UNIVERSITY OF ALGARVE

Development of active packaging systems to improve and extend food products shelf-life

MARGARIDA VIEIRA

DEPARTMENT OF FOOD ENGINEERING, INSTITUTE OF ENGINEERING, UNIVERSITY OF ALGARVE, CAMPUS DA PENHA, 8005-139 FARO, PORTUGAL, MVIEIRA@UALG.PT

COST is supported by
The EU Framework Programme
Horizon 2020

INSTITUTO SUPERIOR DE ENGENHARIA/UNIVERSITY OF ALGARVE

Development of active packaging systems to improve and extend food products shelf-life

1-Development of films with Chitosan

2-Development of chitosan films with microcapsules

3-Development of films from plant origin

MARGARIDA VIEIRA, DEPARTMENT OF FOOD ENGINEERING, INSTITUTE OF ENGINEERING, UNIVERSITY OF ALGARVE, CAMPUS DA PENHA, 8005-139 FARO, PORTUGAL, MVIEIRA@UALG.PT

COST EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

COST is supported by
The EU Framework Programme
Horizon 2020

- 1-Development of packaging films with Chitosan
- Incorporation :
- -Grape Seed Extract a rich source of polyphenolic compounds, mainly monomeric catechin and epicatechin, gallic acid, and polymeric and oligomeric procyanidins
- -Carvacrol is a monoterpenoid phenol with hydroxyl group and exists in the essential oil of Thyme (*Thymus vulgaris*) and oil of Oregano (*Origanum vulgare*)
-
- -Film development with mixture optimization by response surface methodology for antimicrobial wide spectrum of action

COST is supported by
The EU Framework Programme
Horizon 2020

Materials and methods

Carvacrol

GSE

} [ ] Value selection MIC

Microorganisms (M.O.)	Carvacrol [ppm]	Grape seed extract [ppm]
Listeria innocua (10^6 CFU/ml)	**300**	**2000**
Staphylococcus aureus (10^6 CFU/ml)	225	500
Enterococcus faecalis (10^6 CFU/ml)	**300**	**2000**
Pseudomonas aeruginosa (10^4 CFU/ml)	1500	4000
Saccharomyces cerevisiae (10^6 CFU/ml)	150	>16000

Optimization of the combination of carvacrol, grape seed extract and chitosan for all microorganism

- **Carvacrol: 0 ppm – 300 ppm**
- **GSE: 0 ppm - 2000 ppm**
- **Chitosan: 0% w/v - 2% w/v**

} **Mixture design between carvacrol, GSE and chitosan (RSM)**

cost EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

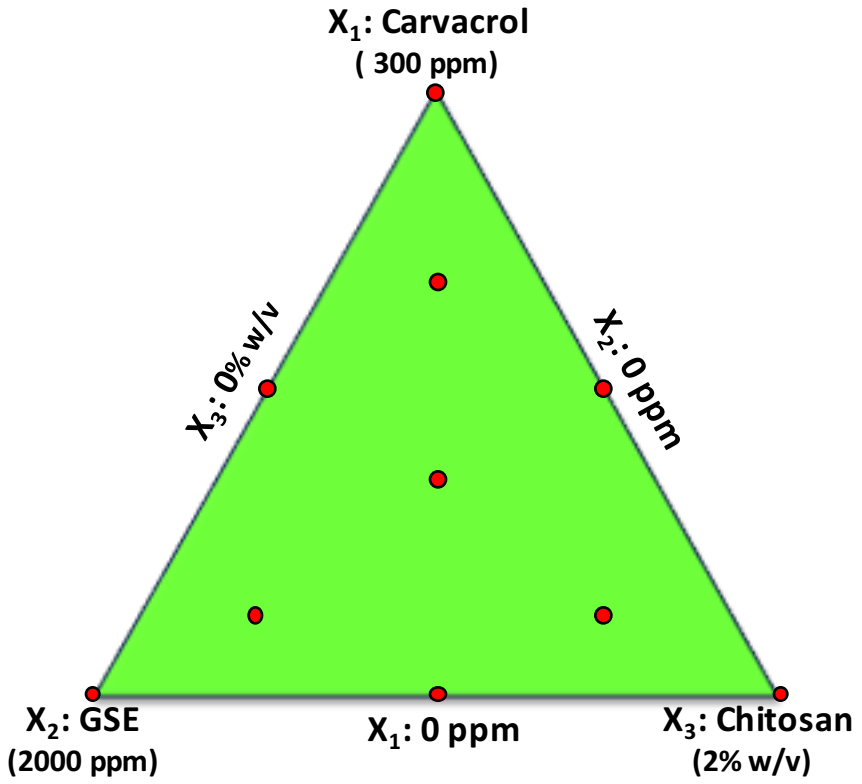
COST is supported by
The EU Framework Programme
Horizon 2020

Materials and methods

Optimization of the combination of carvacrol, grape seed extract and chitosan for all microorganisms

- Series of combinations between 3 natural agents for all microorganism

Carvacrol %	Grape Seed Extract %	Chitosan %
66.67	16.67	16.67
16.67	16.67	66.67
0	50	50
16.67	66.67	16.67
50	0	50
0	100 (2000 ppm)	0
0	0	100 (2% w/v)
100 (300 ppm)	0	0
33.33	33.33	33.33
50	50	0



