

INCORPORATION OF NATURAL COMPOUNDS INTO POLYMERIC MATRIX: DEVELOPMENT OF ANTIMICROBIAL FILMS

ANTONIA NOSTRO



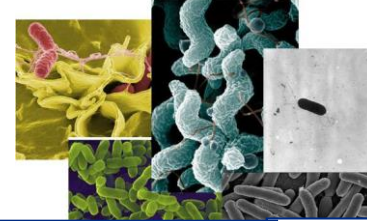
**DIPARTIMENTO DI SCIENZE CHIMICHE, BIOLOGICHE, FARMACEUTICHE ED AMBIENTALI
UNIVERSITÀ DEGLI STUDI DI MESSINA**



UNIVERSITÀ DEGLI STUDI DI MESSINA
Tradizione e cambiamento al centro del Mediterraneo

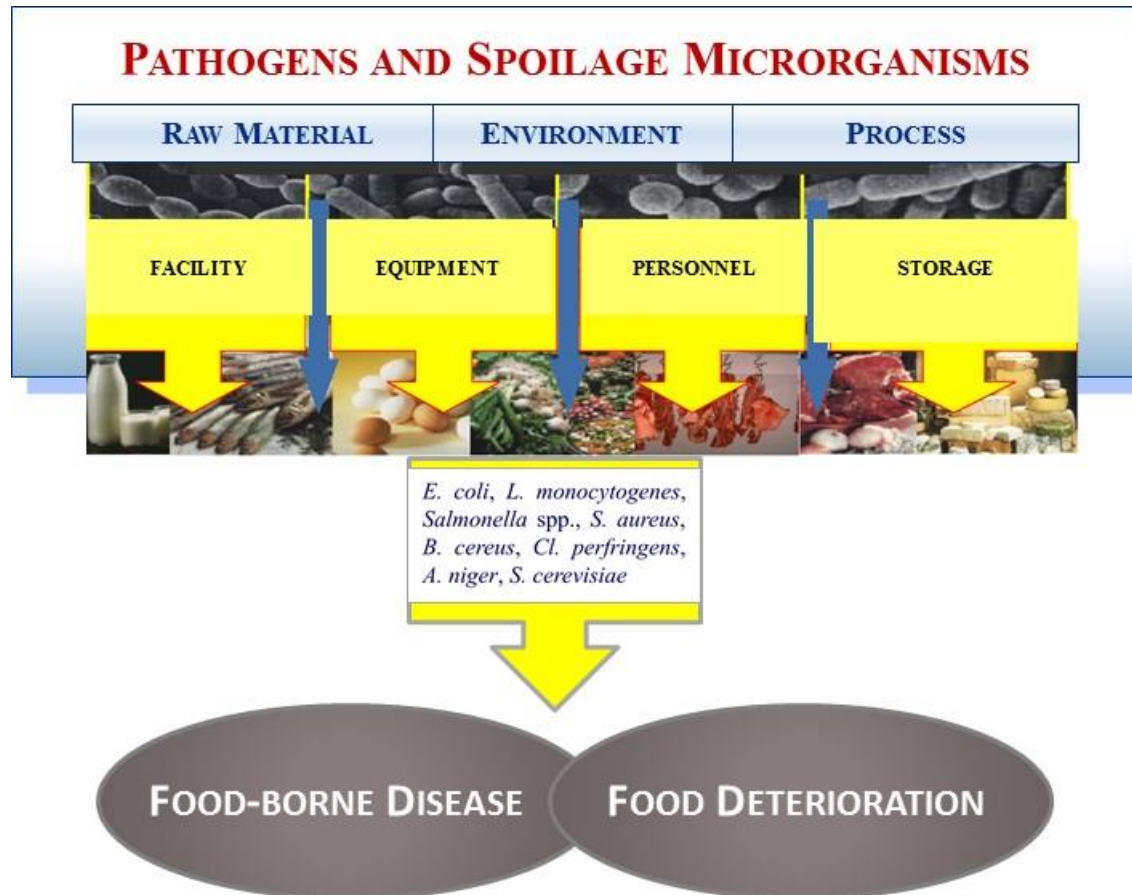


FOOD MICROBIAL CONTAMINATION

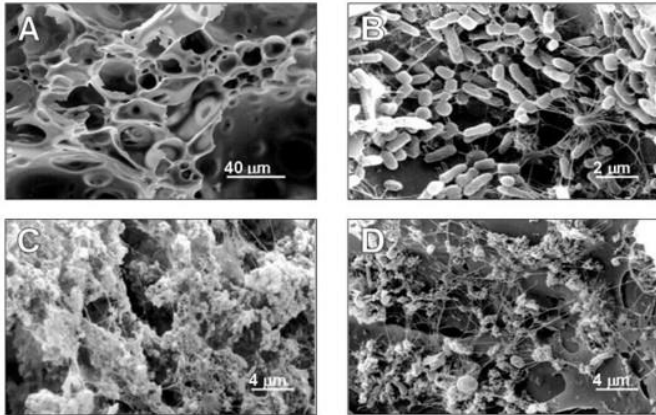


BACTERIA

YEASTS AND MOULDS



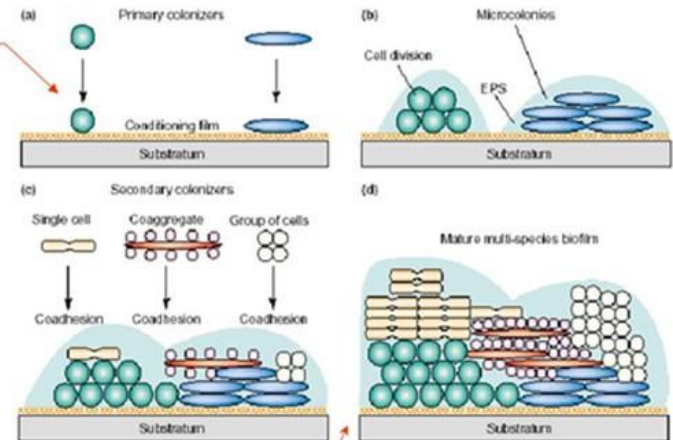
MICROBIAL BIOFILM



A BIOFILM CAN BE VERY DIFFICULT TO ERADICATE BECAUSE POOR SENSIBILITY TO CONVENTIONAL ANTIMICROBIAL AGENTS

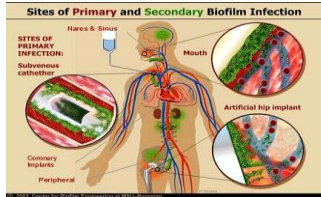
INITIAL ADHESION

- van der Waals interactions
- Electrostatic forces
- Lewis acid-base
- Hydrophobic interactions



MATURE BIOFILM

- Bacterial proliferation
- Exopolysaccharide matrix production
- Intercellular adhesion



ESSENTIAL OILS AND FOOD CONTROL



INCREASE IN CONSUMER DEMAND FOR MINIMALLY PROCESSED FOODS WITH REDUCED CHEMICAL ADDITIVES



