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Methyl cellulose films containing natural extracts: antibacterial properties

Débora Campos,[1] Clara Piccirillo,[1] Robert C. Pullar,[2]
Paula M.L. Castro,[1] Manuela E. Pintado[1]

[1]: CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, PORTUGAL
[2]: Departemento de Engenharia de Materiais e Cerâmica / CICECO, Universidade de Aveiro, Aveiro, PORTUGAL

Outline

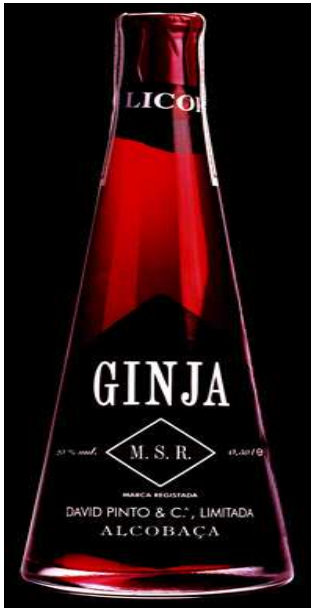
- Introduction – reason for work, use of natural extracts.
- Experimental – preparation of the extracts and the films.
- Results – antibacterial activity, film morphology.
- Conclusions and future work.

Reason for work

- Food microbial contamination
  - Contact with pathogen agents / food degradation.
- Packaging: very important function.
  - Barrier / protection from external agents and/or vapours, oxygen, etc.
- Incorporation of antimicrobial agents in the packaging.
- Use of natural antibacterial compounds (i.e. plant extracts, essential oils).
  - More “accepted”.

Extracts of Ginja cherry stems

- Ginja cherry: traditional Portuguese cherry (*Prunus cerasus*, L. rosaceae).
  - Used to make Ginjinha.
- By products: stems and leaves.
- Previous work: study of the antibacterial and antioxidant properties of the extracts.
- Present work: incorporation of the extracts in food packaging.



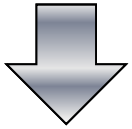
Stem extracts in ethyl acetate

- Extracts with the highest antibacterial activity.
- Rich in terpenes and polyphenols.
  - α-pinene, eugenol, α-terpineol, linalool.
- Active towards Gram-positive and Gram-negative strains.
- *MRSA*, *MSSA*, *Bacillus subtilis*, *Salmonella*, *E. coli*.

Film preparation

Methyl cellulose + glycerol + extract

Smooth and homogenous solution



Solution on a levelled plate

Drying of the solution
(controlled atmosphere, 37 ºC)

Different extract concentration
(3, 4 and 5 % w/v)

Film characteristics

3 % (MC3)

4 % (MC4)

5 % (MC5)

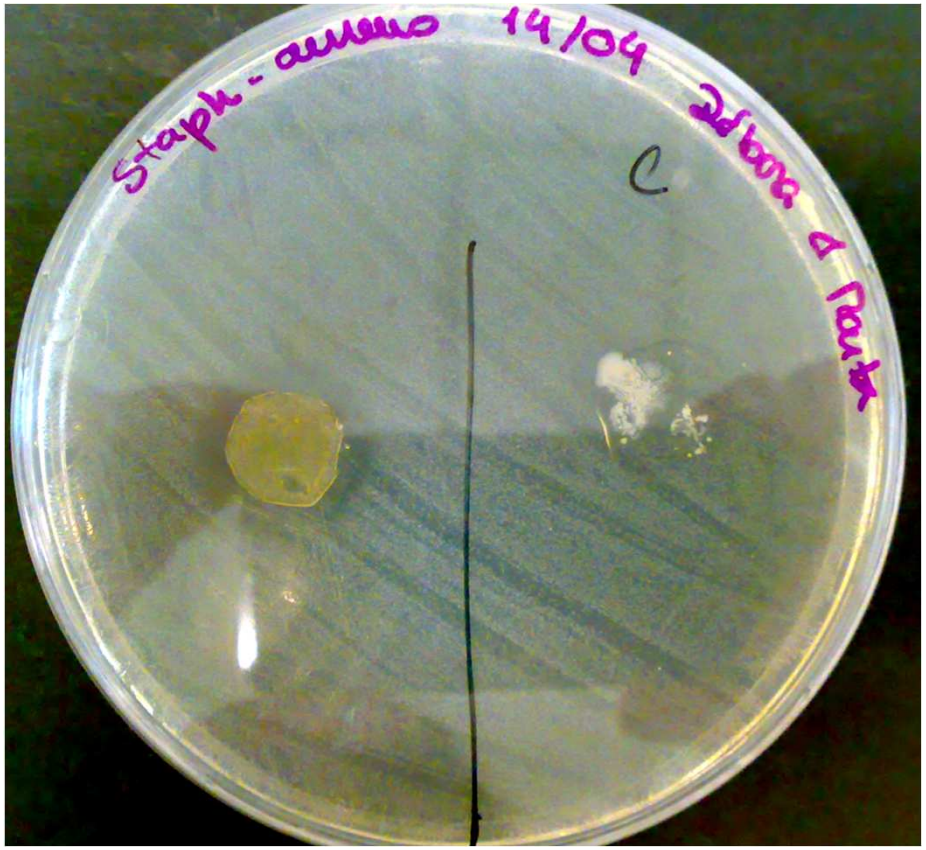
Sample	Control	MC3	MC4	MC5
Thickness (mm)	0.083	0.341	0.396	0.418
Weight* (mg)	4.93	10.56	13.03	14.23
Extract content* (mg)	-	3.99	6.00	6.90

