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Biomimetic Surfaces from Cabbage Leaves: A Novel Approach to Prevent Biofilm Formation on Food Environments

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1. Introduction

Contamination of food contact surfaces is a significant public health concern with severe economic impacts. The effectiveness of standard hygiene procedures is often limited against foodborne pathogens due to their high resistance. Therefore, there is an urgent need for new strategies capable of restricting bacterial proliferation. One approach that has recently gained attention is the development of antifouling surfaces that delay bacterial adhesion, either by antimicrobial coatings or modification of the surface properties. This study aimed to (1) produce and characterize biomimetic surfaces inspired by the topographies and physicochemical characteristics of various plant leaves with self-cleaning and superhydrophobic properties – White cabbage, Tenderheart, Cauliflower and Leek – and (2) evaluate their antifouling activity by attachment, adhesion, and retention assays, the three key phenomena involved in the initiation of biofilm formation. Furthermore, the effect of casein conditioning films on biofilm formation was evaluated as the accumulation of organic matter on surfaces can alter their chemical, physicochemical, and topographic properties.

2. Materials and Methods

The biomimetic surface replicas were synthesised by a moulding technique using wax and characterised by water contact angle measurements (hydrophobicity), Optical Profilometry (roughness), Scanning Electron Microscopy (morphology), and Fourier Transform Infrared Spectroscopy (chemistry). For the microbiological analysis, attachment (spray plus wash assay), adhesion (spray assay), and retention (1-h static incubation) assays were performed using *Escherichia coli*. The culturable cells were enumerated by colony-forming units (CFU), and the surfaces were analysed by Scanning Electron Microscopy. Additionally, 72-h biofilms were formed in 12-well microtiter plates under static conditions and were analysed for the number of culturable cells by CFU, biofilm thickness by Optical Coherence Tomography, and biofilm architecture by Confocal Laser Scanning Microscopy.

3. Discussion

The results showed that, although the macro- and micro-topographies of the original surfaces were well reproduced on the biomimetic surfaces, the presence of nanotopographies was less evident. However, all biomimetic surfaces were more efficient at reducing the number of bacteria bound to the surface than the corresponding original leaves. On the attachment of *E. coli* to biomimetic surfaces, the White Cabbage (the roughest and most hydrophobic surface) showed the highest reduction in cell numbers, approximately 1 Log CFU/cm^2, compared with the natural White Cabbage, followed by Tenderheart and Cauliflower; biomimetic and natural Leek surfaces did not show significant differences. For the adhesion assays, Tenderheart was the biomimetic surface with the lowest number of *E. coli* cells, and the only one presenting significant reductions from the natural leaves. Following the retention assays, all biomimetic surfaces were significantly more efficient than natural leaves, exhibiting a reduction of approximately 2 Log CFU/cm^2. Also, White Cabbage, Tenderheart, and Leek replicas showed lower *E. coli* numbers than the flat control surface. Regarding the biofilm assays, a significant decrease in the number of culturable cells was observed on *E. coli* biofilms formed on both 5% (w/v) casein-conditioned surfaces (up to 50% reduction) and non-conditioned biomimetic surfaces (up to 89% reduction) compared to the flat control, revealing their antifouling properties. Moreover, both unconditioned and conditioned biomimetic surfaces were able to reduce biofilm thickness by up to 26% and 35%, respectively, compared to unconditioned and conditioned flat surfaces.

4. Conclusions

This study highlights moulding as a reliable technique for reproducing the structure of vegetable leaves. The results suggest that these biomimetic surfaces are promising for preventing the initial settlement of bacteria and the development of mature biofilms. The findings of this study have important implications for developing more effective food contact surfaces to enhance food safety and combat foodborne pathogens.

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