Developing artificial biofilm-imitates to establish a novel biofilm cleaning test system

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Introduction and objective
Biofilms are the most frequent cause of food contamination and thus a leading health and safety concern. To develop suitable sanitation methods against biofilms, a fundamental understanding of their growth behavior as well as their behavior during cleaning and removal is essential. However, studying biofilms remains challenging due to their heterogeneous and complex inherent properties. Hence, the aim of the present study was to develop and characterize simplified artificial biofilm imitates.

Methods
The imitates were based on single or mixed polysaccharide hydrogels (18 imitates) or on polysaccharide-protein hydrogels (34 imitates). They were analyzed based on rheological properties (G' and G'') and texture profile analysis, both associated with the behavior of biofilms during cleaning and removal, and compared to the characteristics of bacterial biofilms based on literature.

Results
For rheological properties, the overall lowest G' and G'' values of all imitates were measured for agar-carob (AC) imitates. AC imitates reported similar G' and G'' values (G': max. 500 Pa; G'': max. 100 Pa) to *Pseudomonas aeruginosa, Streptococcus mutants* and *Staphylococcus aureu*s biofilms. The texture profile of polysaccharide mixed hydrogels depended not only on the type but also on the concentration of the polysaccharide. When adding proteins, the imitates became firmer and more cohesive, but at the same time, adhesion was decreased. AC imitates showed high firmness and consistency, making good candidates for biofilm imitates, however, comparison to literature is challenging due to missing data.

Conclusion
The artificial biofilm imitates represent simplifications of native biofilms with similar mechanical properties and may prove helpful in developing a novel biofilm cleaning test system. Hence, current findings prove helpful for testing the efficiency of cleaning procedures or to develop mechanical removal strategies against native biofilms within the food industry. Further studies must include more complex imitate compositions to get closer to the native biofilm structure with greater mechanical heterogeneity and more complex geometries.

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