Average of 20 values in 2 independent experiments; error < 5 %.
*: Values referring to a 1 cm diametre disc of film.

Antibacterial activity: first screening

- Disc test for samples MC4 and MC5.
- Bacterial inoculum solution spread onto Mueller Hinton agar petri dish.
- Film placed on the dish and incubated at 37 ^{o}C for 24 hours.
- Inhibition area (halo) around the film.

Antibacterial activity – disc test

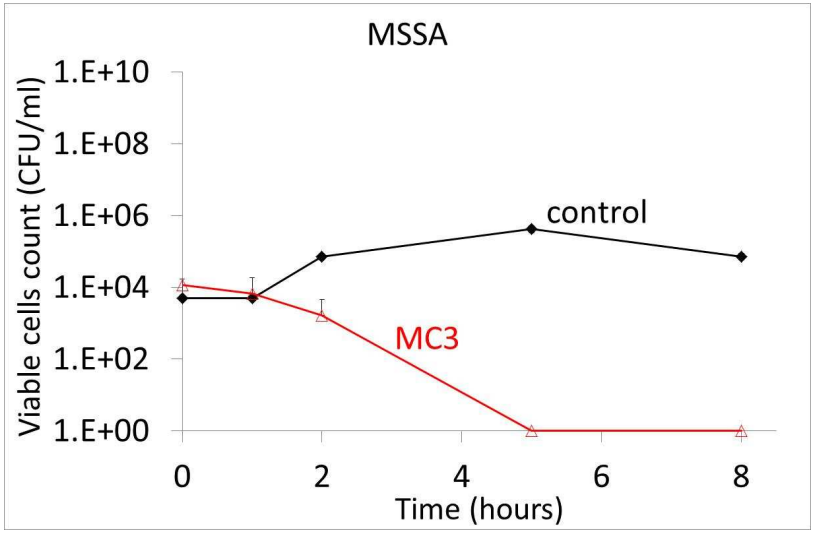


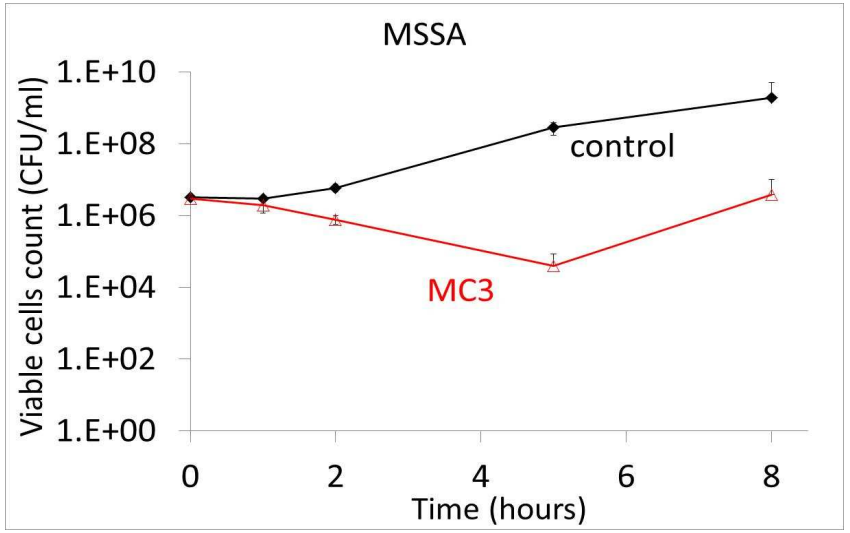
Example of positive results (MRSA).

