









Biomimetic surfaces inspired by cabbage leaves for Escherichia coli biofilm prevention in the food industry

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INTRODUCTION

Biofilms formed by **foodborne pathogens** that occur on food industry equipment are a major problem since they are a frequent source of product contamination, resulting in economic losses for processors and posing serious health concerns for consumers. Safer food production may entail high cleaning costs and severe environmental impacts to reduce contamination¹. One way to reduce the amount of cleaning is to design naturally cleaning surfaces based on **biomimetic designs**².

This work aimed to (i) produce and characterize biomimetic replicates of four self-cleaning cabbage leaves (Tenderheart, Cauliflower, White cabbage and Leek), and (ii) compare the antifouling performance of leaves and their artificial replicates through retention assays using *Escherichia coli*.

MATERIAL AND METHODS

Surface production

Original leaves:



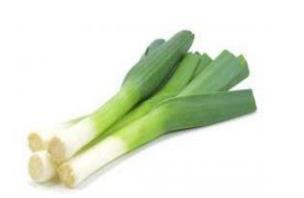
Tenderheart (Brassica oleracea)



Cauliflower (Brassica oleracea var. botrytis)

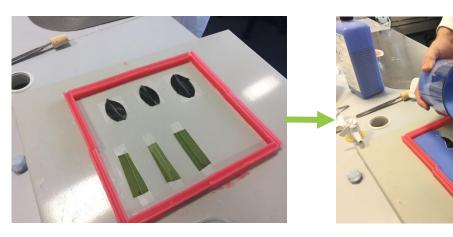


White cabbage (Brassica oleracea capitate)



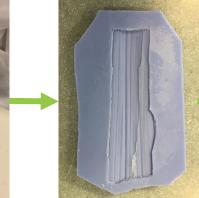
Leek (Allium ampeloprasu)

Casting & Moulding:



Leaf fixation

Silicone pouring

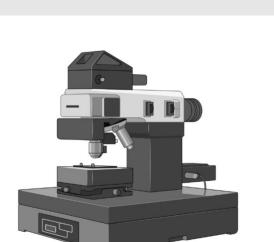


Wax pouring

Negative silicone

Biomimetic replicates

Surface characterization



Optical profilometry - Surface roughness



Contact angle determination



Scanning Electron Microscopy

- Surface hydrophobicity

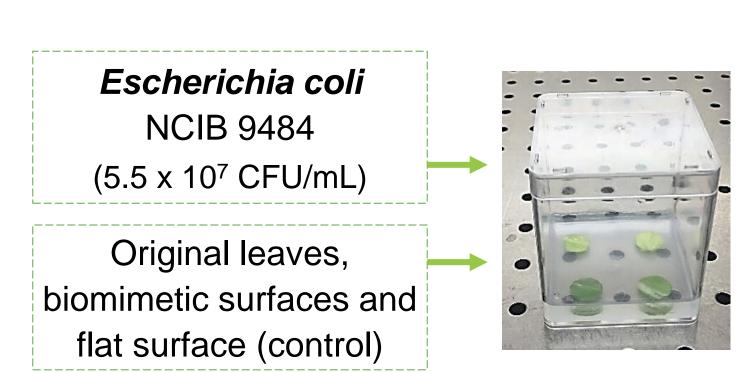
- Surface morphology

Binding assay

Retention assay

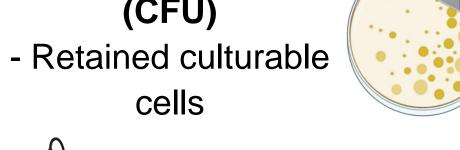
Static incubation in 25 mL of cell suspension for **1 h** at **37** °C

Final step before biofilm formation



Retention analysis

Colony-forming units (CFU)





Scanning Electron Microscopy

- Spatial distribution of retained cells

ACKNOWLEDGMENTS:

This work was financially supported by: LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC), and by the European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 952471 (SurfSAFE). L.C. Gomes thanks to the Portuguese Foundation for Science and Technology (FCT) for the financial support through the Scientific Employment Stimulus - Individual Call - [CEECIND/01700/2017].



