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From chicken feathers to antimicrobial peptides for smart, self-disinfecting nanocoating

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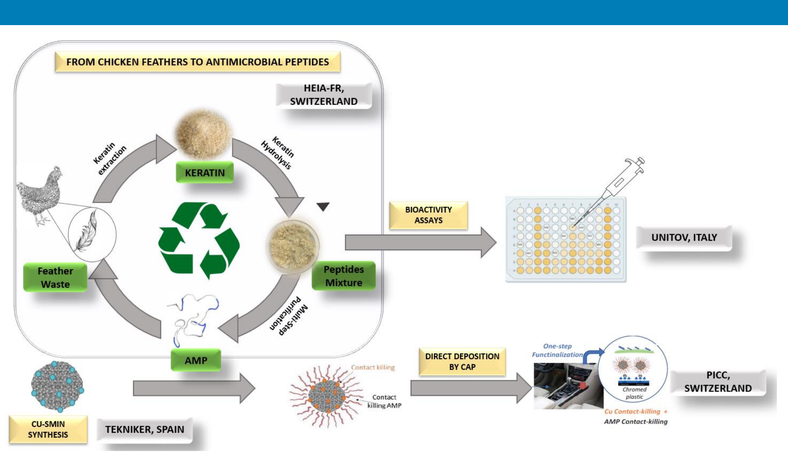
Introduction

Microbial colonization of high traffic surfaces burdens our society by causing significant cost to human lives and economy. The prevention of microbes transmission remains a global challenge. The EU project RELIANCE aims to design and develop an innovative smart response self-disinfectant antimicrobial coatings that act by contact killing and reduce therefore the spread of infections.

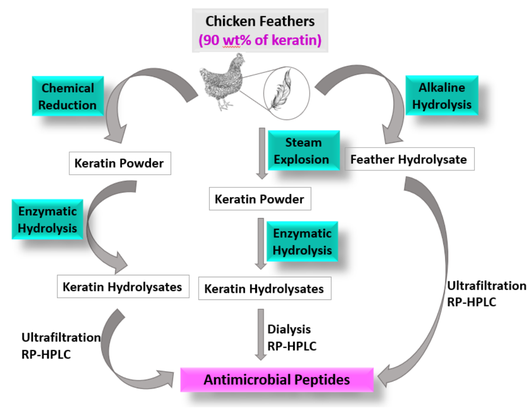


The nanocoating consists of copper-functionalized mesoporous silica nanoparticles modified with **Antimicrobial Compounds** such as peptides extracted from keratin-rich waste streams.

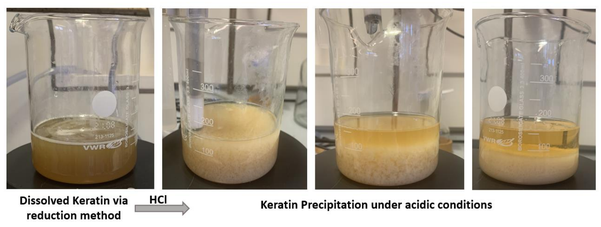
At HEIA-FR, we are deeply involved in the extraction, isolation and purification of **Antimicrobial Peptides** (AMP) from chicken feathers evaluating multiple extraction and purification approaches.

Keratin Extraction, AMPs Isolation & Characterization

Keratin extraction and AMPs isolation

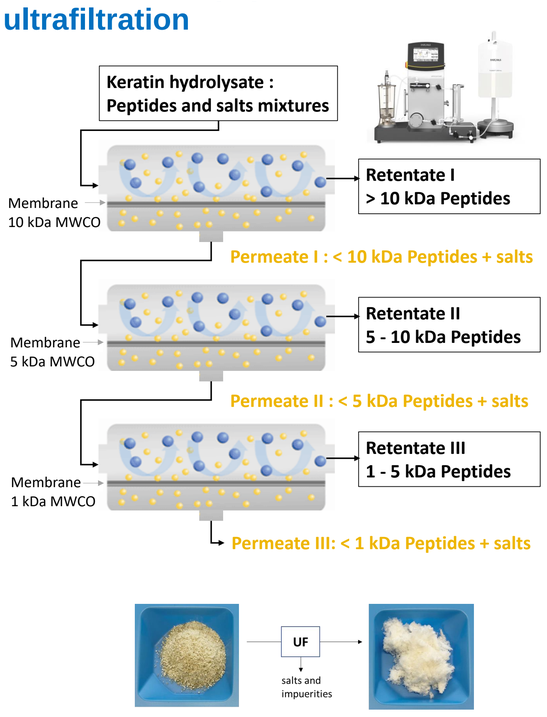


Scheme 1. Overview of the selected Keratin hydrolysis methods



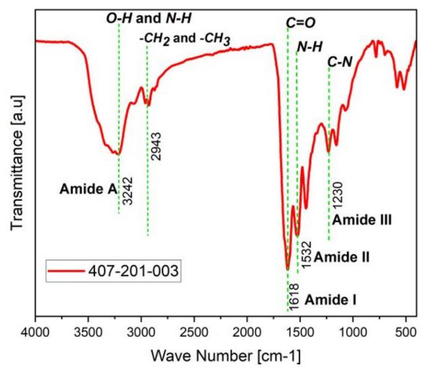
Precipitation of dissolved keratin extracted via L-cysteine reduction method, under acidic conditions

