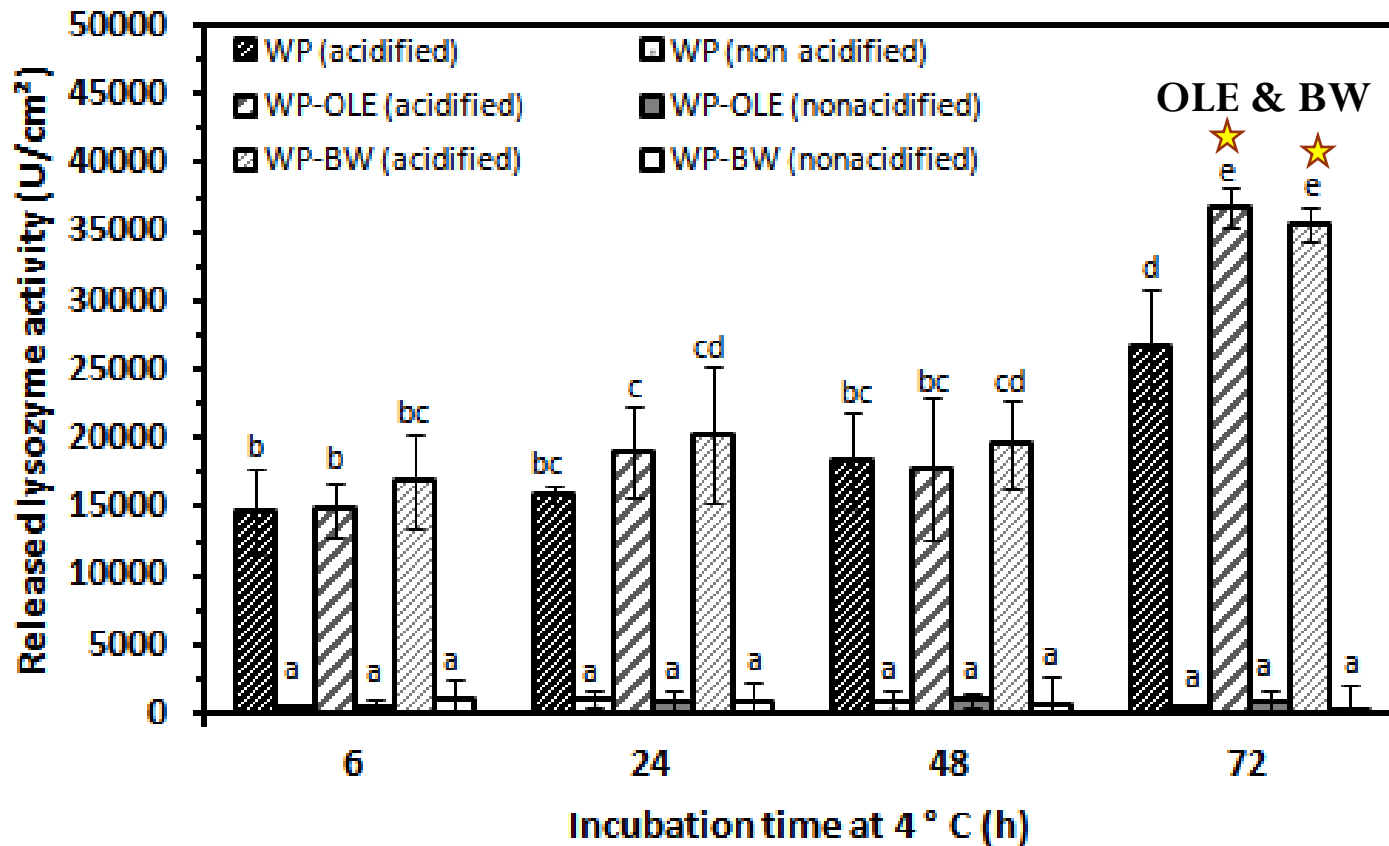
WPI + Oleic acid



WPI + bees wax



Release on Smoked Salmon



Antimicrobial Activity against *L. innocua*

Film composition				Average zone area (mm ²) at 4°C	
LYZ (mg/cm ²)	OLE (%) ^a	BW (%) ^a	Acidification (5% citric acid)	24 h	48 h
-	-	-	-	Partial zones	Partial zones
-	-	-	+	Partial zones	Partial zones
0.7	-	-	-	51.7 ± 8.5 ^d	63.5 ± 13.9 ^c
0.7	-	-	+	101.1 ± 15.3 ^c	84.6 ± 22.5 ^b
0.7	9	-	-	121.5 ± 14.9 ^b	93.9 ± 17.4 ^b
0.7	9	-	+	141.6 ± 26.6 ^a	124.3 ± 32.7 ^a
0.7	-	30	-	54.5 ± 7.4 ^d	55.6 ± 11.04 ^c
0.7	-	30	+	58.5 ± 21.2 ^d	88.11 ± 55.6 ^c

Antimicrobial Activity against *L. innocua*

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

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

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!