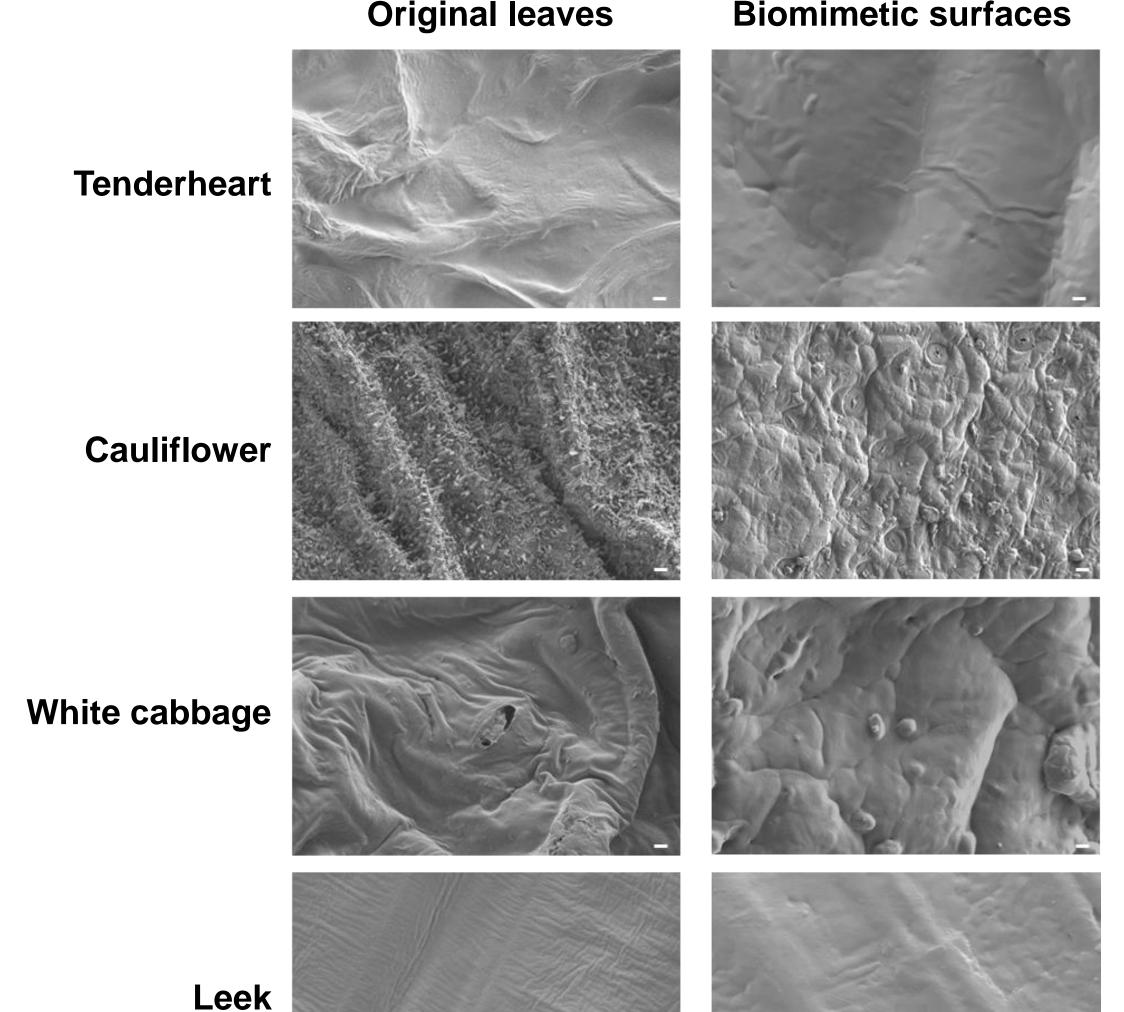




ASM Conference on Biofilms, November 13-16, 2022 – Charlotte, NC, USA



RESULTS



The macro- and microtopographies of the original leaves were well reproduced in wax

