Promising hurdle technologies to minimise the survival and growth of pathogens and spoilage bacteria in seafood during processing

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Introduction

- **Preservative factor = hurdle**
- **Most important hurdles in food**

  temperature, water activity, pH, redox potential, preservatives (nitrite, sorbate, sulphite)
- **More than 60 hurdles described, including novel decontamination technology**

- **Hurdle technology = combination of hurdles (Leisner, 1985)**

HURDLETECH

Hurdle technology, including minimal processing, to ensure quality and safety of convenience seafood

Project leader : Dr Françoise Leroi (Ifremer)

Partners

- Ifremer — France
- enitiaa — France
- Fiskeriforskning — Norway
- azti tecnalia — Spain
- RF — Island
- RIVO — Netherlands

- **LPFPs : lightly preserved fish products (salt <6% WP, pH >5) : cold-smoked fish, carpaccio, middly cooked shrimp …**
  - ***Listeria monocytogenes***
  - **spoiling micro-organisms**

- **PSFPs : preserved semi finished products : salted cured-desalted cod, klipfish, frozen-thawed cod …**
  - ***Listeria monocytogenes, Staphylococcus aureus***
  - **spoiling micro-organisms**

Selected hurdles

1. Technological hurdles (salt, freezing, smoke …)
2. Competitive micro-organisms (biopreservation)
3. Chitosan and bioactive packaging
4. Pulsed light

Common collection of target micro-organisms isolated from fish products

- Pathogenic or surrogates micro-organisms
  - ***Listeria monocytogenes* and *innocua*, *Staphylococcus aureus and xylosus, Bacillus subtilis, Clostridium sporogenes***
- Spoiling micro-organisms
  - ***Photobacterium phosphoreum, Shewanella putrefaciens, Pseudomonas* spp., *Serratia liquefaciens, Psychrobacter* spp., *Lactobacillus sakei, Brochothrix thermosphacta***

Technological hurdles

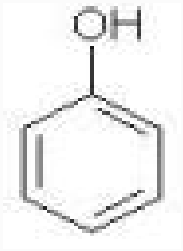
Technological hurdles in cold-smoked salmon

Salt | smoke | vacuum packaging | storage T°C

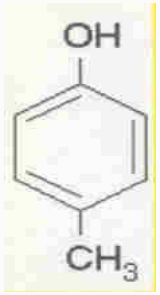
Smoke

- **Smoke = mixture of volatile compounds**

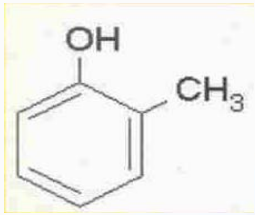
• Antimicrobial properties of phenolic compounds



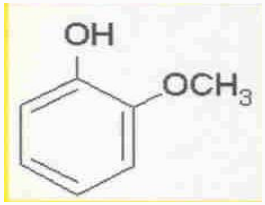
Phénol



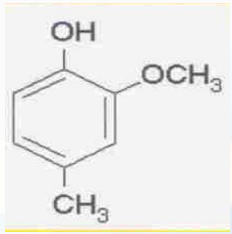
o-crésol



p-crésol

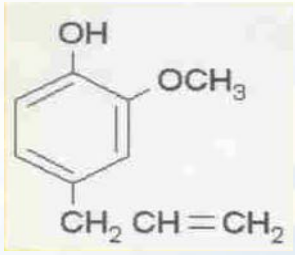


Guaïacol

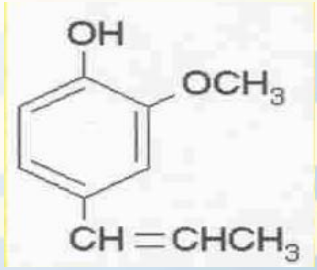


4-methyl guaïacol

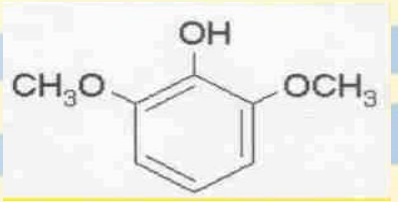
4-ethyl guaïacol



Eugenol



Isoeugenol



Syringol

Main results

- **Different sensitivity within *Lm* strains to phenolic compounds (factor 10)**
- **Anti-listeria effect varies from one compound to another (MIC : 322 to 8600 ppm)**
- **At high concentrations, synergy between the phenolic compounds (MIC = 1500 ppm)**
- **In CSS, 20 ppm**
  - **Other inhibitory compounds**
  - **Interaction with pH, aw …**

