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Keratin-based antimicrobial peptides for smart response self-desinfected surfaces – Isolation & Characterization

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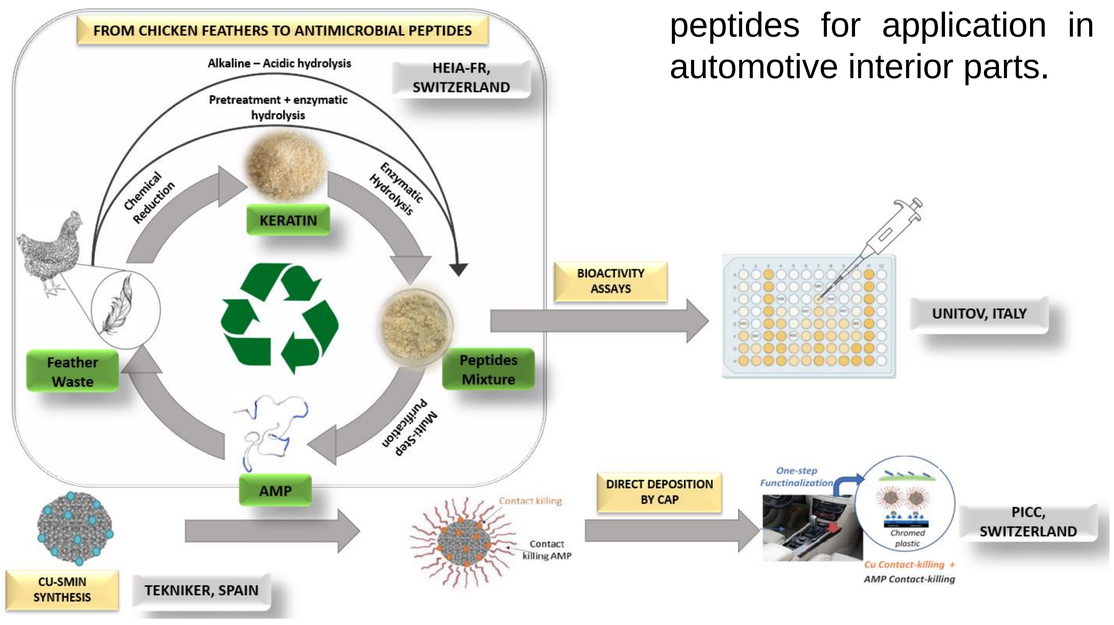
Introduction

Microbial colonization of frequently touched surfaces burdens our society by causing significant cost to human lives and economy. The EU project RELIANCE aims to design and develop an innovative smart response self-disinfectant antimicrobial coatings that act by contact killing and prevent therefore the spread of infections. The nanocoating consists of copper-functionalized mesoporous silica nanoparticles modified with **Antimicrobial Compounds** such as peptides extracted from protein-containing waste streams.

At HEIA-FR, we are deeply involved in the extraction, isolation and identification of **Antimicrobial Peptides** (AMP) from chicken feathers. Multiple extraction approaches are investigated and a spectrum of analytical methods are used for the purification and characterization of the extracted peptides.

Overview

Chicken feathers, a keratinous waste source, very abundant in nature but underexploited due to the high stability of keratin. Therefore, various promising approaches are employed in the extraction and isolation of sustainable antimicrobial peptides for application in automotive interior parts.



Peptide's isolation and purification

In ultrafiltration process, a semipermeable membrane with defined pore sizes allowing smaller molecules, such as salts and contaminants, to pass through while retaining peptides.

This method not only ensures the purity of peptides by eliminating impurities but also allows to separate peptides into distinct ranges, such as 1-5 kDa, 5-10 kDa, and >10 kDa, enabling precise isolation according to size.

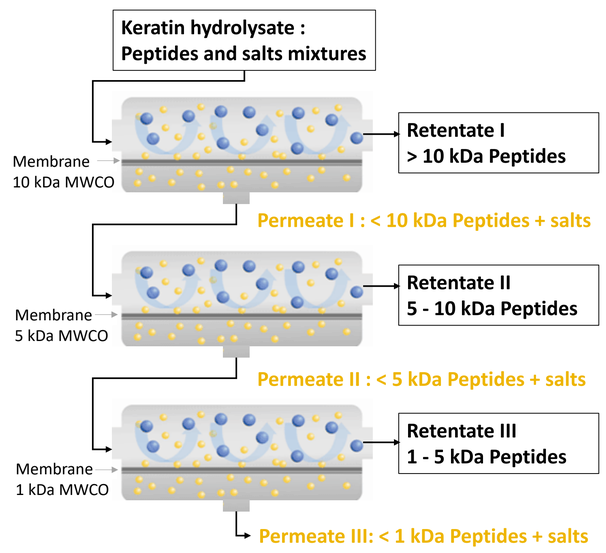


Figure 1. Overview of our global UF approach.

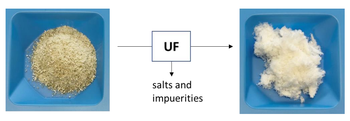


Figure 2. Keratin extract : crude (left), and after desalting by UF with a 2 kDa MWCO (right).