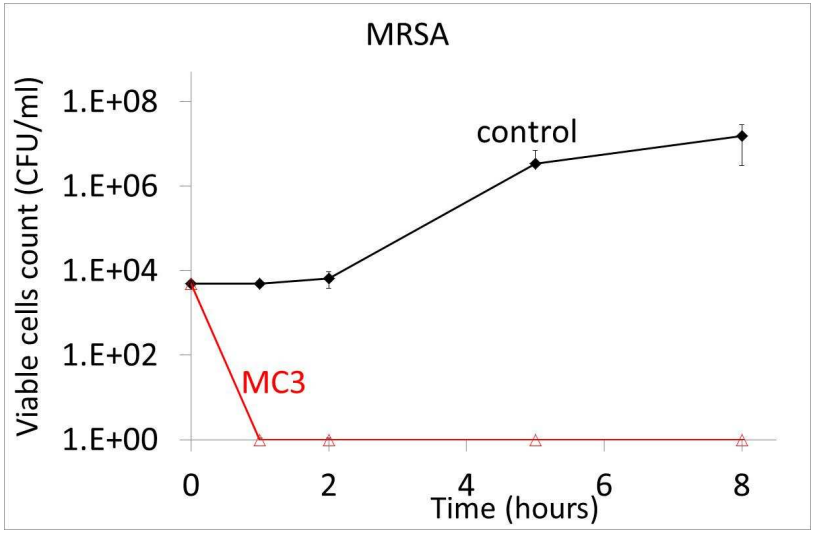
Antibacterial activity: viable cells count assay

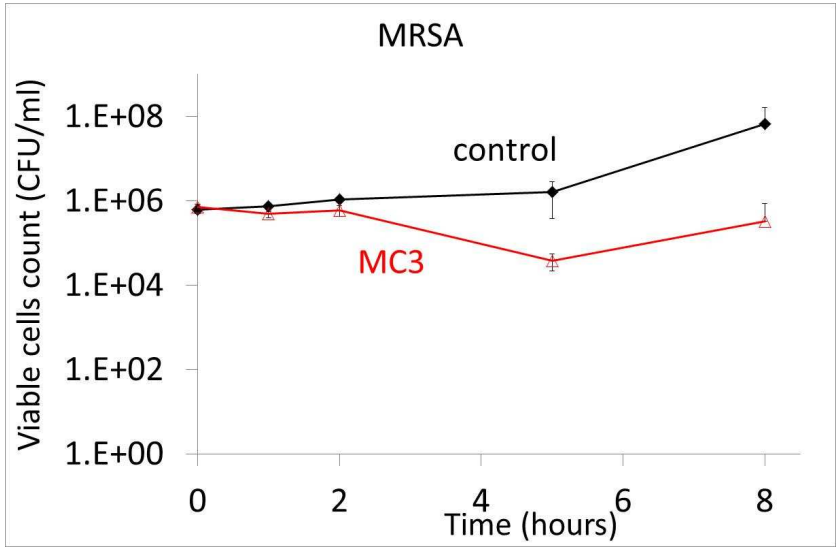
- Solution of the bacterial strain (growth overnight at 37 ^{o}C).
- Inoculum concentration adjusted with peptone water.
  - Approximately $10^{+4}/10^{+5}$ and $10^{+6}/10^{+7}$ CFU/ml.
- 200 µl of inoculum solution in contact with the films for different lengths of time (0, 1, 2, 5 and 8 hours).
- Appropriate dilution, plating and counting.