Results

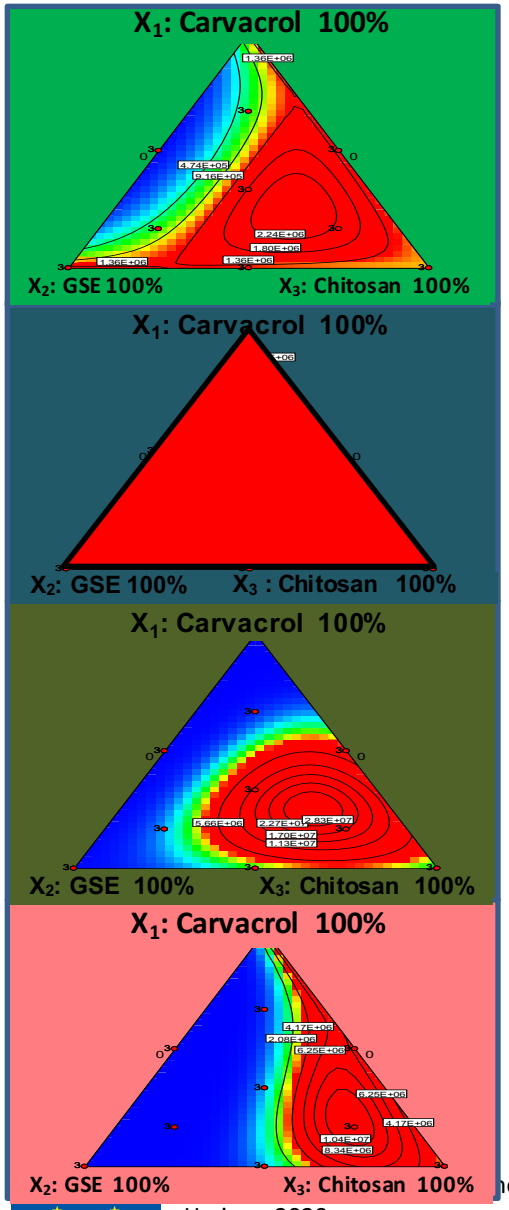
Optimization of the combination of Carvacrol, GSE and chitosan for all microorganisms

6 log cycles reduction

4 log cycles reduction

Microorganism

Listeria innocua
Staphylococcus aureus
Enterococcus faecalis
Pseudomonas aeruginosa
Saccharomyces cerevisiae



Results

MODEL

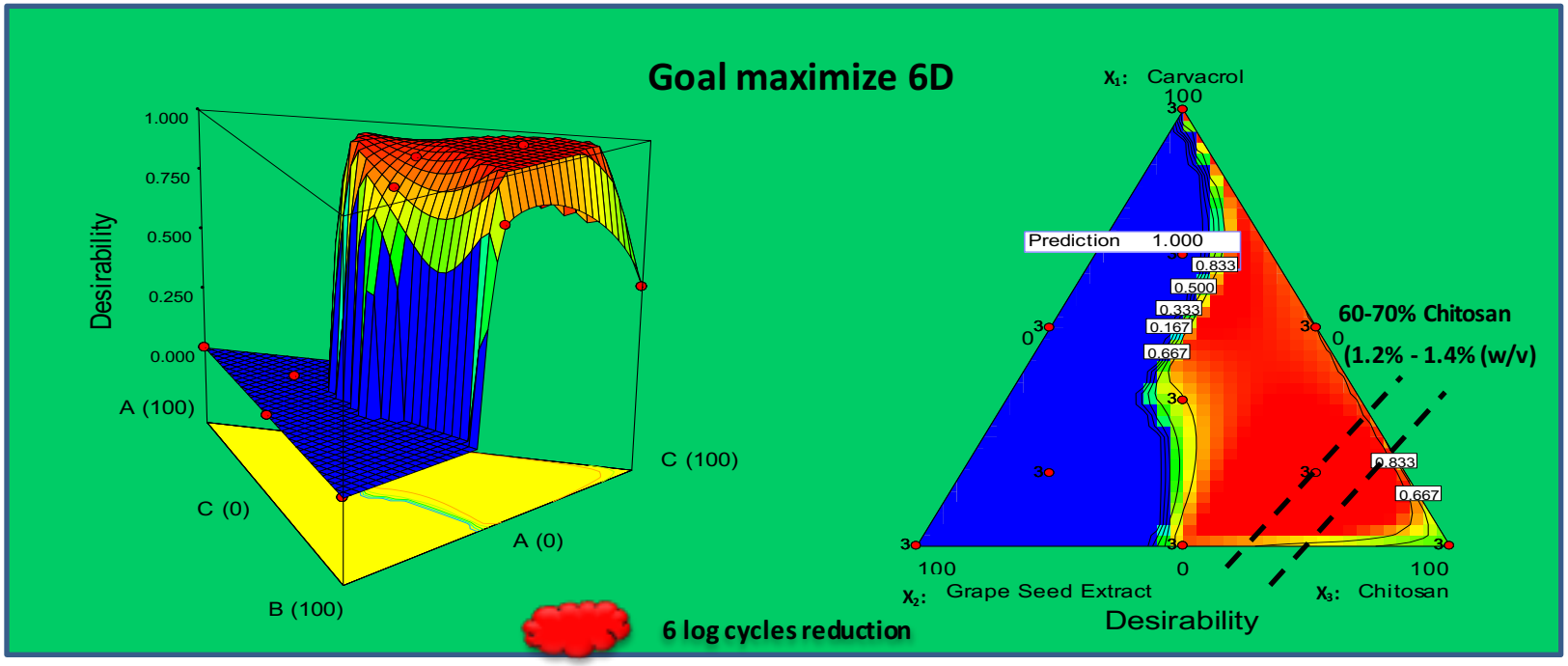
$$\text{Log}_{10}(\text{microorganism}) = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3$$

Coefficients	Microorganisms				
	L. innocua	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*S. cerevisiae*
β_1	+6.16	+6.48	+6.33	+1.55	+6.37
β_2	+6.15	+6.48	+6.33	+4.17	+0.40
β_3	+6.06	+6.48	+6.10	+6.26	+6.35
β_{12}	-6.83	+0.00	-4.48	+2.31	+11.98
β_{13}	-0.026	+0.00	-0.035	+10.34	-0.10
β_{23}	-0.029	+0.00	-0.035	+4.47	+11.94
β_{123}	+24.16	+0.00	+18.34	+33.39	-21.83

Microorganism	Source	R- Squared	R- Squared Adjust	Lack of Fit	F-Value	P- Value
L. innocua	Special Cubic	0.9106	0.8872	0.74	39.02	< 0.0001
E. faecalis	Special Cubic	0.9010	0.8751	0.30	34.87	< 0.0001
P. aeruginosa	Special Cubic	0.8598	0.8232	13.23	23.51	< 0.0001
S. cerevisiae	Special Cubic	0.9952	0.9939	0.44	791.12	< 0.0001

Results

Optimization of the combination of carvacrol, grape seed extract and chitosan for all microorganisms