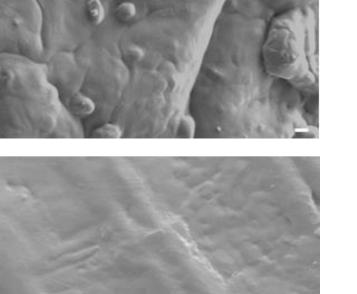
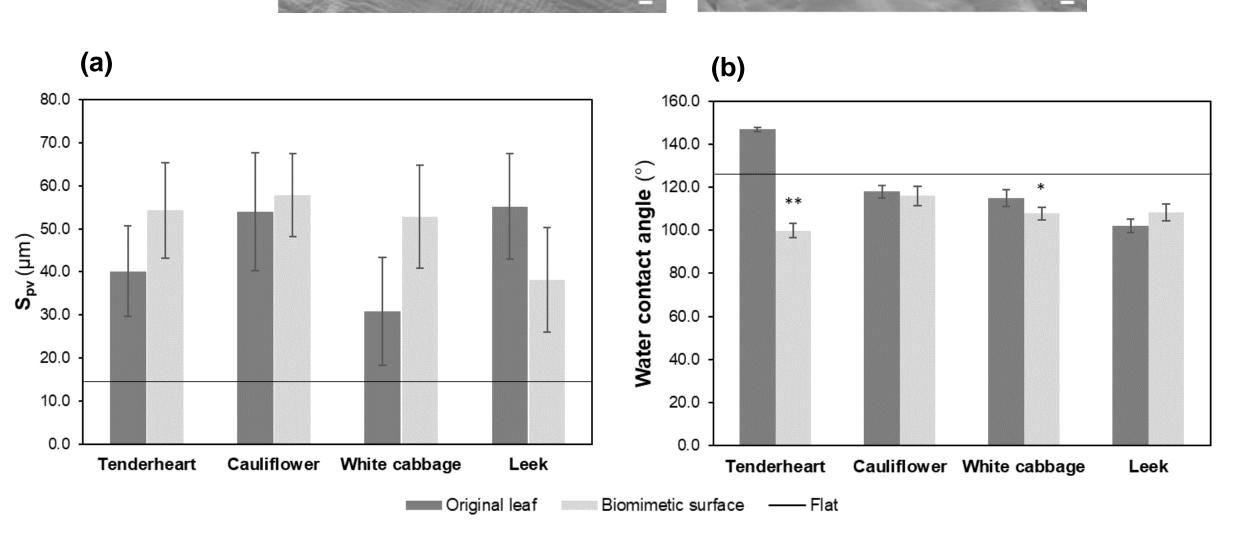


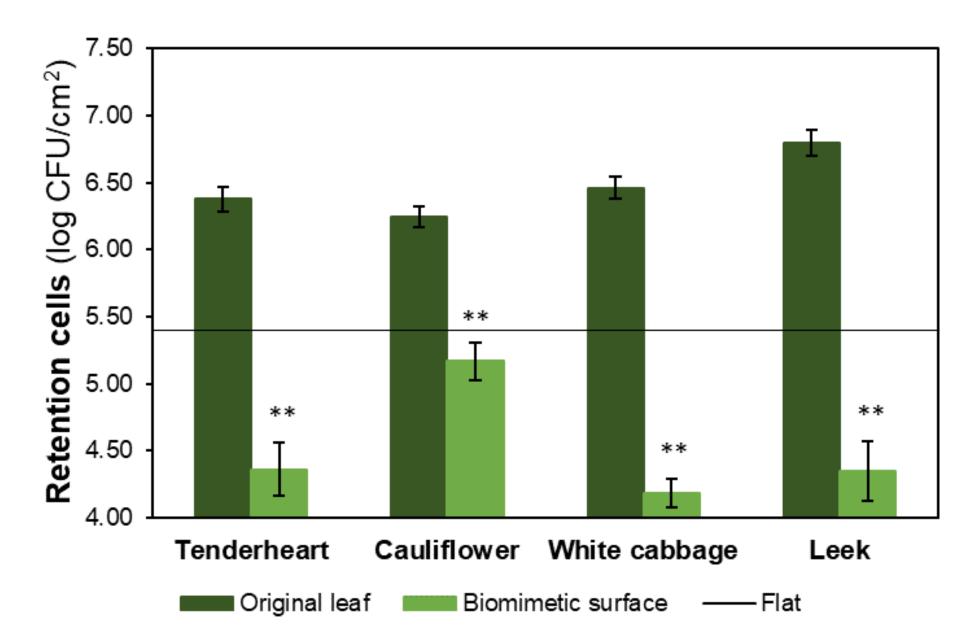
Figure 1. SEM micrographs of the original leaves and biomimetic surfaces. Magnification = $5000 \times$, scale bar = $2 \mu m$.



The natural White cabbage had the lowest roughness values, but its replica is one of the roughest surfaces.

All the surfaces were non-wettable $(WCA > 100^{\circ}).$

Figure 2. (a) Peak to valley height (S_{nv}) and **(b)** water contact angles. Asterisks denote significant differences between the original and replicate of the same leaf (* p < 0.05 and ** p < 0.01). Black lines are the values of the flat control.



The biomimetic surfaces were more efficient at avoiding retention than natural leaves.

The biomimetic replicas of White cabbage and Leek were the most promising surfaces (reductions of $\sim 2 \text{ Log}$).

Figure 3. Culturable cells on the original leaf and corresponding biomimetic surface. The means \pm SDs for three independent experiments are presented. Asterisks denote significant differences between the original and replicate of the same leaf (** p < 0.01). The black line indicates the value of the flat control.

CONCLUSIONS

- o These results showed that via a casting approach wax surfaces mimicking the structure of cabbage leaves could be prepared.
- The nano-scale topography and wettability associated with the natural leaves are not essential for bacterial retention in the tested conditions.
- Biomimetic surfaces inspired by self-cleaning leaves have potential for the development of industrial antifouling surfaces.

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