PLANT ESSENTIAL OILS WITH A BROAD SPECTRUM OF ANTIMICROBIAL ACTIVITY

EFFICACY AGAINST PATHOGENS AND FOOD-BORNE MICROORGANISMS ALSO IN **FOOD MATRIX**

THEIR DIRECT ADDITION STILL ENCOUNTERS LIMITATIONS

- EVAPORATION
- HYDROPHOBICITY
- SENSORIAL CHARACTERISTICS

DUE TO



NEW ANTIMICROBIAL SYSTEMS

INCORPORATION OF ESSENTIAL OILS INTO POLYMERIC MATERIALS



ACTIVE PACKAGING



ACTIVE PACKAGING
PROLONGED *SHELF LIFE*



SYNTHETIC POLYMER

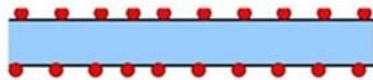
LOW DENSITY POLYETHYLENE (**LDPE**)
 HIGH DENSITY POLYETHYLENE (**HDPE**)
 POLYSTYRENE (**PS**)
 POLYPROPYLENE (**PP**)
 ETHYLENE VINYL ACETATE (**EVA**)



NATURAL POLYMER

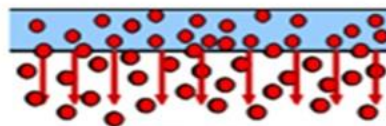
PROTEINS, STARCH, ALGINATE,
 CELLULOSE, CHITOSAN

COATING



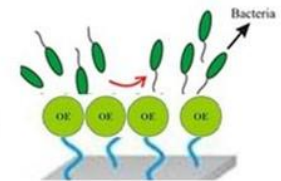
ACTIVITY BY CONTACT

INCORPORATION

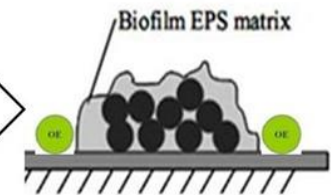


ACTIVITY BY RELEASE

CONTAMINATION CONTROL



BIOFILM CONTROL



AIMS

Study on carvacrol and cinnamaldehyde polymeric films: mechanical properties, release kinetics and antibacterial and antibiofilm activities