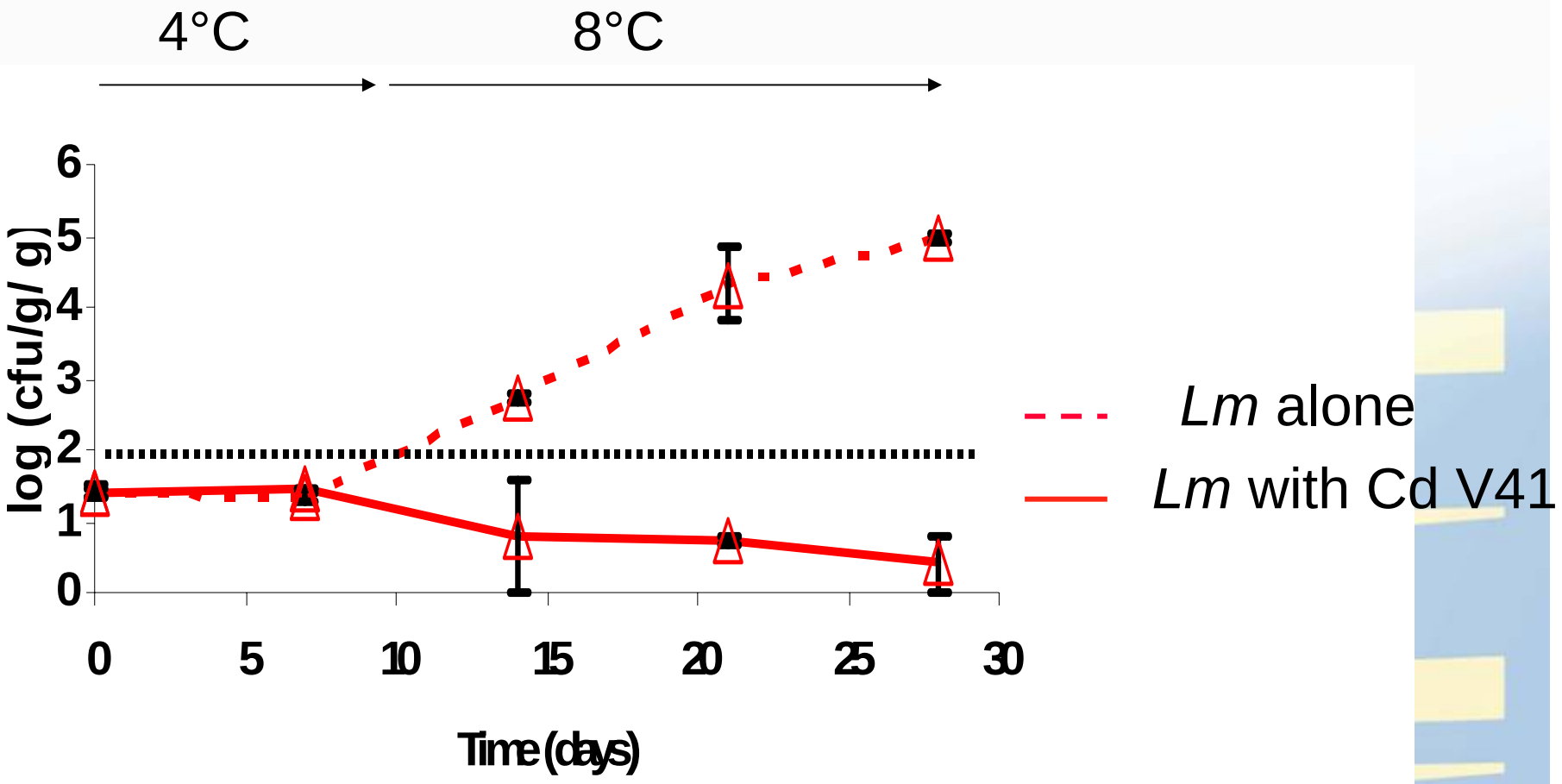
Competitive micro-organisms

Biopreservation

- **Biopreservation is the extension of storage life and enhancing of safety of food using the natural or controlled microflora and/or their antimicrobial products (Stiles, 1996)**
- **Lactic acid bacteria (LAB) are good candidates**