Viable cells count assay

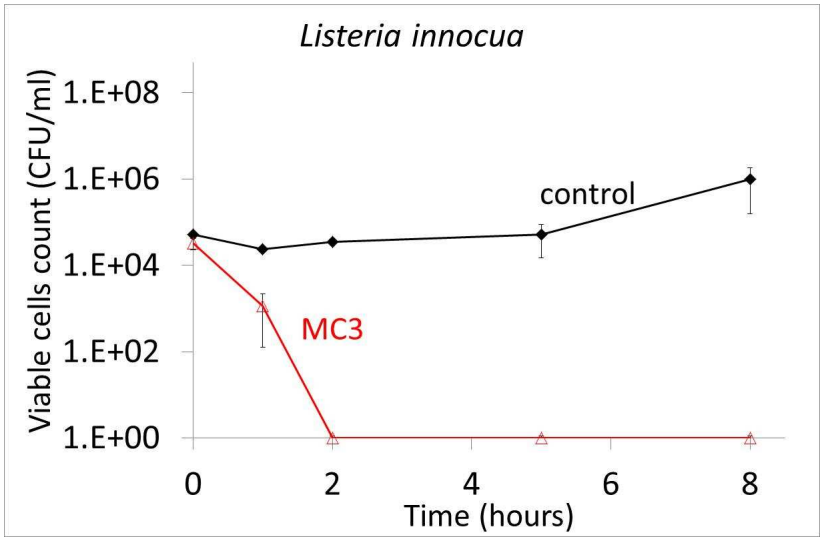


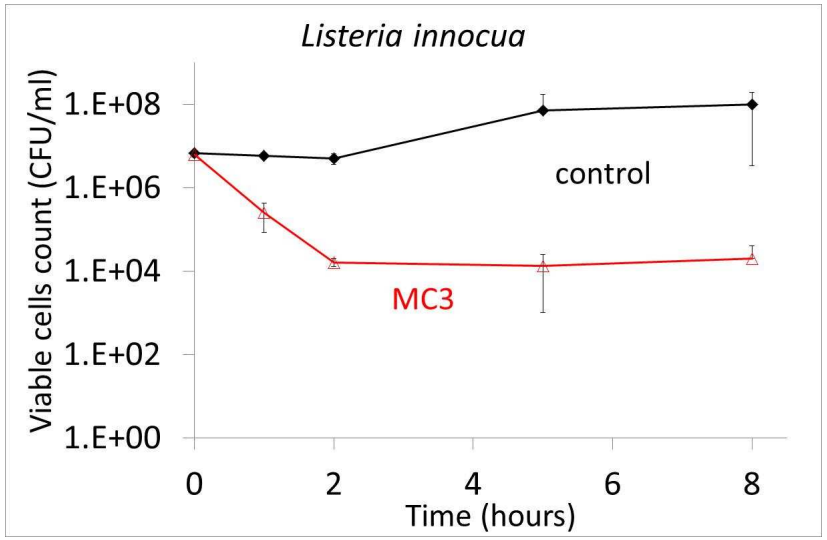


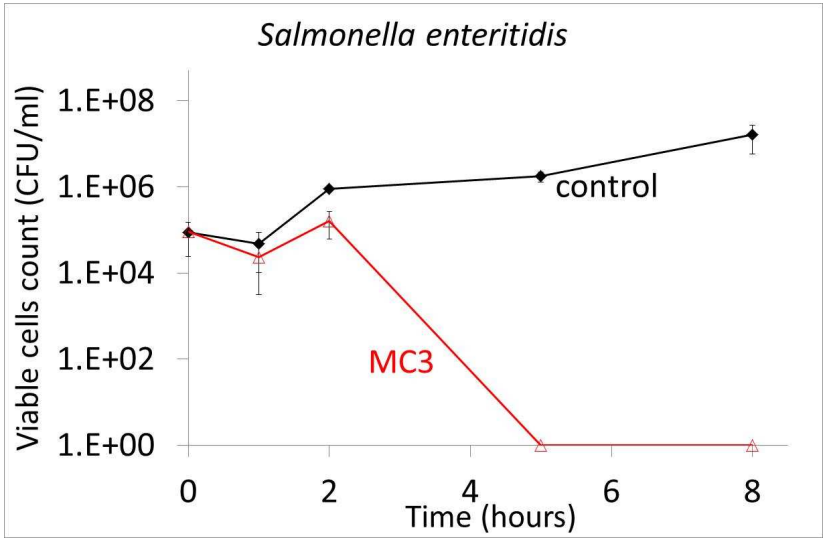


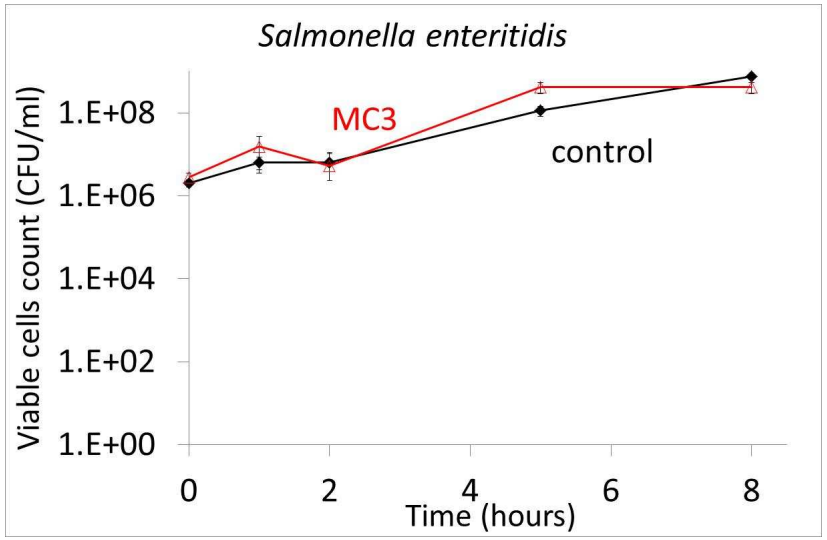


Viable cells count assay





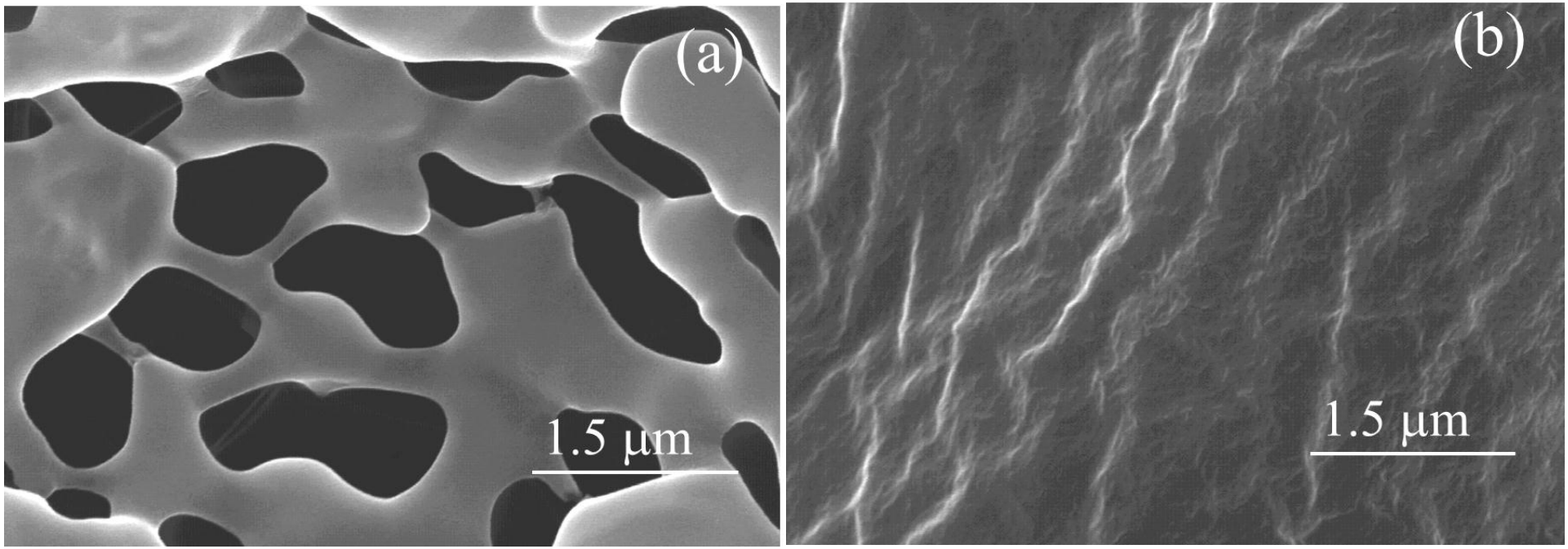




Viable cells count assay: summary

- Sample MC3: active towards both Gram-positive and Gram-negative strains for lower inoculum concentration.
- Active towards Gram-positive strains for higher inoculum concentration.
- Sample MC4: active with all strains for both concentration – complete inhibition of the growth.
  - Curves not shown.

Films morphology: SEM micrographies



Control film: non-continuous structure, pores

Sample MC3: continuous structure

Effective barrier/protection for external contaminating agents.

Conclusions / Future work

- MC films containing Ginja cherry stem extract successfully prepared.
- Antibacterial activity towards Gram-positive and Gram-negative strains.
- Activity function of inoculum concentration (for lower extract concentration in the films).
- Films with a continuous structure – effective protective barrier.
- More characterisation of the films; tests on food to see the effect on preventing spoilage.

References

- Piccirillo, C. *et al.* (2013). Chemical composition and antibacterial properties of stem and leaf extracts from Ginja cherry plants. *Industrial Crops and Products.* **43(1)**:562-569.
- Campos, D. et al., Characterization and antimicrobial properties of methylcellulose films enriched with stems extract of Ginja cherry . *J. Applied Microbiology*, submitted.

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Thank you for your attention

cpiccirillo@porto.ucp.pt