A. Nostro • R. Scaffaro • M. D'Arrigo • L. Botta •
A. Filocamo • A. Marino • G. Bisignano



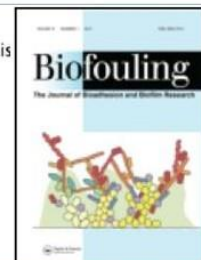
*Applied
and
Microbiology
Biotechnology*

Biofouling, 2015

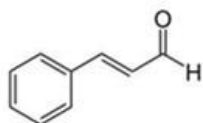
Vol. 31, No. 8, 639–649, <http://dx.doi.org/10.1080/08927014.2015.1079703>

Effect of temperature on the release of carvacrol and cinnamaldehyde incorporated into polymeric systems to control growth and biofilms of *Escherichia coli* and *Staphylococcus aureus*

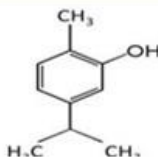
A. Nostro^{a*}, R. Scaffaro^b, L. Botta^b, A. Filocamo^a, A. Marino^a and G. Bisignano^a



ANTIMICROBIAL AGENT

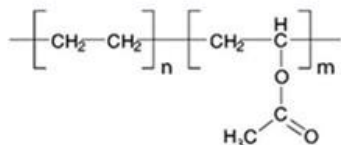


Cinnamaldehyde

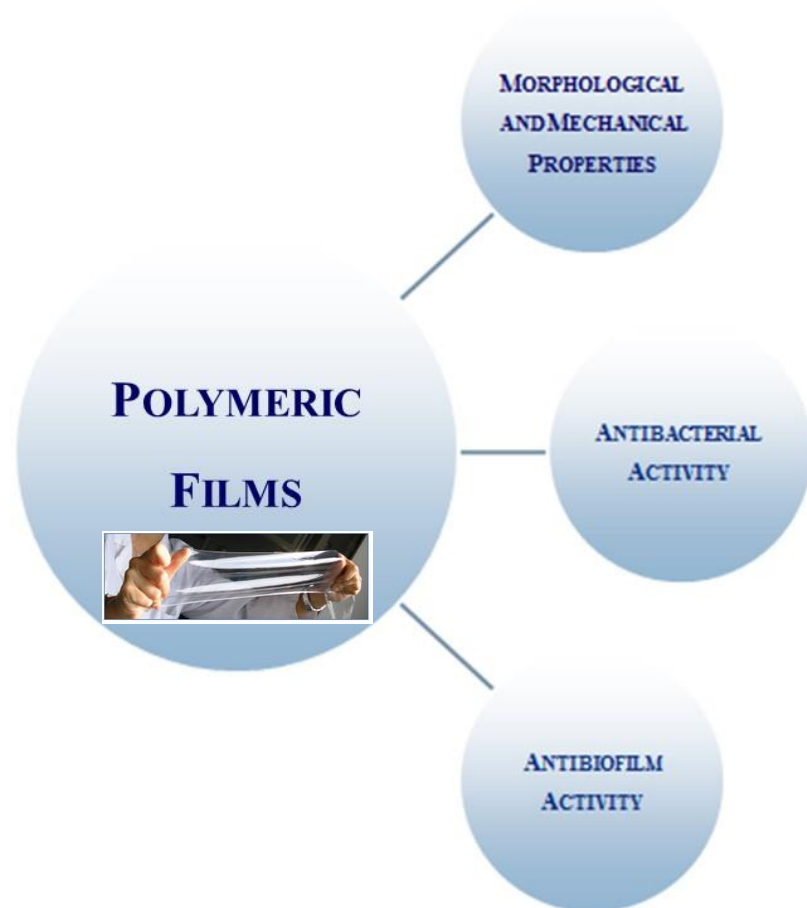


Carvacrol

POLYMERIC MATERIAL



Ethylene-vinyl-acetate (EVA14)



PREPARATION OF ANTIMICROBIAL POLYMERIC FILM

POLYMERIC MATERIAL



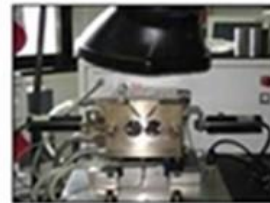
EVA14

EVA containing 14% of vinyl acetate
Greenflex FC 45
Melt flow index 0.3 dg/min,
Melting T 93°C