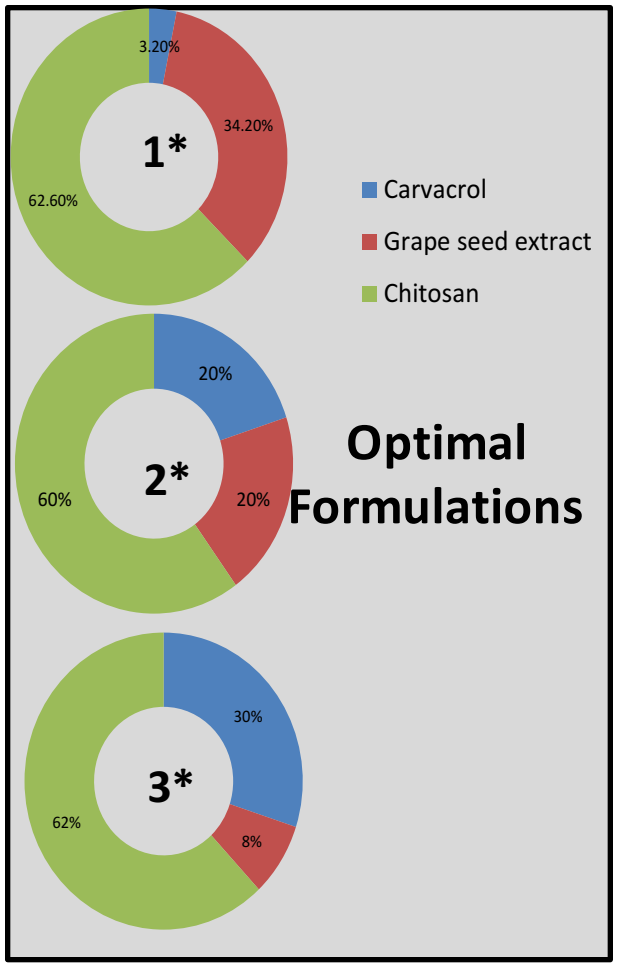
Results

Optimal	AM Agents	Formulation
1*	Carvacrol	3.2% (9.6 ppm)
	GSE	34.2% (684 ppm)
	Chitosan	62.6% (1.25% w/v)

Optimal	AM Agents	Formulation
2*	Carvacrol	20% (60 ppm)
	GSE	20% (400 ppm)
	Chitosan	60% (1.2% w/v)

Optimal	AM Agents	Formulation
3*	Carvacrol	30% (90 ppm)
	GSE	8% (160 ppm)
	Chitosan	62% (1.24% w/v)

Experimental validation



COST is supported by
The EU Framework Programme
Horizon 2020

Conclusions

1. Response Surfaces → Showed → Synergistic effect → Between → Carvacrol, GSE, Chitosan → For all microorganisms

2. Experimental Validation → Confirmed → Optimal solution selected by the polynomials models → Can predict with success → The inhibition for all microorganisms

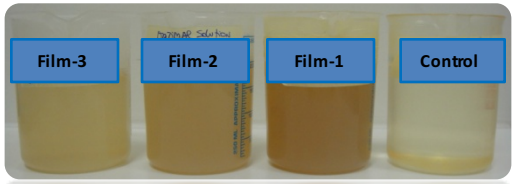
The developed formulations with Carvacrol, GSE and Chitosan, allow the reduction of at least 6 log cycles of all the studied microorganisms

cost EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

COST is supported by
The EU Framework Programme
Horizon 2020

- -Physico-mechanical properties of chitosan films with carvacrol and GSE

Materials and methods



Solvent casting technique

48 h 25 ºC

Physical properties

- Thickness
- Water solubility
- Moisture content
- Colour
- Fourier-transform infrared (FTIR) spectroscopy
- Scanning electron microscopy (SEM)
- X-ray

Barrier properties

- Water vapour permeability (*WVP*)
- Carbon dioxide permeabilities (CO_2P)
- Oxygen permeabilities (O_2P)

Mechanical properties

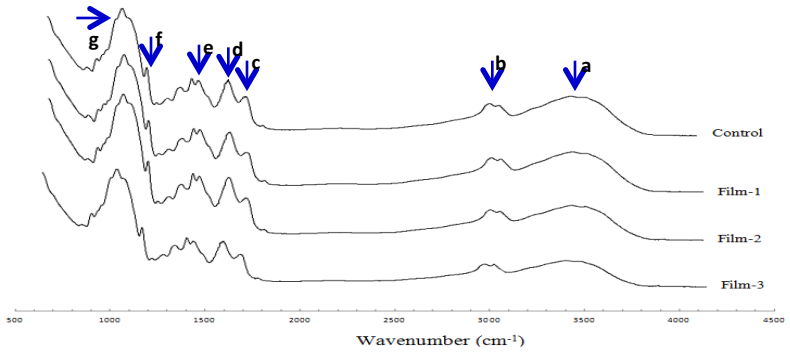
- Tensile strength (*TS*)
- Elongation-at-break (*EB*)

cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

COST is supported by
The EU Framework Programme
Horizon 2020

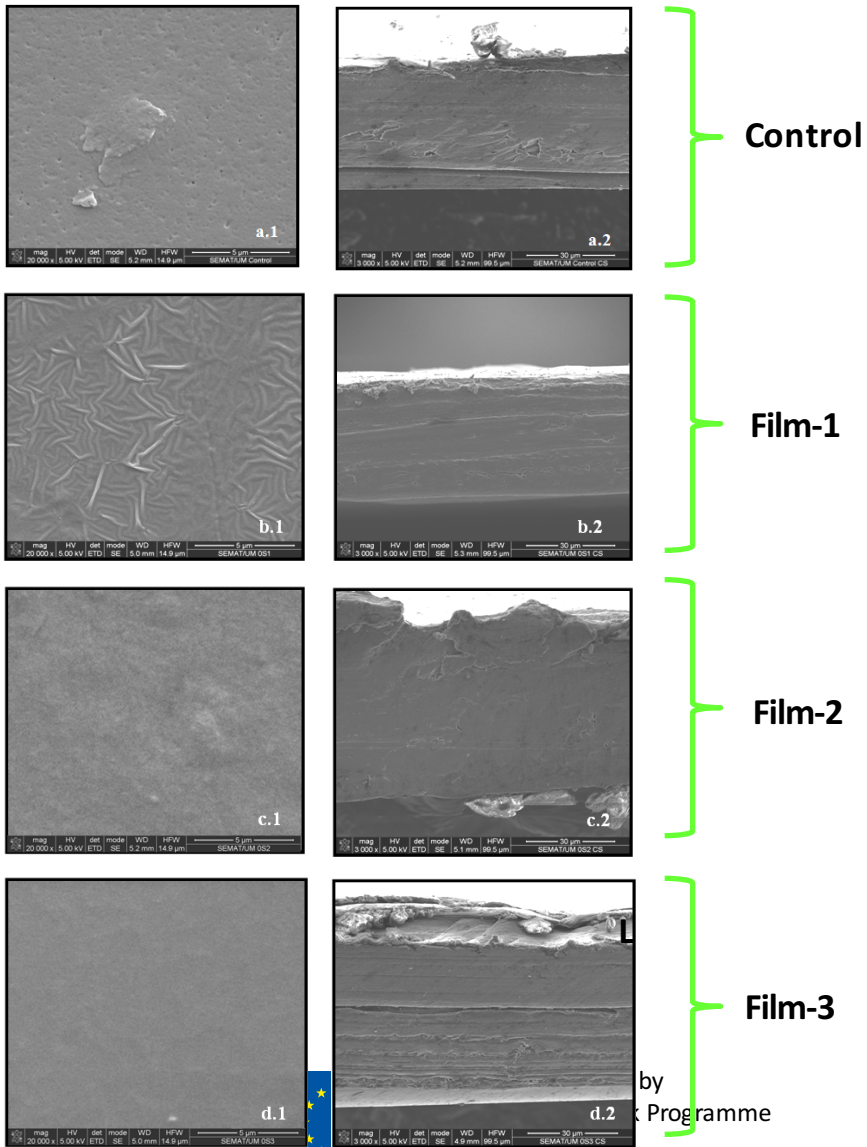
Results

F-TIR



(a) 3500 cm^{-1} : O-H stretching
(b) 3000 cm^{-1} : C-H stretching
(c) 1740 cm^{-1} : Presence of a carbonyl group in the film.
(d) 1655 cm^{-1} : Amide I
(e) 1560 cm^{-1} : Amide II vibrational mode
(f) 1160 cm^{-1} : Bridge oxygen stretching
(g) 1070 cm^{-1} :C-O stretching

SEM



Surface film

control	Smooth and continuous structure	
Film-1	Higher [ ] GSE (Hydrophilic)	→ Rugous surface
Film-2	Medium [ ] GSE and carvacrol	→ Less Rugous surface than Film-1
Film-3	Higher [ ] carvacrol (Hydrophobic)	→ Smooth surface

Cross-section film

All cross sections showed GSE and carvacrol (in different concentrations) uniformly incorporatedin the chitosan matrix.

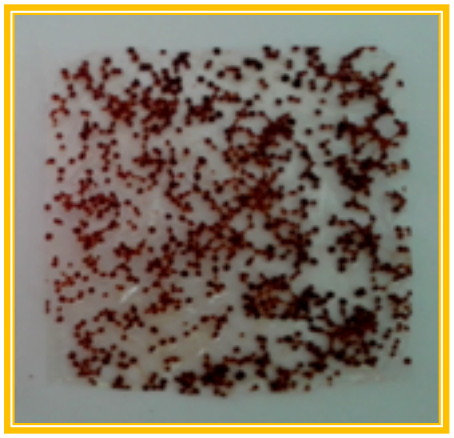
- 2-Development of chitosan packaging films with microcapsules and study its effect on refrigerated salmon shelf-life surface

COST is supported by
The EU Framework Programme
Horizon 2020

Materials and methods

Chitosan film with carvacrol and grape seed extract microcapsules:

- Chitosan (1.25%) films were prepared in acetic acid solution (1%) and the microcapsules (62,6% Chitosan, 3.2% carvacrol and 34.2% GSE) by ion gelation with sodium trypolyphosphate (0.8%)



Salmon samples

- **Control**
- **Chitosan film**
- **Chitosan film with microcapsules**

Materials and methods

Study conditions

Storage temperature → **5 ºC**

Time → **14 days**

Materials and methods

Parameters

Physico-chemical

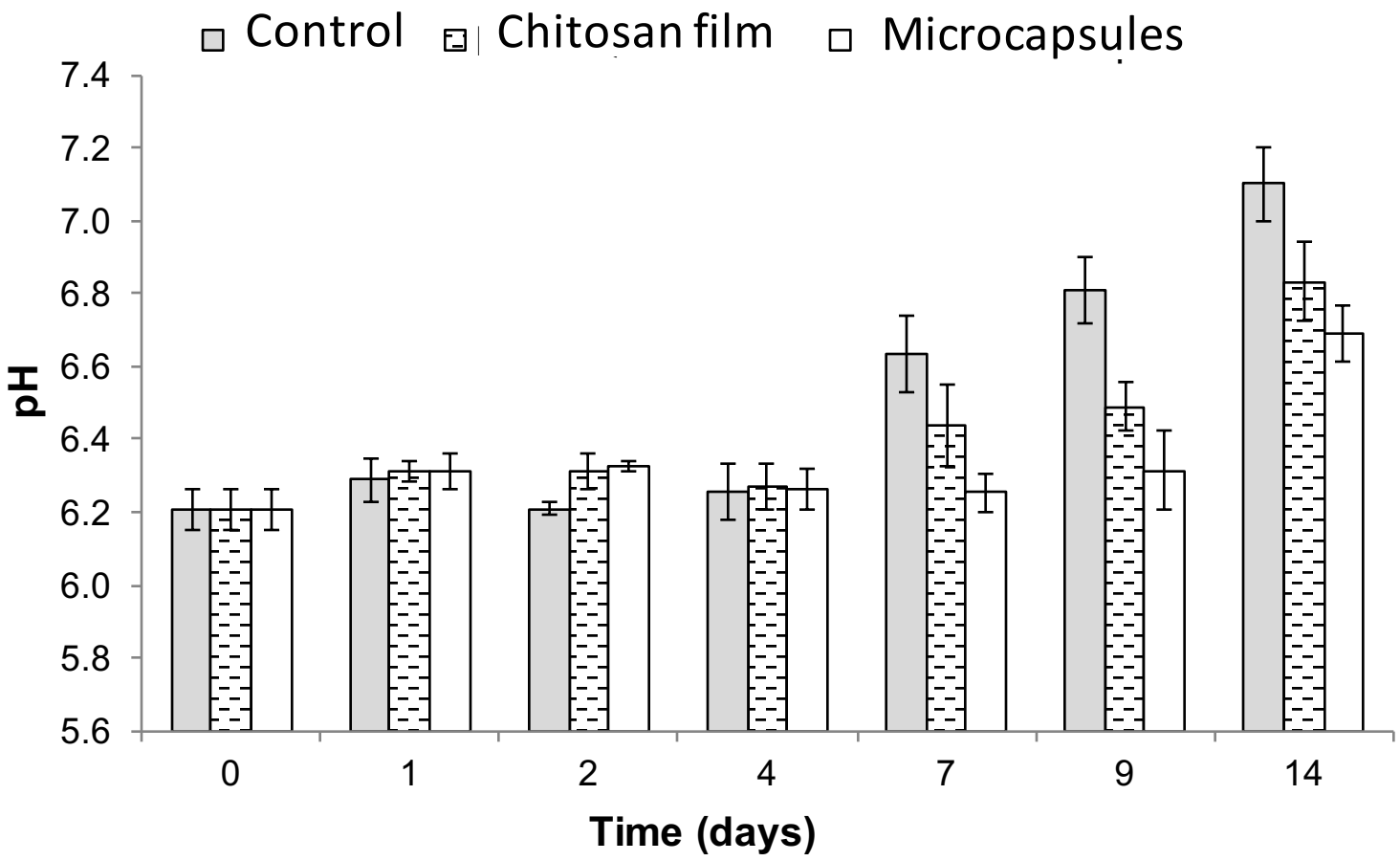
- ✓ **pH**
- ✓ **Total volatile basic nitrogen (TVB-N):** norma NP 2930 (2009).