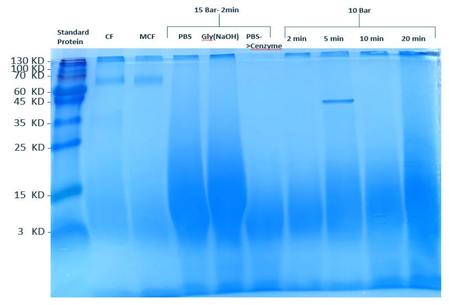
Purification of peptides' mixture via ultrafiltration



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

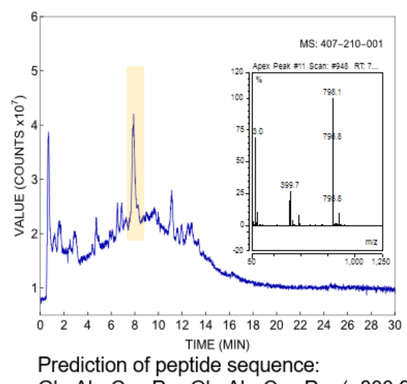
Characterization by FTIR and SDS-PAGE





SDS-PAGE is used to obtain high resolution separation of a complex mixture of proteins and peptides based on their molecular weight (MW). The figure shows SDS-PAGE profiles of different peptide mixture illustrating that peptides' MW are smaller at higher treatment conditions.

Purification & identification using RP-HPLC-UV and LC-MS

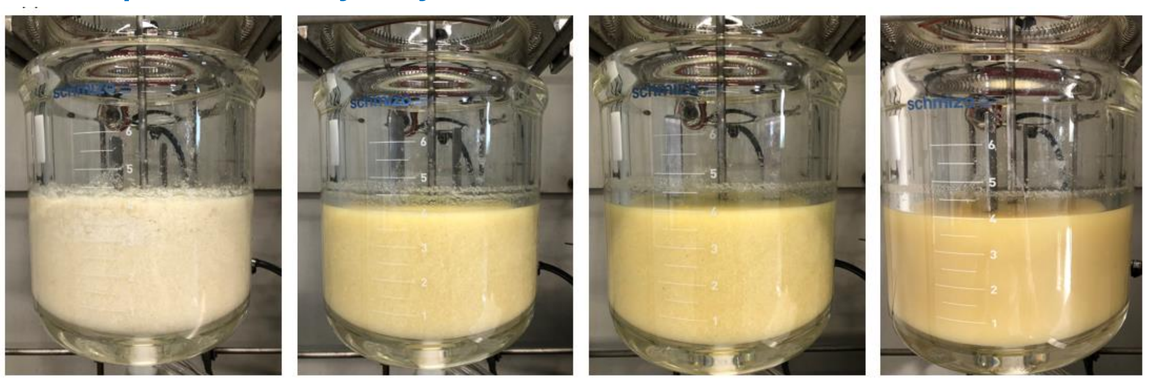


Prediction of peptide sequence:
Gly-Ala-Cys-Pro-Gly-Ala-Cys-Pro (~800.9 Da)

Up-scaling of AMPs extraction

All peptides mixtures obtained through the selected hydrolysis methods were tested on *E. coli* and *S. Aureus* bacteria, as well as on *CHIKV* virus. Several extracted AMP mixtures demonstrate antimicrobial activity. Based on these bioactivity results, two scalable and sustainable hydrolysis methods were selected for the production of AMPs at a larger scale.

Scale-up of alkaline hydrolysis

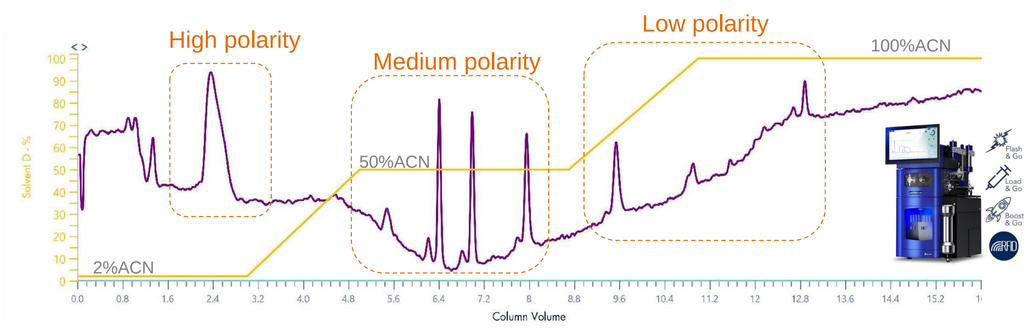


Scale-up of alkaline hydrolysis in a 5 L reactor – Appearance of the reaction mixture over time

Freeze-drying of the different ultrafiltrated fractions

Freeze-drying of the different peptide fractions of the scaled-up hydrolysates to gently remove water and obtain stable peptide format

Scaling-up separation by preparative HPLC



Chromatograms from preparative HPLC using a C18 column (US5C183-150/300). 20 mL/min flow rate, mobile phase gradient of water and acetonitrile. 40 mg of injected sample at 275 nm

Summary & Outlook

- Various hydrolysis methods are evaluated on poultry waste and the best approaches for optimal antimicrobial activity are selected for larger scale production.
- Several extracted AMP mixtures demonstrate antimicrobial activity and are further purified to isolate the most active peptide fractions.
- Studies are ongoing to explore the anchoring of bioactive keratin-based peptides on the surface of mesoporous copper-silica nanoparticles.

Acknowledgements

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