ANTIMICROBIAL AGENT

CARVACROL
OR
CINNAMALDEHYDE
3.5 wt% -7wt%

MIXER IN A SINGLE-STEP PROCESS



BATCH MIXER

T 120 °C
Rotational speed 64 rpm
Time ~ 1 min
Immersed in liquid nitrogen to reduce the evaporation

COMPRESSION



T 120 °C
100 bar
Time ~ 2 min
Thickness 200 µm



Storage at 0 °C in vacuum packed plastic bags until their final use

POLYMERIC FILM

SURFACE AND MECHANICAL PROPERTIES



Dinamometro



UV-Spettofotometro



Angolo di Contatto



Microscopio Elettronico

SEM

CONTROL

CARV

ALD



Samples 10×90 mm	E (Mpa)	TS (Mpa)	EB (%)	Contact Angle (°)
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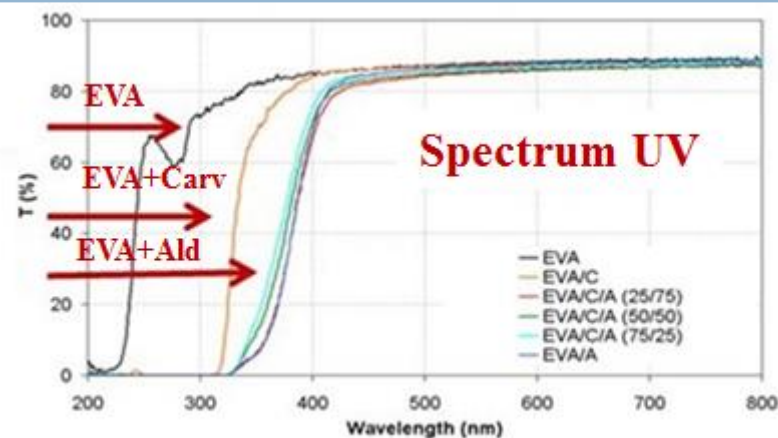
CONTROL 46.8 ± 1.9 24.8 ± 1.2 590 ± 20 92 ± 1.2

ALD 40.6 ± 1.5 17.1 ± 0.8 680 ± 28 79.4 ± 1.1

CARV 39.9 ± 1.6 16.4 ± 0.8 680 ± 30 75.3 ± 1.0

(E) Elastic modulus (TS) Tensile stress (EB) Elongation at break

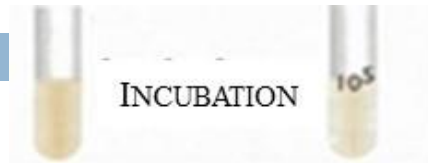
Measured by a Dynamometer on rectangular shaped specimens (10×90 mm) cut off from films



Release in water solution buffered

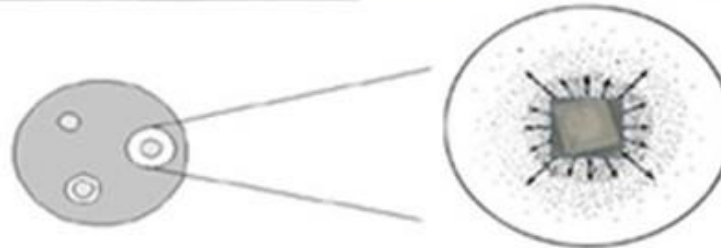
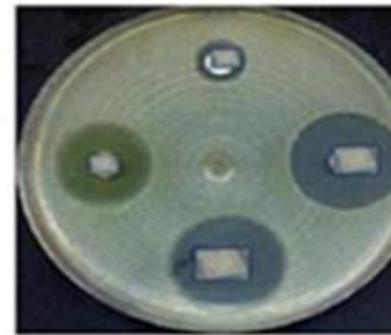
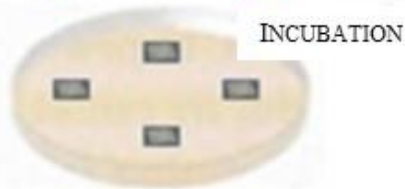
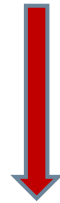
Time (h)	Carvacrol		Cinnamaldehyde	
	3.5 wt%	7 wt%	3.5 wt%	7 wt%
mg/l				
0.5	1.57	3.36	0.41	0.61
1	1.83	3.88	0.53	0.65
2	2.21	4.73	0.62	0.68
4	3.27	6.54	0.75	0.79
16	13.30	28.24	10.00	12.80
24	13.91	29.56	10.36	13.00
48	14.20	30.10	12.50	16.60