Microbiological

- ✓ **Total viable count:** 37 ºC, 48 h, norma NP 4405 (2002).
- ✓ **Psychrotrophic:** 6,5 ºC, 10 days , norma ISO 17410 (2001).
- ✓ ***Pseudomonas spp.*:** 44 ºC, 48 h.

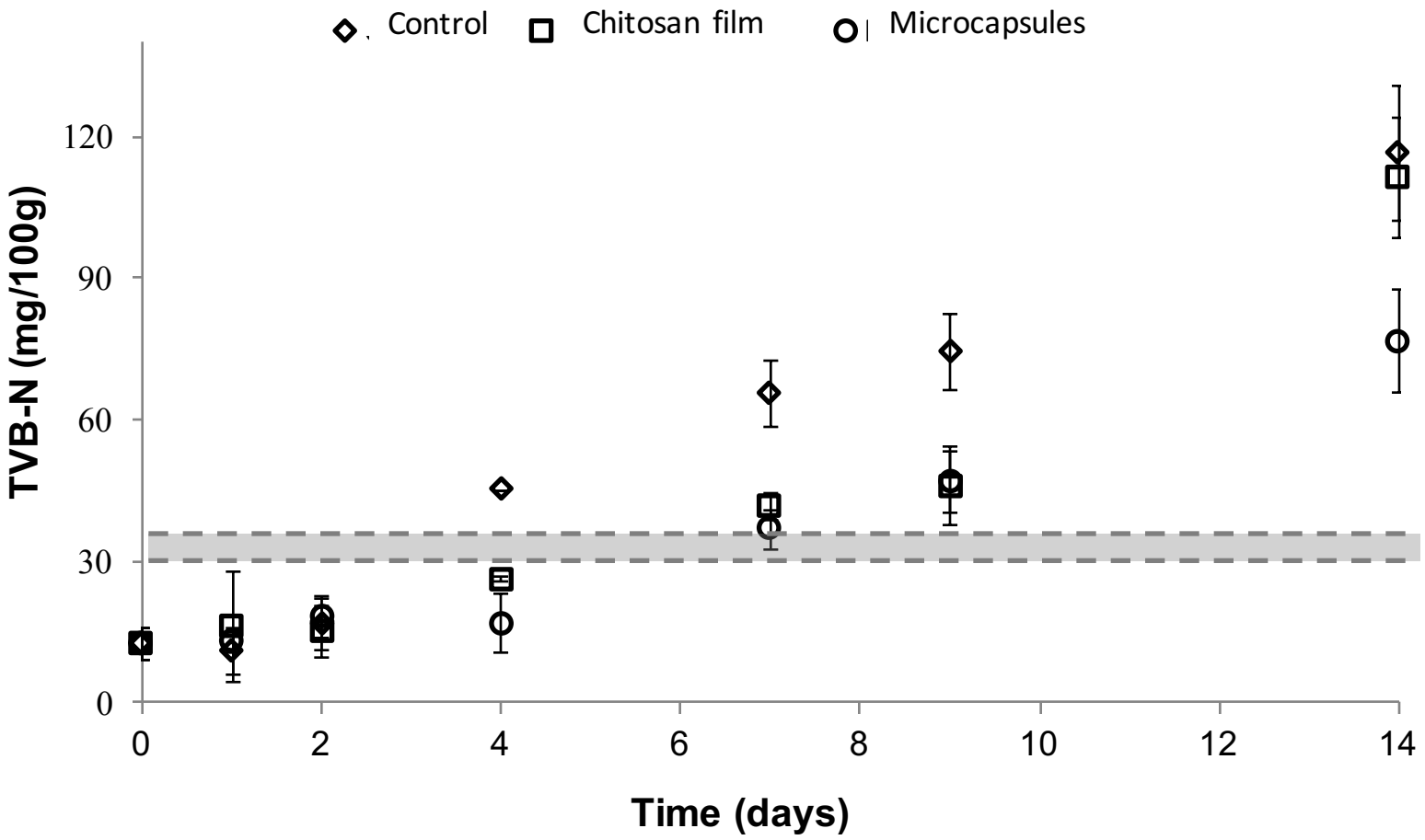
Results

pH


Control
Chitosan film
Microcapsules
pH
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
0
1
2
4
7
9
14
Time (days)

Results

Total volatile basic nitrogen (TVB-N)