Previous results

- ***Carnobacterium divergens*** **V41 isolated from salmon**
- **Active against a wide collection of *Listeria monocytogenes***
- **Activity confirmed in cold-smoked salmon during 28 days of storage**


4°C
8°C
log (cfu/g/ g)
0
1
2
3
4
5
6
0
5
10
15
20
25
30
Time (days)
Lm alone
Lm with Cd V41

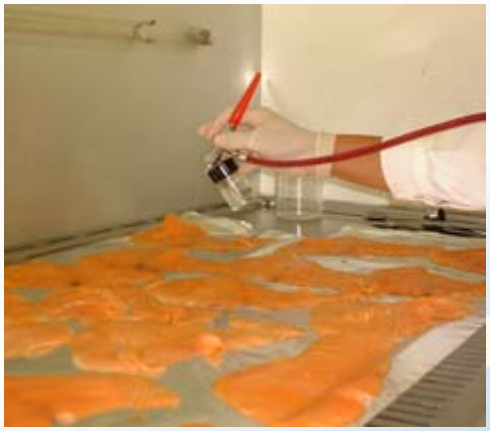
Can we use *C. divergens* V41 as a protective strain ?

- Do not spoil the product
- Do not produce toxic compounds
- Easy to produce

4 batches coming from 4 industries

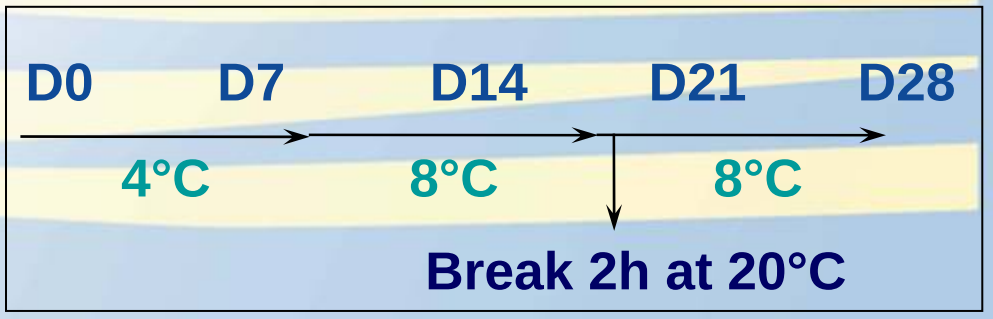
Slices of cold smoked salmon (40-50 g)

Inoculation by spraying *C. divergens* V41 (10^5 UFC/g)



Vacuum-package

Storage conditions



- **No or low production of TVBN**
- **No acidification**
- **No sensory modification (odour, taste, texture, aspect)**
- **No production of histamine**
- ***C. divergens* easy to produce fermentor**
- **High inibition of *Listeria monocytogenes***

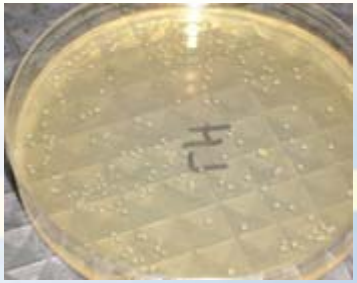
Carnobacterium divergens V41 good candidate for an application in CSS

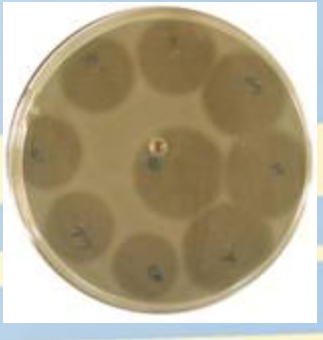
New protective cultures

Active against other pathogenic bacteria and/or spoiling micro-organisms

51 seafood products

stored at 8°C





- presence of LAB strains

- active at least against one out of four target strains

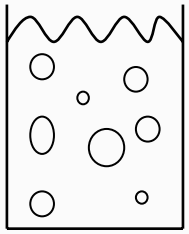
- able to grow at 15°C but not 30°C

- **52 strains were selected**
- **Inhibition spectrum enlarged against 14 target strains**
- **Clustered in 7 groups with hierarchical classification methods**