ANTIBACTERIAL ACTIVITY



DISCS (1 cm²)

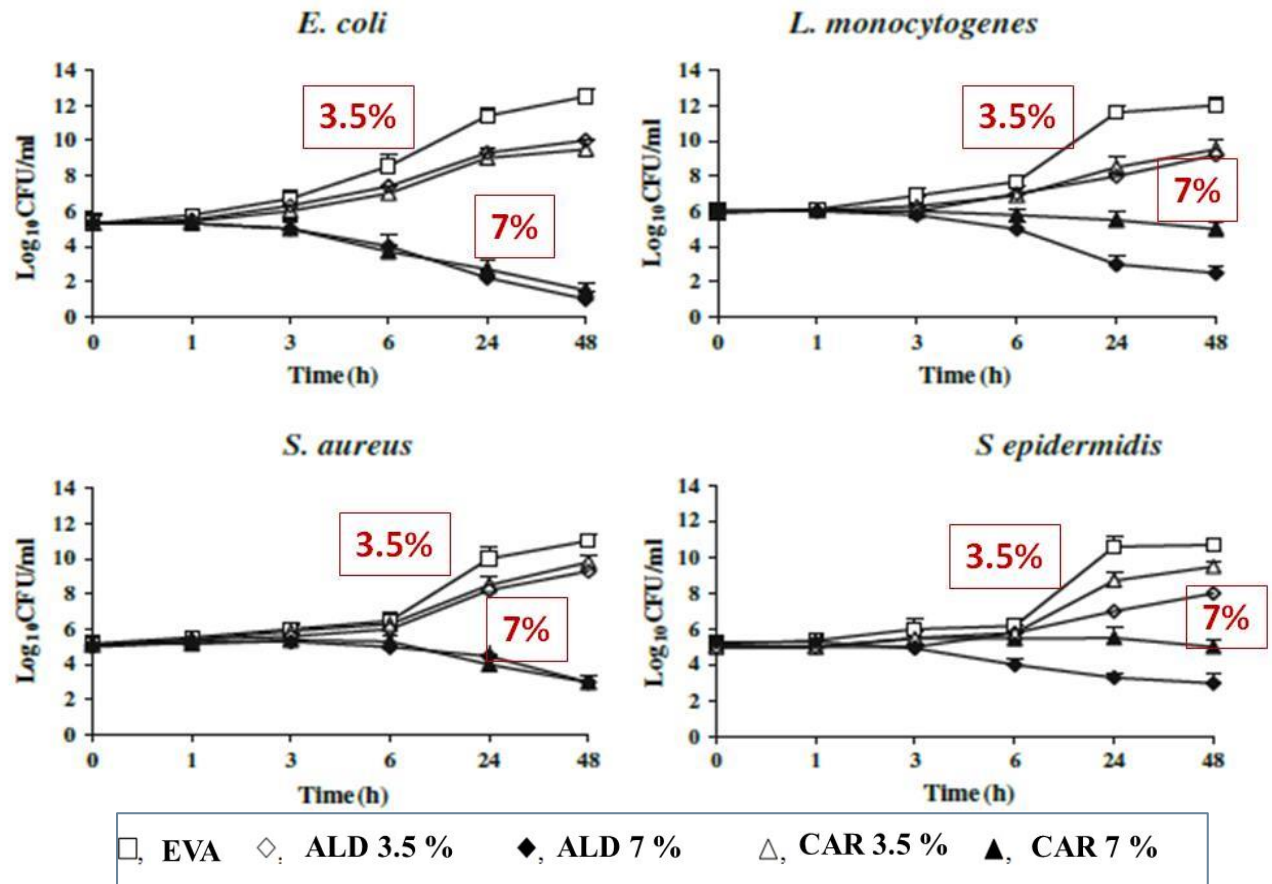
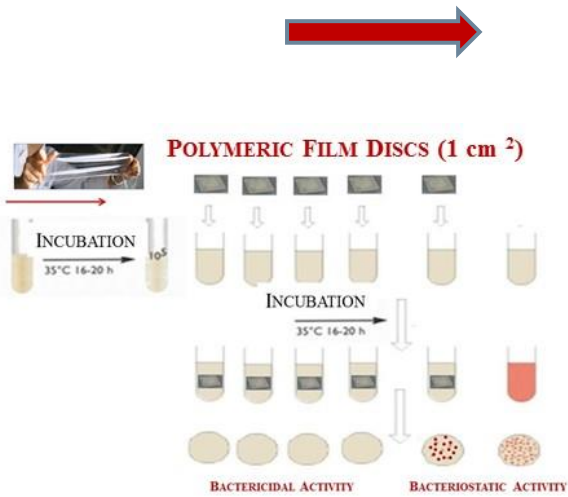
DISC DIFFUSION TEST



