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

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Abstract

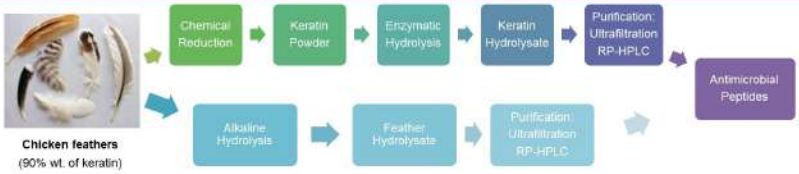
We are focused on the development of innovative self-disinfectant nano-coatings designed with unique antimicrobial nanoparticles, incorporating bioactive elements such as Antimicrobial Peptides (AMPs) sourced from chicken feathers. At the current preliminary stage, we are actively engaged in the extraction, characterization, and optimization process of obtaining these AMPs. The extraction process of keratin employs enzymatic and alkaline hydrolysis methods, while the resulting peptides are being characterized using a spectrum of analytical techniques which will be further presented and discussed.

Introduction

The widespread presence of microbes on surfaces carries significant annual costs for human health and the economy. In response, RELIANCE aims to pioneer innovative self-disinfectant antimicrobial surfaces. These surfaces integrate Antimicrobial Peptides (AMPs) from keratin-rich chicken feathers.

HEIA-FR, as the partner in the RELIANCE project, is responsible for AMP extraction using enzymatic and alkaline hydrolysis, followed by characterization. This initiative echoes our mission to reduce the impact of microbial colonization and introduce novel methods to promote surface hygiene and well-being.

Chicken feathers to AMPs



Scheme 1. Overview of the selected keratin hydrolysis approaches.

Keratin is often under-utilized due to its robust structure stemming from high cystine cross-link content. This makes keratin stable and resistant to degradation by proteases and chemicals. AMPs are short protein fragments that exhibit antimicrobial properties by disrupting the membranes of bacteria, fungi, and even viruses. AMPs range from around 5 to 50 amino acids in length about 500 – 6,000 Da.

AMPs Extraction & Characterization

Peptides obtained by the alkaline hydrolysis

Feathers were hydrolyzed into keratin hydrolysate using NaOH, where higher NaOH concentrations yield smaller peptide fragments. Bioactivity testing is ongoing to evaluate the hydrolysate's antimicrobial properties. The results will guide optimization of the hydrolysis process based on observed biological effects.

NaOH (M):	0 (bulk)	0.5	1.0	2.0
Yield (%w/w):	0	24.9%	74.0%	75.9%
GPC (Mn/ kDa)			8.67 kDa	

Scheme 2. Images of the feathers which were hydrolyzed by NaOH. The quantity of solubilized peptides exhibited a proportional relationship with the increased concentration of NaOH.

GPC chromatogram

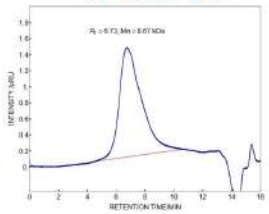


Figure 1. The GPC chromatogram of the peptide hydrolyzed by 1.0 M NaOH. The molecular number (Mn) was observed at 8.67 kDa. The GPC calibration curves (not shown) were fitted with a fifth-order polynomial function using PMMA as standards.

Keratin hydrolysates structures using FTIR

FTIR analysis of keratin hydrolysis displays peak shifts, confirming structural modifications with cleaved disulfide bonds, observed through S-H bonding (2,600 cm^{-1}) and sulfur oxides (1,400 cm^{-1}). Secondary structure assessment indicates preserved β-sheets in higher MW hydrolysates and α-helix prevalence in lower MW ones. Enzymatic hydrolysis significantly influences keratin's molecular arrangement, relevant in various applications.

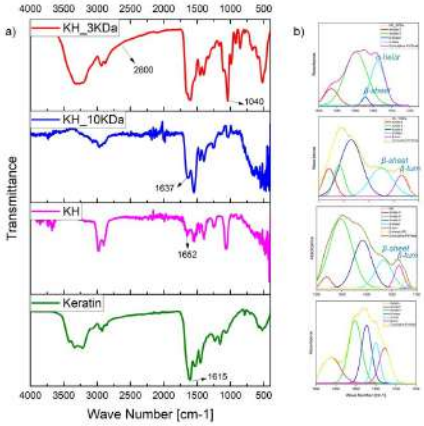


Figure 2. a) FT-IR spectra of keratin, keratin hydrolysate (KH), KH of ~3 kDa and KH of ~10 kDa. b) Deconvolution of *amide I* region (1,700-1,800 cm^{-1}). The α-helices and β-sheets are formed due to hydrogen bonding interactions between amino acid residues in the protein chain.

Characteristic bands of keratin provided by FTIR

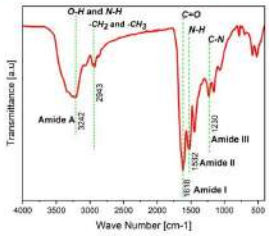


Figure 3. FTIR spectra of keratin. *Amide I* band (~ 1,600 cm^{-1}) contains significant details about the secondary structure of keratin.

Characterization using LC-MS

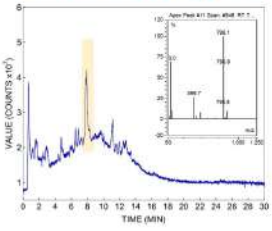


Figure 4. LC-MS analysis of a hydrolysate peptide with molecular weight of 798.1 Da, an insert chromatogram is a total ion count (TIC). This led to identification of the peptide with the amino acid sequence (prediction): Gly-Ala-Cys-Pro-Gly-Ala-Cys-Pro (~800.9 Da).

Acknowledgements

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Summary & Outlook

AMPs were extracted from chicken feathers using the hydrolysis methods that break them into smaller units. These peptides are undergoing further purification, detailed characterization and bioactivity assays. We're currently investigating various hydrolysis methods to refine the process for broader production and to acquire fully active AMPs. These AMPs will be integrated into our materials, driving advancements in self-disinfectant applications