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Bio-based antimicrobial peptides for smart response self-disinfected surfaces

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Introduction

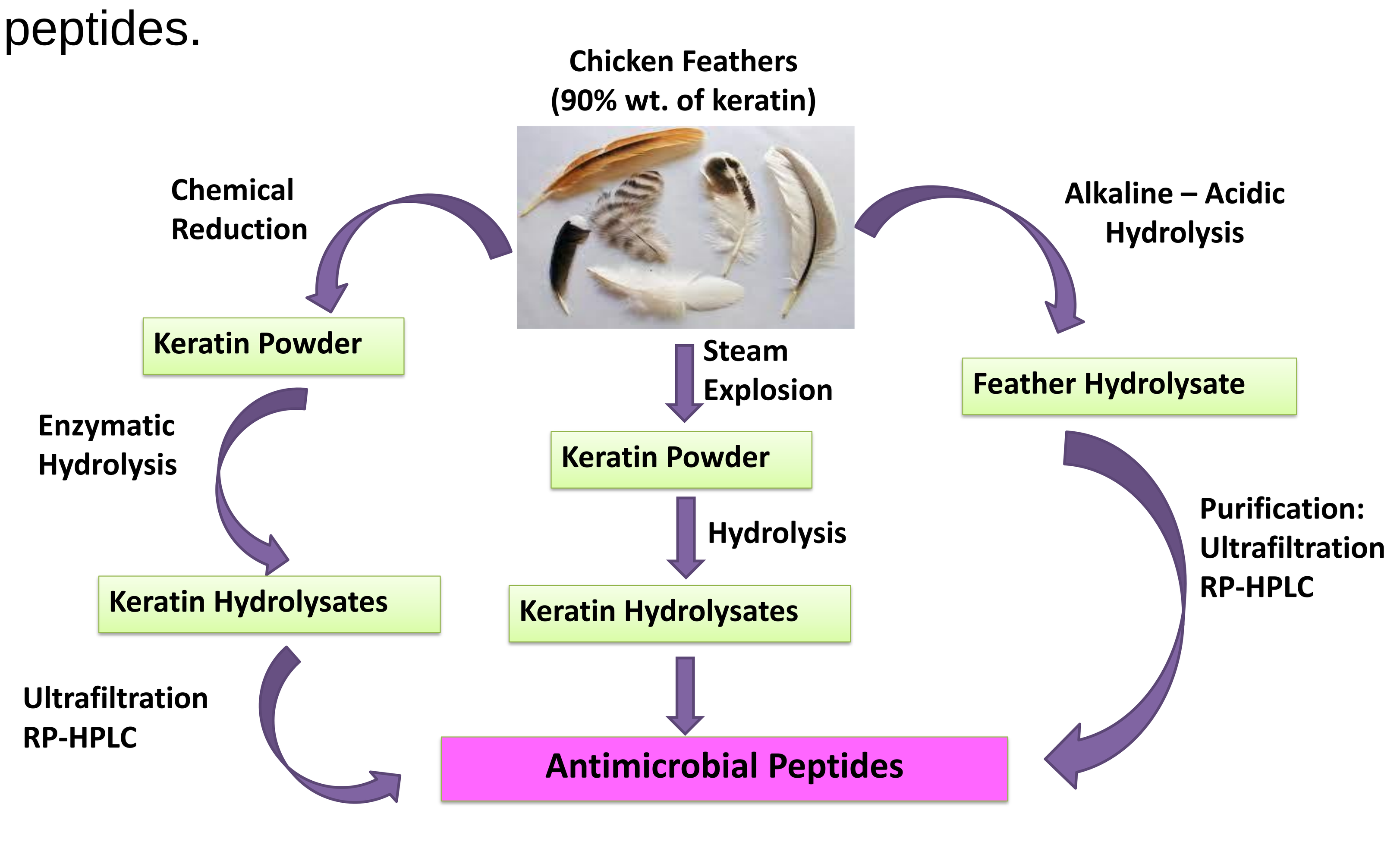
Significant cost of human life and economy is caused every year by microbial colonization of surfaces. In this context arises the Horizon Europe interdisciplinary project RELIANCE. RELIANCE aims to design and develop smart response self-disinfectant antimicrobial surfaces based on a new range of smart antimicrobial nanocoating consisting of copper doped mesoporous silica nanoparticles. These nanoparticles are modified with **Antimicrobial Compounds** based on either essential oils or peptides from protein-containing waste streams.

As RELIANCE's partner, HEIA-FR is responsible for the isolation of **Antimicrobial Peptides** (AMP) from non-valorized keratinous waste, such as chicken feathers. Here, we present the different hydrolysis approaches being currently investigated to obtain AMPs with optimal antimicrobial activity.

From Chicken Feathers to AMP

Keratin Hydrolysis Approaches

Although keratin is the third most abundant polymer in nature after cellulose and chitin, it is still underexploited due to its high cystine cross-link content which makes it highly stable and resistant to most proteases and reagents. Scheme 1 shows the most promising hydrolysis methods for the isolation of bioactive peptides.



Scheme 1. Overview of the selected Keratin hydrolysis approaches

Summary and outlook

The isolation of AMP requires a deep understanding of the structure-function relationship for that the use multiple characterization techniques is needed. These first investigations on the enzymatic hydrolysis approach are followed by bioactivity assays, which are being carried out by our partner UNITOV.

Studies on the alkaline hydrolysis are currently being performed. According to bioactivity results of the different hydrolysis methods, further purification using RP-HPLC and characterization will be performed.