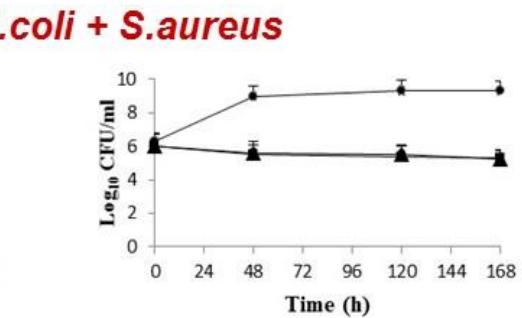
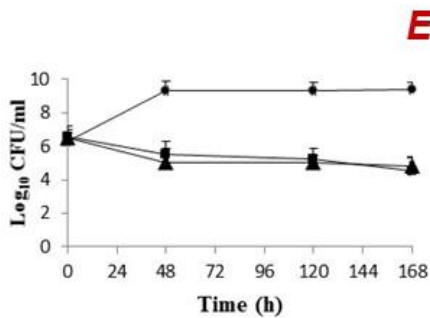
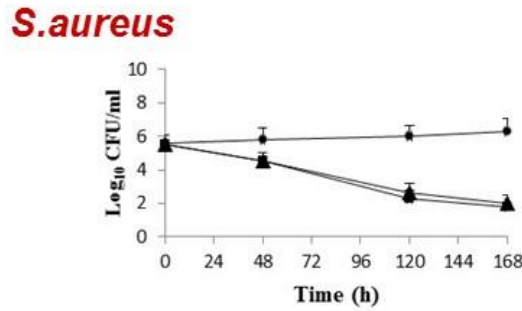
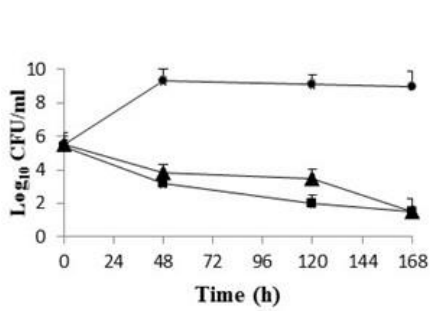
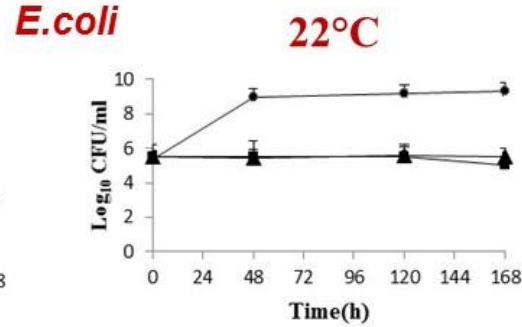
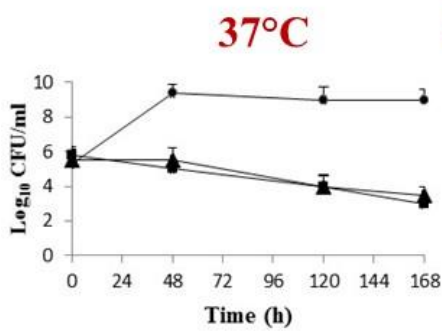
Samples		E. coli	L. monocytogenes	S.aureus	S.epidermidis
		Inibition alone (mm)			
EVA	-	-	-	-	-
EVA + ALD	7%	12	-	17	-
EVA +CARV	7%	14	15	15	17