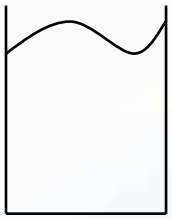
➔ **Selection of 7 representative strains**

Technological application

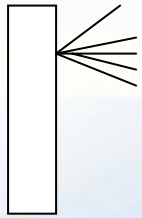
Frozen shrimps



2 minutes in boiling water + NaCl



Cooling at 0°C



10^{5-6} ufc/g

Inoculating with the 7 selected strains

Vacuum packing

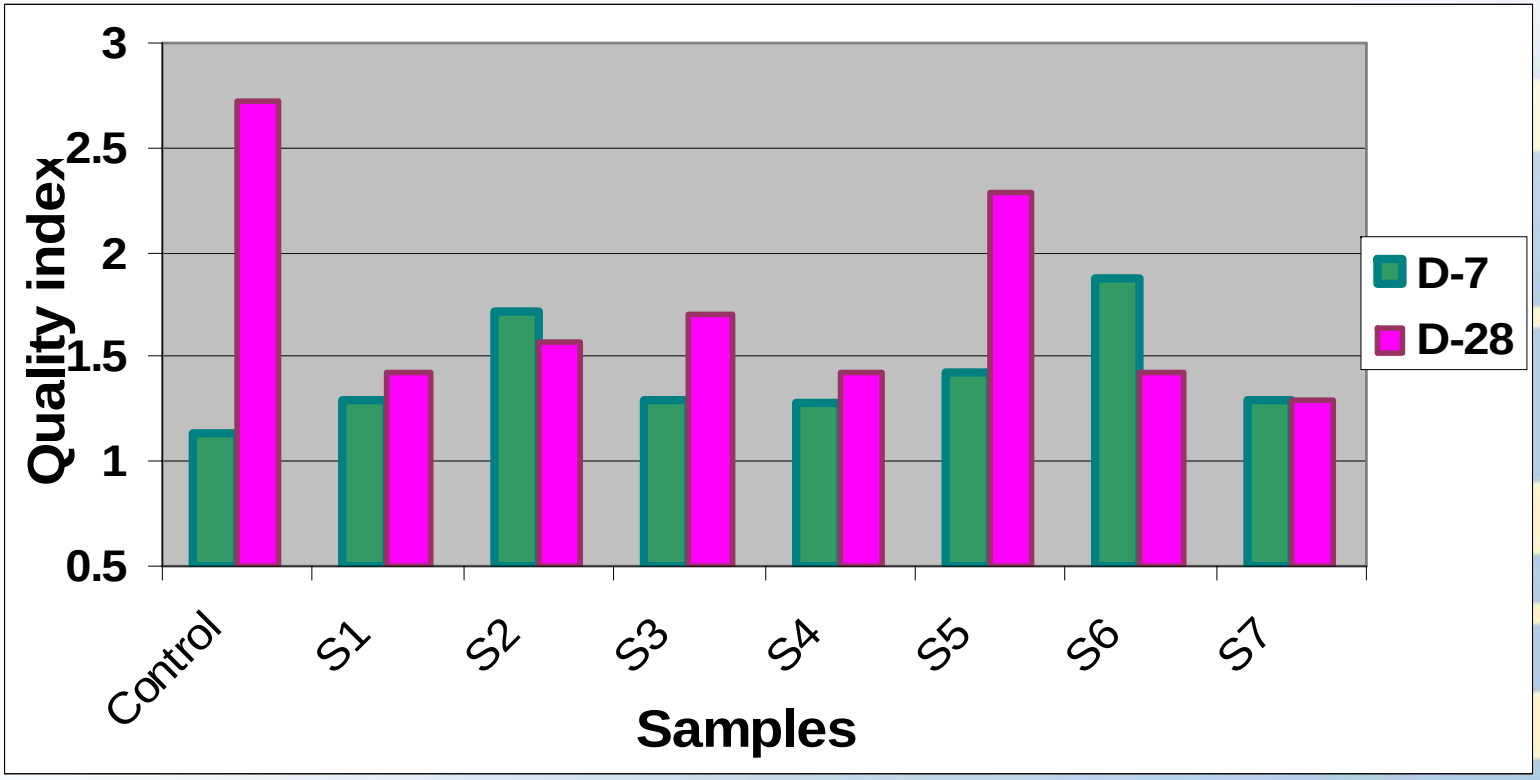
Storage at chilled temperature : 8°C for 28 d