Characterization by FTIR and SDS-PAGE

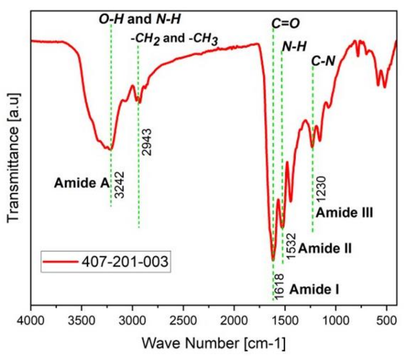


Figure 3. FTIR spectra of keratin.

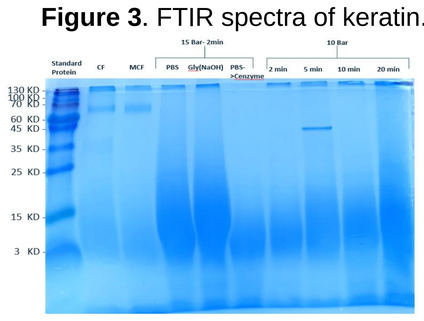


Figure 4. SDS-PAGE profiles of various peptides mixtures

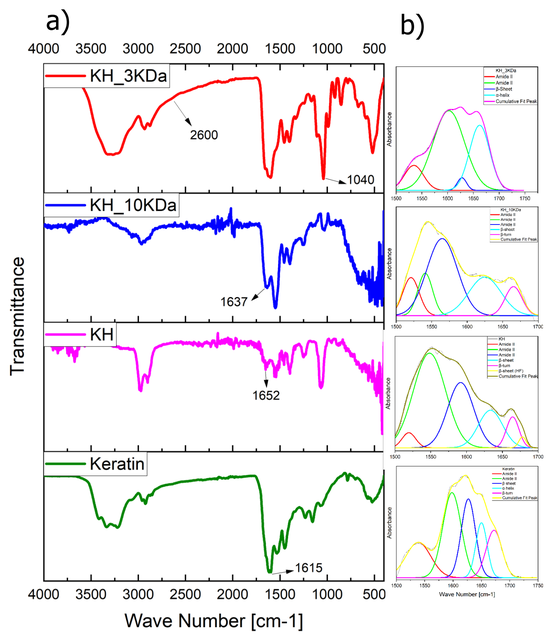


Figure 5. a) FT-IR spectra of Keratin and Keratin hydrolysates, b) Deconvolution of the amide I region for four samples

Purification and characterization by HPLC

HPLC-CAD separates peptides for bioactivity testing. HPLC-MS provides molecular weight data. These analyses contribute to the comprehensive characterization of peptide composition and bioactive potential.

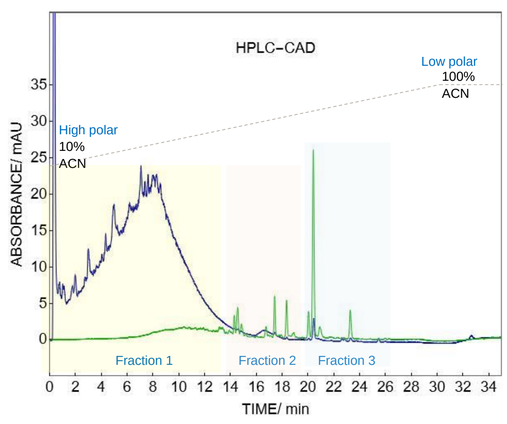


Figure 6. Chromatograms obtained by the HPLC-CAD. The peptide hydrolysate will be fractionated based on their polarity. The bioactive test will be done using each separated fraction.

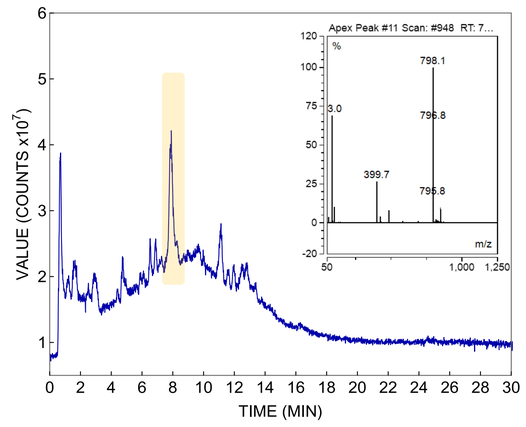


Figure 7. Chromatograms obtained by a HPLC-MS. This could give us the idea of the molecular weight of the peptides.

Summary & Outlook

This work brings variety in the evaluation of the best extraction approach for the production of peptides with optimal antimicrobial activity from poultry waste. Several extracted AMP mixtures revealed an antimicrobial activity and are then subjected to further purification to isolate the most active peptide fraction. This challenging process will contribute to advancing the understanding of the activity-structure relationship for an optimized sustainable keratin-based AMPs process production.

Acknowledgements

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