KILLING CURVES

BACTERICIDAL ACTIVITY



KILLING CURVES



EVA
 ALD
 CAR

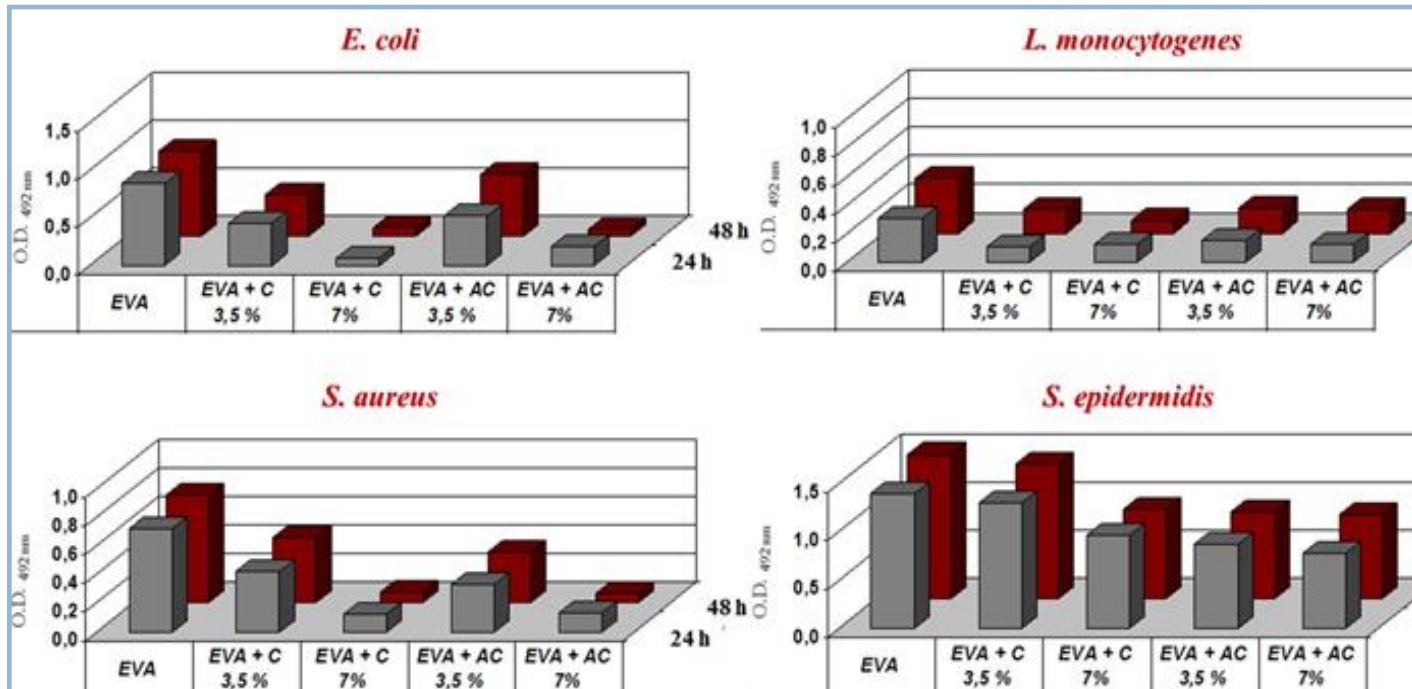
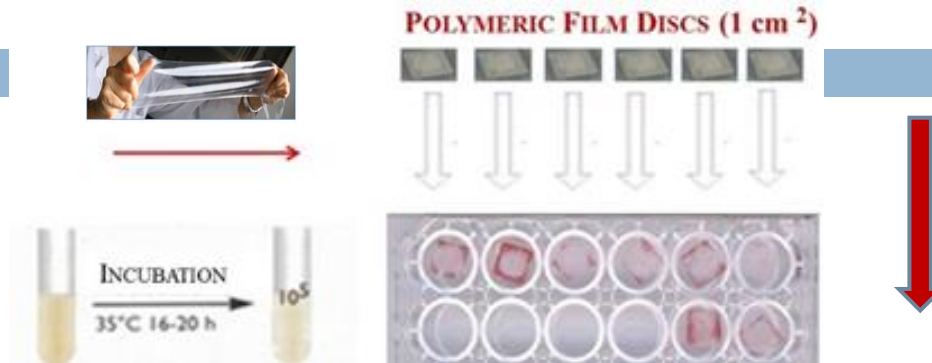
BACTERICIDAL ACTIVITY

4 °C

37 °C

Sample	Temperature 4°C				Temperature 37°C 48 h
	0 h	48 h	120 h	168 h	
EVA	5.56±0.55 ^a	5.75±0.53 ^a	5.69±0.38 ^a	5.51±0.39 ^a	9.24±1.04 ^a
ALD	5.12±0.18 ^a	5.00±0.25 ^a	4.88±0.42 ^a	4.55±0.43 ^a	3.69±0.22 ^b
CAR	5.52±0.32 ^a	5.41±0.23 ^a	5.56±0.45 ^a	5.23±0.19 ^a	3.82±0.54 ^b