Microbial analysis

- Good implantation of the PC
- No inhibitory effect on mesophilic total flora and enterobacteriaceae

Sensory analysis (7 trained panellists)

Quality Indice based on off-odours perception



Promising strains

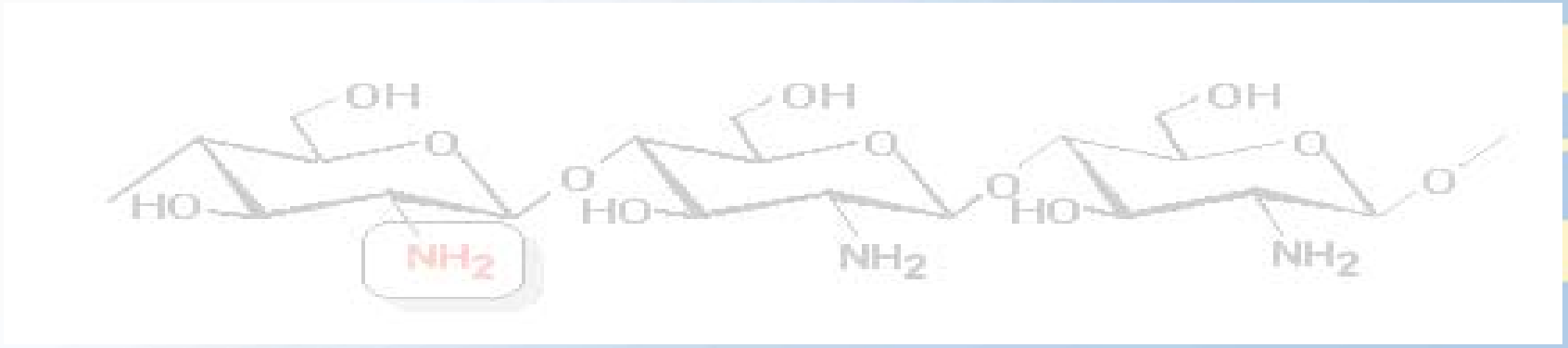
Strain	Analysis of 16S rDNA sequence
S1	*Leuconostoc gelidum/inhae*
S2	*Lactococcus piscium*
S3	*Lactococcus piscium*
S4	*Leuconostoc gelidum/inhae*
S5	*Lactobacillus fuchuensis/plantarum*
S6	*Carnobacterium alterfunditum*
S7	*Leuconostoc gelidum/inhae*

We still need to explain …

- Why PC strains increase sensory quality ?
- How it works …
- Does it work on pathogenic bacteria ?
- Does it work on other fish products ?

Chitosan

- **Chitosan is the principal derivative of chitin and is produced by its alkaline deacetylation**

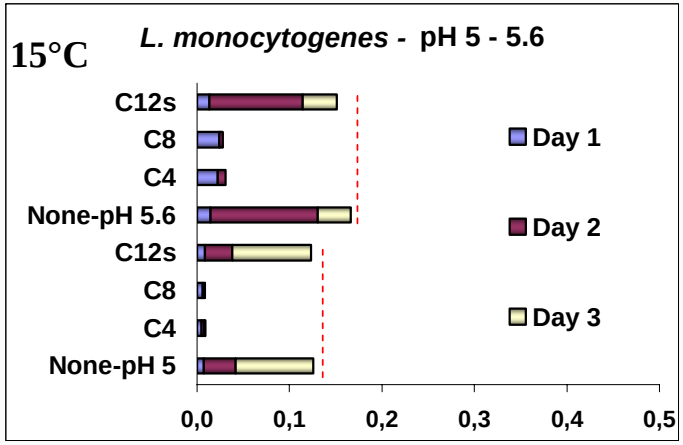


- **Chitosan has been proved to be non-toxic, biodegradable, biocompatible and it has been used in the food industry as safe and natural fat digestion and trapped lipid compound**
- **The antibacterial and antifungal activity of chitosan has been reported widely in the scientific literature (Roller, 2003)**

A novel food preservative material for minimal processed food ?