First Hydrolysis results

Chicken Feathers and keratin extracted under reductive conditions have been subjected to enzymatic hydrolysis. The molecular weight pattern of soluble protein/peptides was analyzed by SDS-PAGE. Figure 2 shows a difference in protein/peptide bands (PBs) between both samples. Feather hydrolysates present bands at 260, 200, 55 and a thicker band at 10 KDa. Keratin hydrolysates reveal various PBs ranging between 100 and 18 KDa and a thicker band at 10 KDa. This indicates that enzymatic hydrolysis of keratin could generate a plethora of various peptides having different lengths and amino acid compositions and owing various bioactivities.

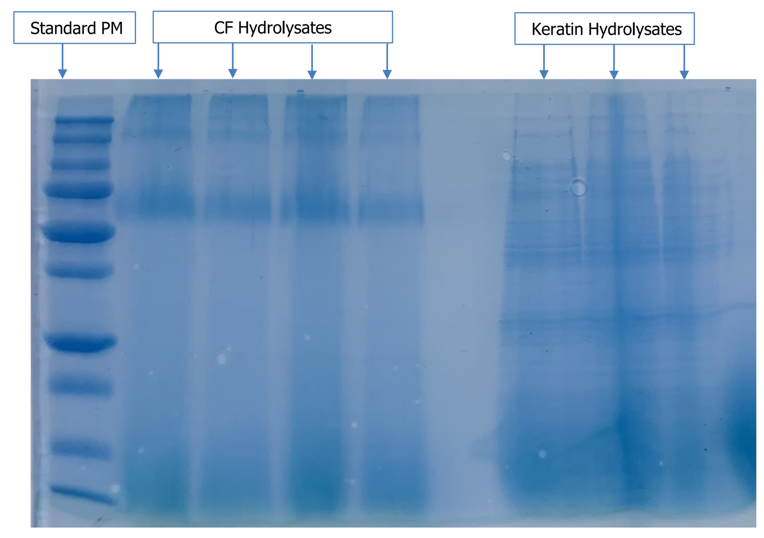


Figure 1. SDS-PAGE of CF and Keratin hydrolysates

Extracted Keratin and its corresponding hydrolysates were characterized by FT-IR spectroscopy. Figure 2a) shows that the hydrolysis of keratin peaks generates a shift of its main characteristic peaks towards higher wave numbers. We also observe a band at 2600 cm^{-1} associated with S-H binding and another at 1040 cm^{-1} characteristic for sulfur oxides, as a result of the cleavage of disulfide bonds. The secondary structure of keratin and KH were determined by deconvolution of FT-IR spectra. Keratin's secondary structure mainly consists of β-sheets coexisting with α-helix and β-turns. Enzymatic hydrolysis retain keratin secondary structure with the presence of a majority of β-sheets for higher MW hydrolysates and α-helix for lower MW hydrolysates.

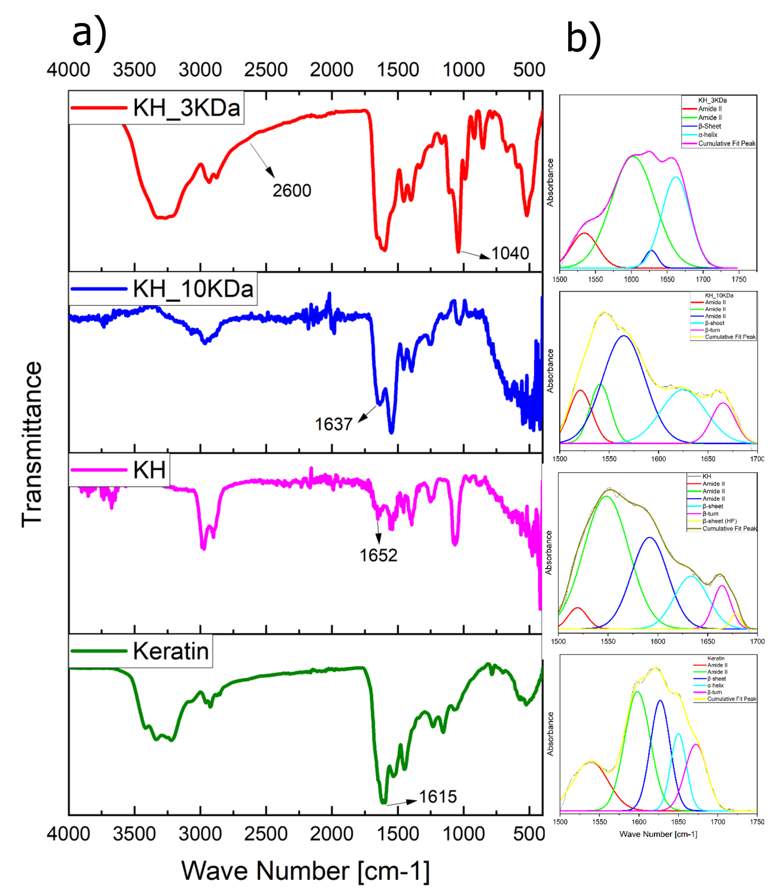


Figure 2. a) FT-IR spectra of Keratin and Keratin hydrolysates, b) Deconvolution of the amide I region for four samples

RELIANCE project partners

RELIANCE consortium is composed of 15 partners from 8 EU and 2 non-EU countries, to include research organizations, universities, SME and large industry partners.

Tekniker, MAIER, MILLIDYNE, POLYRISE, CENTEXBEL, RÉPUBLIQUE FRANÇAISE, INERIS, TOR VERGATA, EUROPROJECT, mpg, Arçelik, alsico, Haute école d'ingénierie et d'architecture Fribourg, UNIVERSITY OF PATRAS

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