EFFICACY AGAINST BACTERIAL BIOFILM



EFFICACY AGAINST BACTERIAL BIOFILM

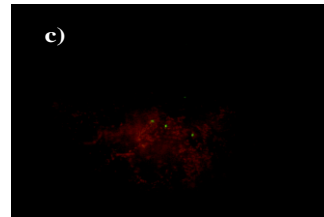
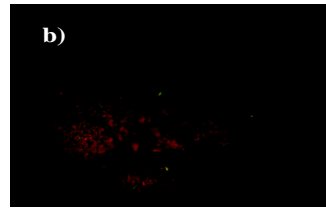
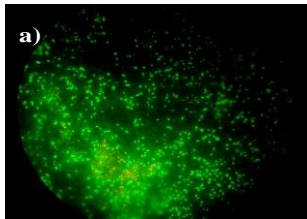
FLUORESCENCE MICROSCOPY IMAGES - LIVE/DEAD STAINING

EVA

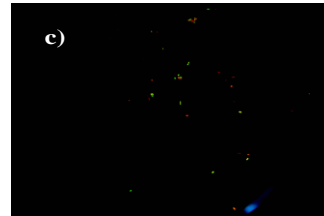
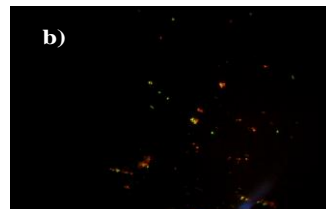
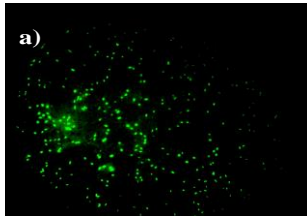
EVA + CAR

EVA + ALD

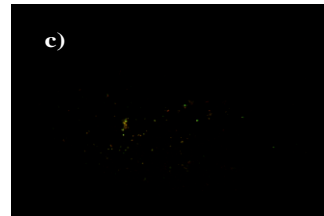
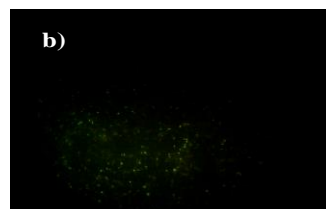
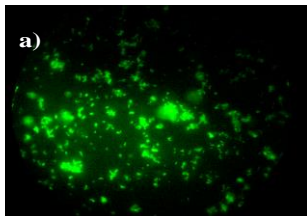
E. coli



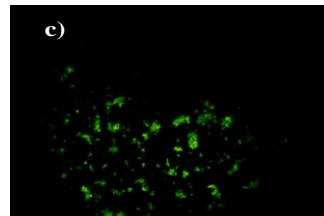
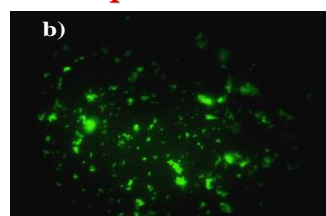
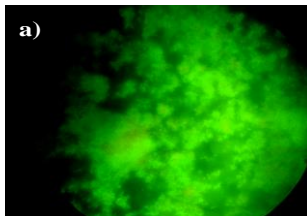
L. monocytogenes



S. aureus



S. epidermidis



BIOFILM REDUCTION

REDUCED SURFACE HYDROPHOBICITY

BACTERICIDAL ACTIVITY



PRESENCE OF VIABLE CELLS



PRESENCE OF DEAD CELLS

CONCLUSION



CARVACROL
AND
CINNAMALDEHYDE
INCORPORATED INTO **EVA FILMS**
MAINTAIN
THEIR ANTIBACTERIAL AND
ANTIBIOFILM ACTIVITIES

- ⇒ Prolonged time and at different T values
- ⇒ Against single and mixed cultures
- ⇒ Either in Planktonic and Biofilm phase



THE RESULTS SUGGEST THEIR POTENTIAL ROLE FOR FUTURE APPLICATIONS AND OPEN UP NEW HORIZONS IN THE FOOD AND INDUSTRIAL AREAS



UNIVERSITÀ DEGLI STUDI DI MESSINA
Tradizione e cambiamento al centro del Mediterraneo



Nano Rome, 20-23 September
2016 Innovation
Conference & Exhibition

**THANK YOU
FOR YOUR
ATTENTION**



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