- 11 chitosan formulations
  - commercial chitosan with different degree of deacetylation and molecular weight
  - Different solvents
  - Different concentrations
- Tested against 14 target bacteria
- Selection of 2 chitosan formulations

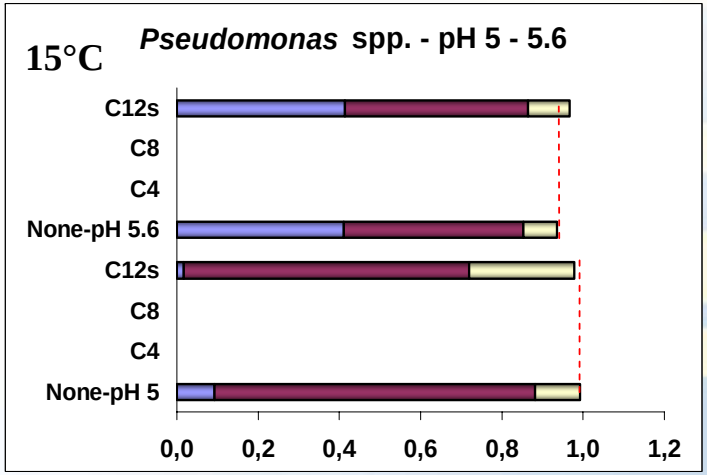
all pathogenic bacteria are inhibited



L. monocytogenes,
innocua
S. aureus
Bacillus subtilis

Different temperature and pH

all spoiling bacteria are inhibited



P. phosphoreum
S. putrefaciens
Pseudomonas
Psychrobacter
S. liquefaciens
L. sakei
B. thermosphacta

Effect on Listeria

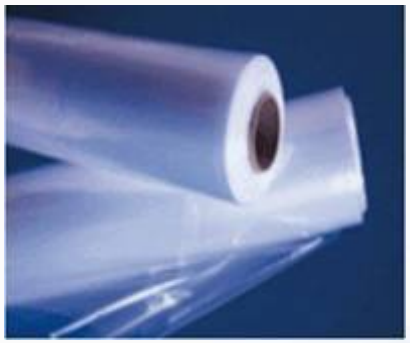
- **Lethal effect of chitosan**
- **Recovery depends on chitosan concentration**
  - **0.2% inhibit growth for 28 days**
- **Chitosan do not inhibit growth of *C. divergens* V41**

Work in progress

- **Validation *in vivo* (LPFPs) of the most effective formulations**
- **Modification of the chitosan formulations to improve their film forming properties**
- ***In vitro* validation of the modified formulations**

Future work

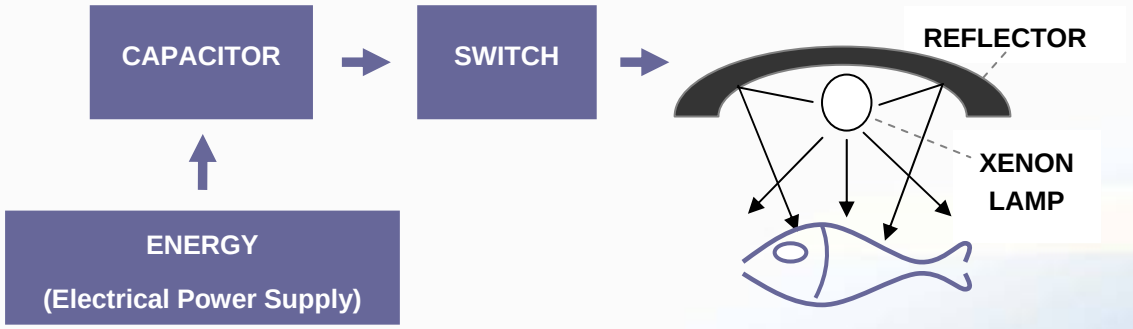
- **Production of commercial plastic/chitosan film with antimicrobial properties.**