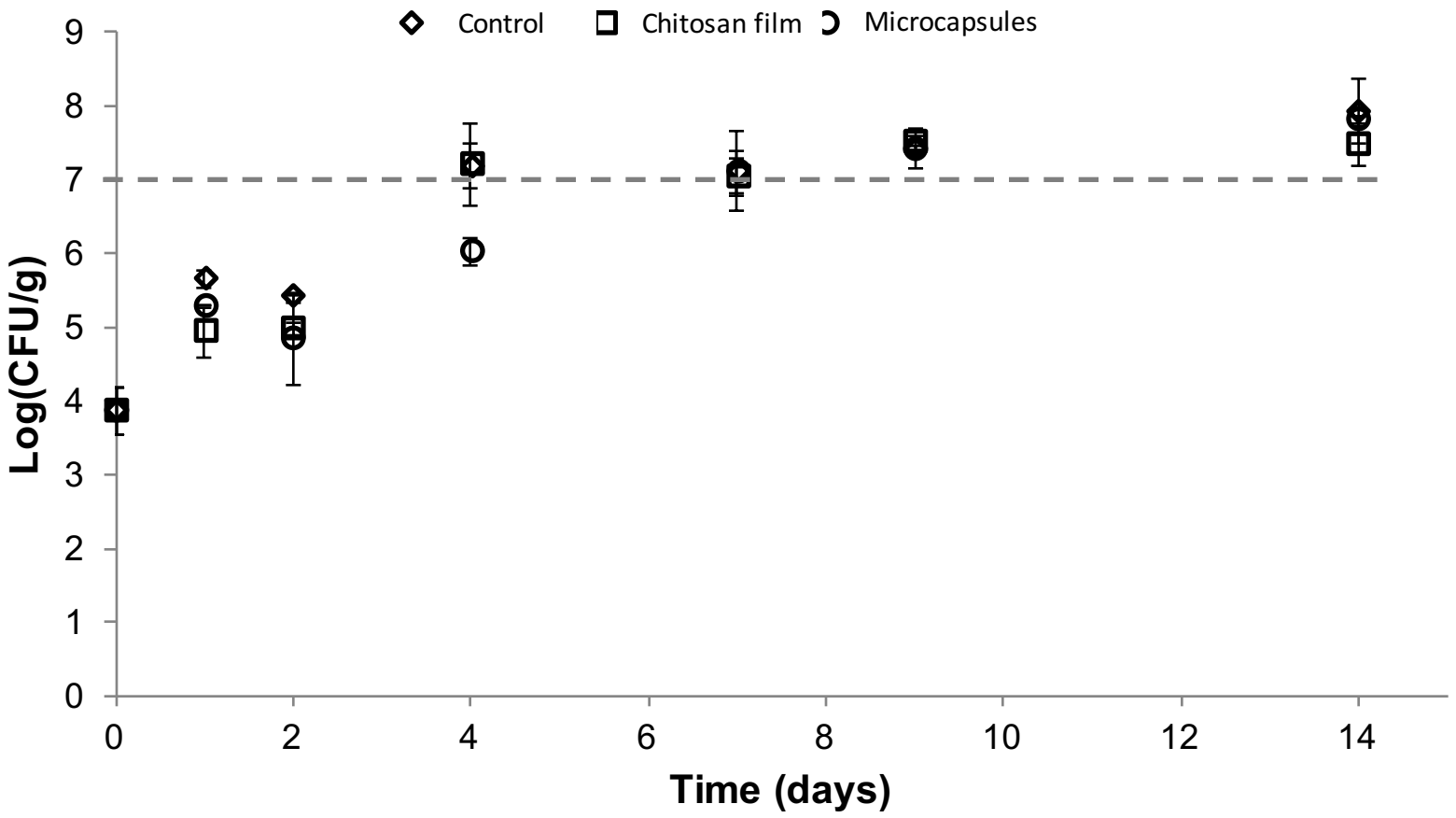
Fish rejection limit 30 to 35 mg (CE) N.º 2074/2005)

cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

COST is supported by
The EU Framework Programme
Horizon 2020

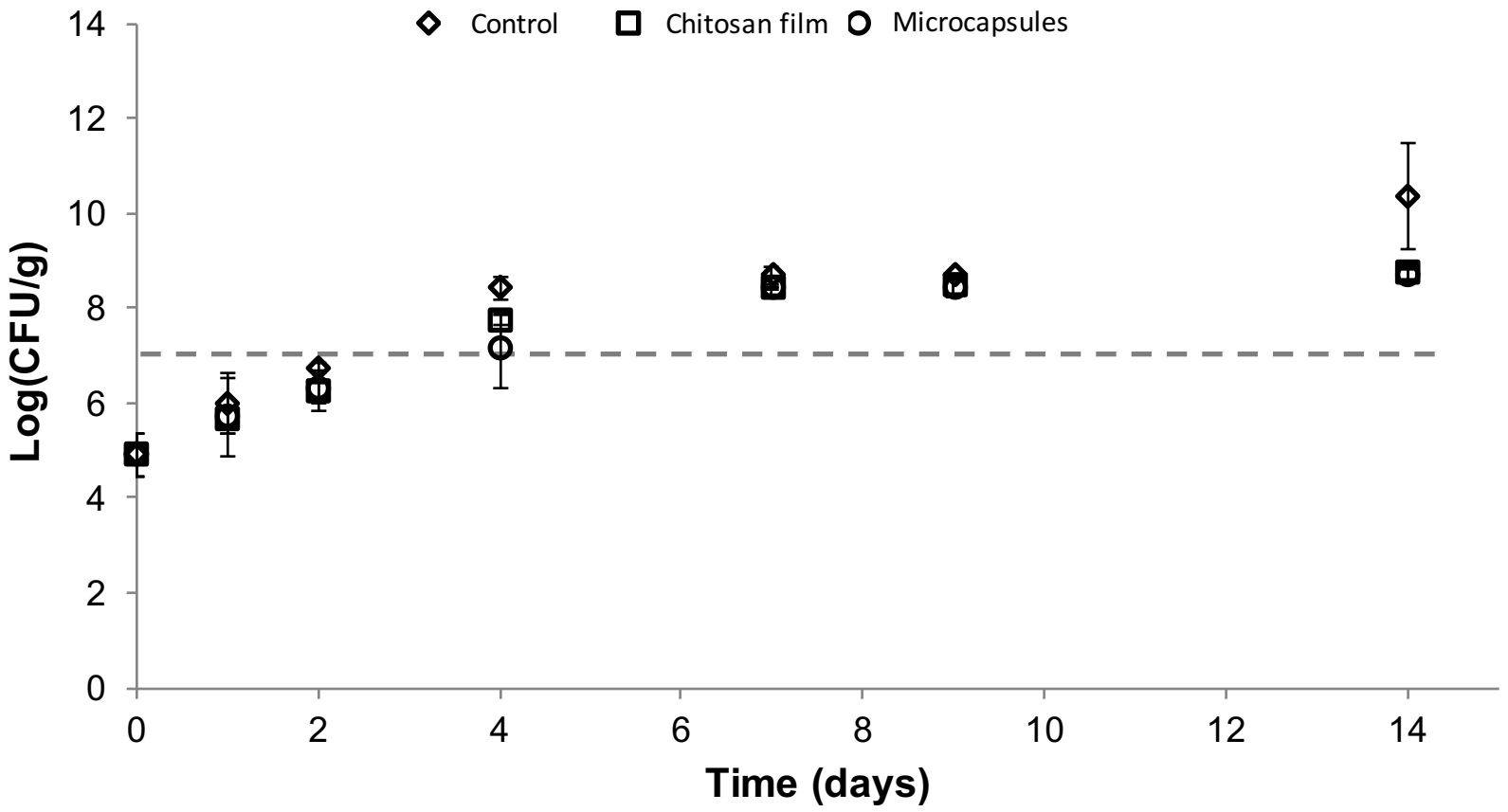
Results

Total viable count



Maximum limit allowed for consumption 7,0 Log(CFU/g)

Psychrotrophic

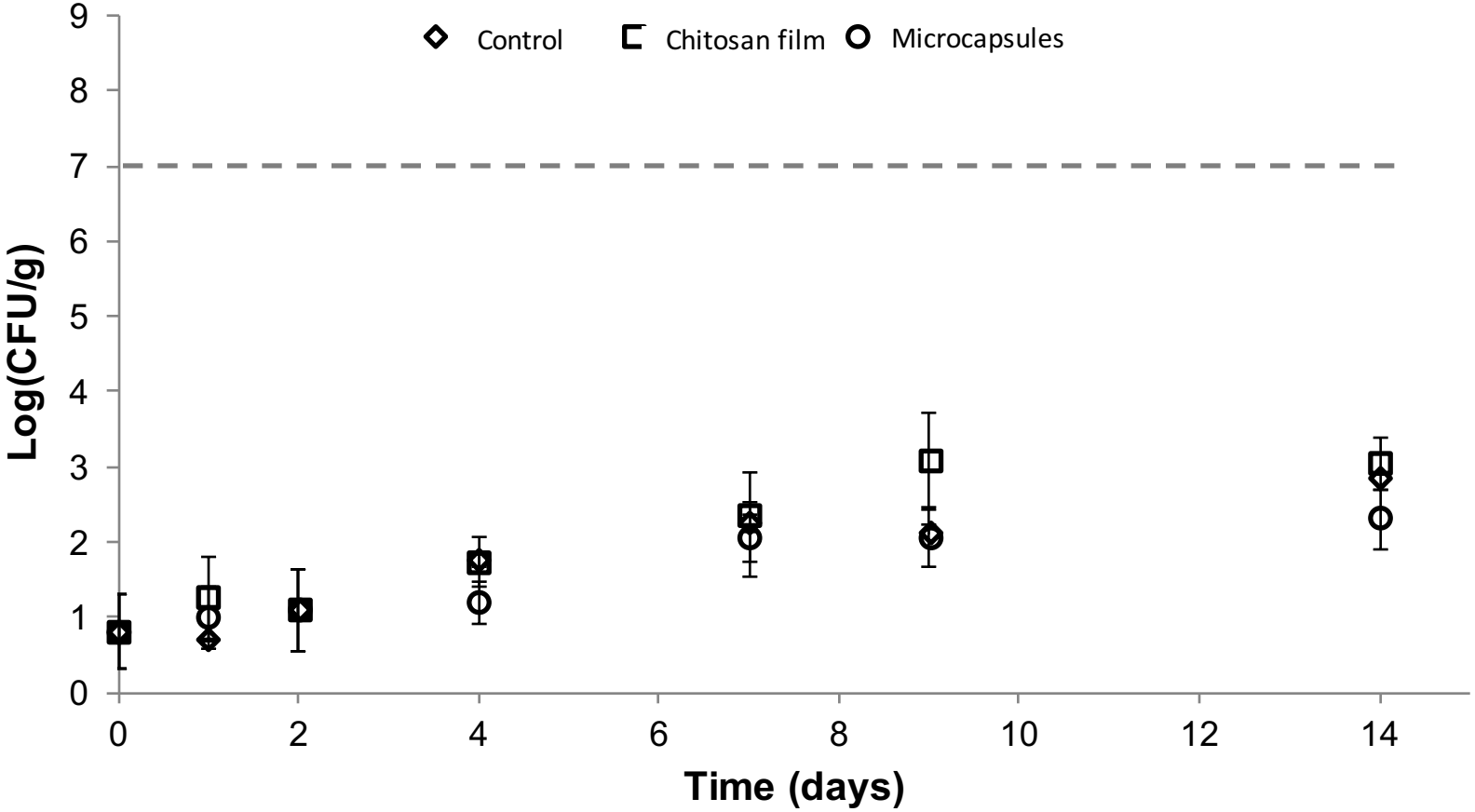


Maximum limit allowed for consumption 7,0 Log(CFU/g)

COST is supported by
The EU Framework Programme
Horizon 2020

Results

Pseudomonas spp.



Maximum limit allowed for consumption 7,0 Log(CFU/g)

cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

COST is supported by
The EU Framework Programme
Horizon 2020

Conclusions

- The film with microcapsules allowed to extend the salmon shelf-life from 2 days (control) to 7 days .

- The antimicrobial properties of the films allowed reducing growth of microorganisms that perform the oxidative deamination of non-protein nitrogen compounds, retaining the fish freshness.