  - **To find an optimal film formation methodology.**
  - **Analysis of the stability, antimicrobial activity and physical properties of the modified chitosan films.**
  - **Adhesion chitosan-commercial plastic films.**

Pulsed light

Pulsed light technology



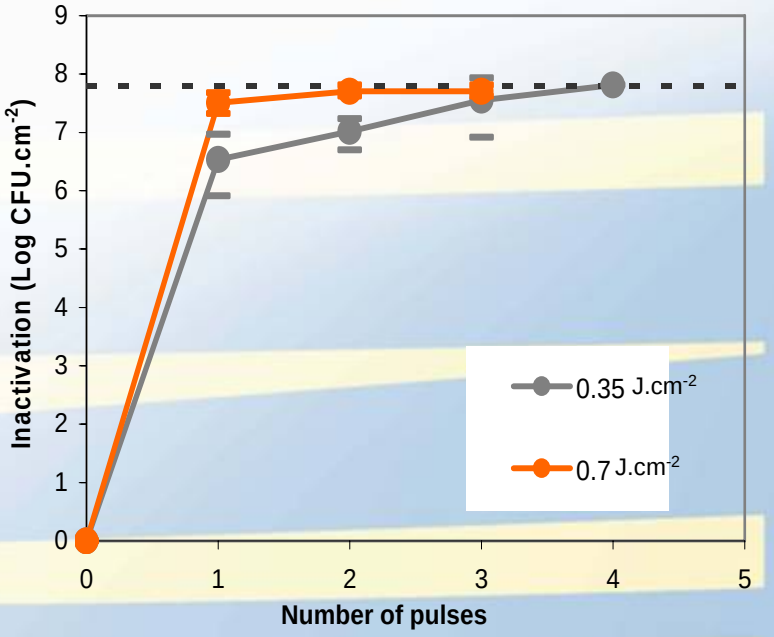
NON-THERMAL PROCESS

- **Broadband light emission (λ = 200-1000nm) Ultraviolet region: 40% of total light**
- **Pulse duration: 325 µs (125 µs to reach the pulse peak). 27 s between consecutive pulses**
- **Approved by the US FDA (21CFR179.41) for applications in the production, processing and handling of food products (12 J.cm^{-2})**

Results

Very efficient process inactivating microorganisms

- Significant inactivation (>7 Log CFU) of spoilage and pathogen strains isolated from LPFPs
- High efficacy at low doses (0.7 J.cm^{-2})
- Short treatment time (< 1s)
- Minimal heating (<3ºC): Non-thermal process



Critical factors of pulsed light process

- Pulse energy
- Distance from the lamp
- Number of pulses

→ **LIGHT DOSE**

Critical factors of product

– ***L. innocua*** **inactivation <u>does not</u> depend on**

Growth temperature

Process temperature

NaCl concentration (up to 5%)

Cell concentration

– ***L. innocua*** **inactivation <u>slightly depends</u> on**

Physiological state

– ***L. innocua*** **inactivation <u>strongly depends</u> on**

Storage temperature

Pulsed light sensibility

- ***L. innocua*** **is one of the most resistant strain among tested spoilage (7) and pathogen microorganisms (6)**
- ***L. innocua*** **could be considered as a surrogate for *L. monocytogenes* and as a reference microorganism for Pulsed Light Treatment optimization in LPFPs**

IMPACT OF PL TECHNOLOGY on LPFPs

- ***L. innocua*** **inactivation is less important *in vivo* than *in vitro***
- ***L. innocua*** **inactivation increases with light dose**
- ***L. innocua*** **inactivation does not depend on initial cell concentration**

➔ **PL technology could be used as a novel process to improve safety and increase shelf life of LPFPs**

Research continues ...

- **Suitability of this process to increase the shelf life of LPFPs**

- **Impact of PL technology on physico-chemical and sensorial characteristics of LPFPs**

Conclusion

- **Wide range of promising hurdle for decontamination and inhibition of growth in fish products**
- **Some of them still need validation in real products**
- **Some of them are at the « demonstration » stage (PL, biopreservation)**

Conclusion

- **Combination those hurdles**
  - **Pulse light and biopreservation**
  - **Pulsed light and bioactive packaging**
  - **Biopreservation and bioactive packaging**
  - **Compatibility/synergy with technological hurdles**

Acknowledgment

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