- This work will contribute to the understanding of the impact of AP films on refrigerated salmon, in order to improve its preservation and obtain shelf-life extension.

cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

COST is supported by
The EU Framework Programme
Horizon 2020

3-Development of packaging films from plant origin

-Film optimization by response surface methodology based in physico-chemical parameters

-Improve heat sealing properties

-Biodegradation test

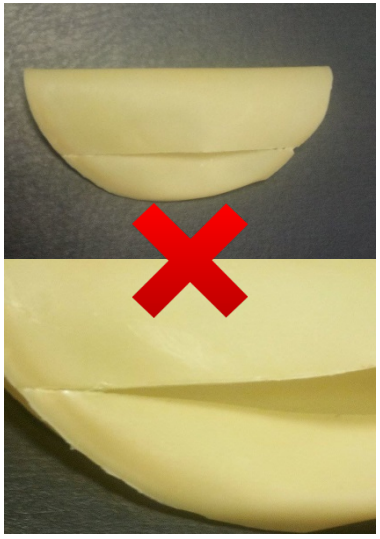
-Film antioxidant and antimicrobial properties characterization

-Study the effect of the developed films in food products' shelf-life

COST is supported by
The EU Framework Programme
Horizon 2020

Preliminary results

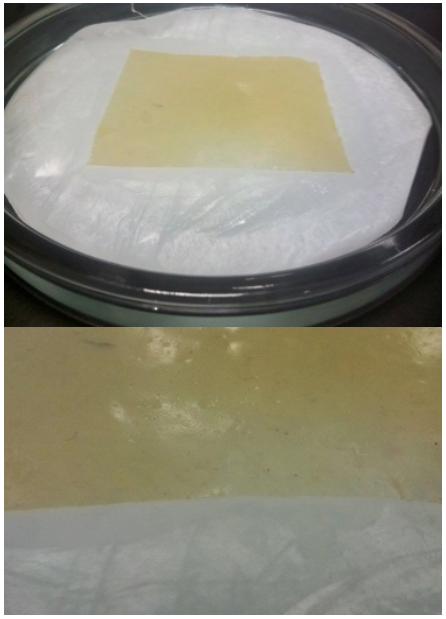
Film from plant origin



Blend with Polyhydroxybutyrate (PHB)

Preliminary results

Heat sealing properties



Bilayer Film from plant origin and PHB

COST is supported by
The EU Framework Programme
Horizon 2020

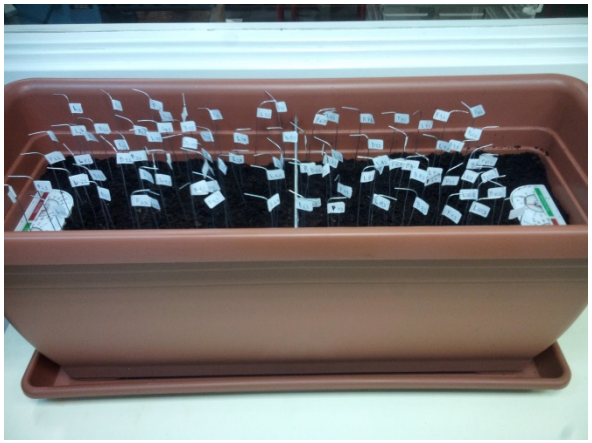
Materials and methods

Films

- ✓ Size 2x3cm

Soil

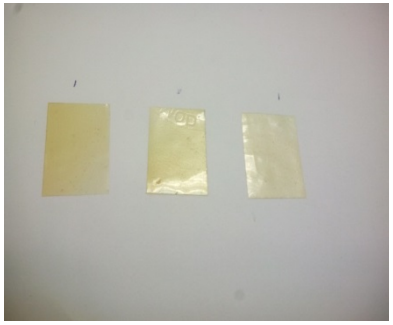
- ✓ pH=6,5
- ✓ Moisture 50%
- ✓ Buried at 11 cm depth
- ✓ Room temperature 20 °C



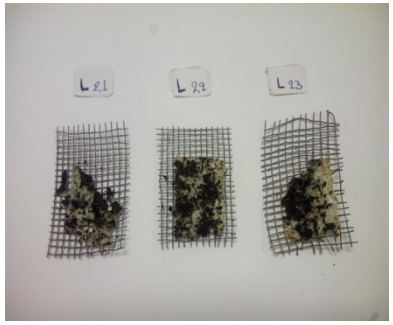
Results

Biodegradation test

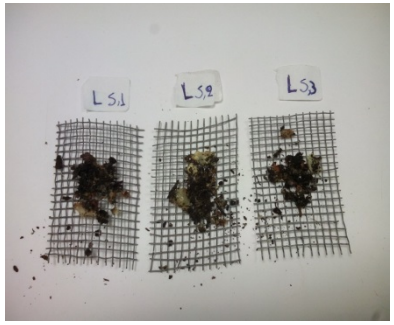
Day 0



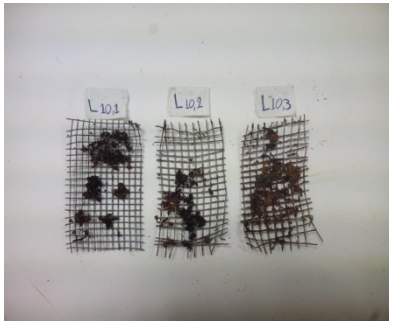
Day 3



Day11



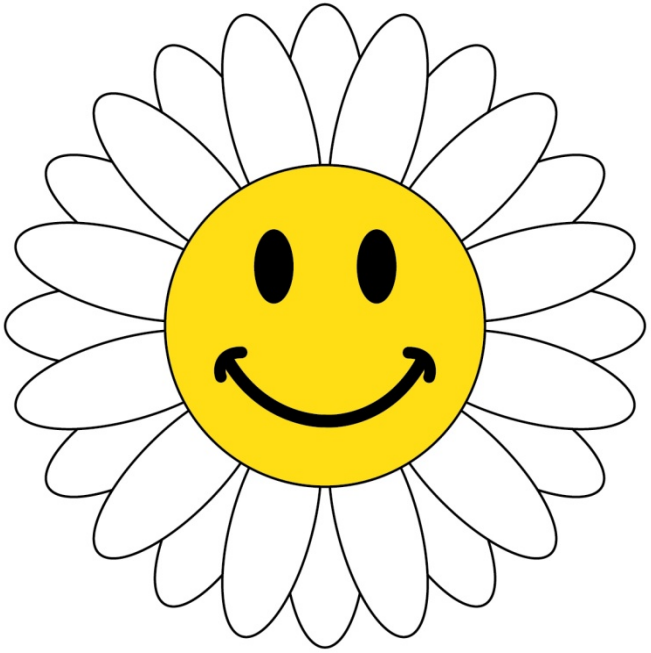
Day 42



EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

COST is supported by
The EU Framework Programme
Horizon 2020

Thank you for your attention!!!



cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

COST is supported by
The EU Framework Programme
Horizon 2020