

Preparation of renewable films based on feather-derived keratin: a comparative investigation of glutaraldehyde and genipin as crosslinkers

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1. Introduction

1.1 Background

Conventional petroleum-based materials remain widely used because they are inexpensive, available at large scale, and can be engineered for a wide range of technical applications. However, their life cycle is associated with several environmental burdens, including dependence on non-renewable resources, greenhouse gas emissions, accumulation of persistent waste, and the release of plastic-associated chemicals. In 2022, approximately 362 million tonnes of virgin plastics were still produced from petroleum feedstocks such as crude oil and natural gas (Houssini et al., 2025). Estimates suggest that primary plastic production generated approximately 2.24 Gt CO₂e in 2019, corresponding to 5.3% of global greenhouse gas emissions (Karali et al., 2024). At the end-of-life stage, plastic waste may accumulate in landfills and natural environments, while an estimated 19-23 million tonnes of plastic waste leak into aquatic ecosystems each year (Borrelle et al., 2020). These concerns have increased the need for materials that are not only functional during use, but also better aligned with sustainable production, processing, and end-of-life strategies.

These limitations are closely related to the linear economic model, in which raw materials are extracted, processed into products, used, and eventually discarded. A circular economy aims to reduce this loss of resources by keeping materials in use for longer and by recovering value from products or residues after their initial use (*Circular economy*, 2026; Kirchherr et al., 2023). For a material to be considered sustainable, it is not sufficient that it is bio-based or derived from a renewable source. Its production, processing, use phase, and end-of-life behaviour must also be evaluated (Rosenboom et al., 2022). Cradle-to-grave thinking is therefore necessary to assess whether a material reduces environmental impact across its full life cycle. Biodegradable materials may contribute to this transition, particularly in applications where recycling is difficult, contamination is likely, or material loss into biological waste streams may occur (Emadian et al., 2017; Rosenboom et al., 2022). However, biodegradability only has practical environmental value when degradation occurs under realistic and controlled conditions without producing harmful residues (Emadian et al., 2017).

Protein-based materials are one group of biomaterials that are promising for sustainable material development. Proteins contain functional chemical groups that can participate in intermolecular interactions, network formation, material behaviour, and biodegradability (Feroz et al., 2020; Lazarus et al., 2021). Commonly studied protein-based materials include collagen, gelatin, silk fibroin, soy protein, and keratin. These materials can be processed into films, coatings, hydrogels, scaffolds, and bioplastics (Feroz et al., 2020; Subramanian & VEDIAPPAN, 2015). Their potential value lies in the combination of renewable or residue-derived origin with functional material properties.

1.2 Keratin waste as a renewable resource

Keratin has been investigated as a group of protein-based materials. It is a structural protein found in biological materials such as wool, hair, nails, feathers, horns, and beaks (Wang et al., 2016). In these tissues, keratin provides mechanical support, protection and resistance to external damage (Lazarus et al., 2021; Wang et al., 2016). Extracted keratin can be regenerated into continuous films and has been studied for applications such as coatings, packaging-related materials, biomedical scaffolds, and other biopolymer systems (Feroz et al., 2020; Lazarus et al., 2021; Poole & Church, 2015; Zhang, Luo, et al., 2024). In many of these systems, keratin is not used as the only structural component but is blended with polymers such as polyvinyl alcohol, chitosan, gelatin, or cellulose to improve film formation and mechanical performance (Polesca et al., 2024; Wu et al., 2018; Zhang, Luo, et al., 2024). This reflects both the potential and the limitation of keratin as a material source: it is chemically versatile and biologically derived, but its performance as a regenerated material often requires additional stabilisation or modification.

Several extraction routes have been investigated to recover keratin from keratin-rich residual streams, including reductive extraction, deep eutectic solvent (DES)-based extraction, and enzymatic hydrolysis (Nuutinen et al., 2019; Poole et al., 2011; Sinkiewicz et al., 2017). These methods differ in extraction efficiency, environmental impact, and the quality of the recovered keratin. Reductive extraction can preserve longer protein chains because it cleaves disulphide bonds without extensively breaking the protein backbone, but it often relies on reducing agents that are difficult to reconcile with sustainable processing (Poole et al., 2011; Shavandi et al., 2017). DES-based extraction has been explored as a more sustainable alternative because these solvents can be prepared from

less volatile or less toxic components, although their environmental value depends on efficient recovery and reuse (Nuutinen et al., 2019; Pääkkönen et al., 2025). Enzymatic hydrolysis uses milder conditions and avoids harsh chemicals, but it generally breaks keratin into shorter peptide fragments or hydrolysates (Sharma et al., 2022; Sinkiewicz et al., 2017). These hydrolysates are easier to dissolve and process than native keratin, but their lower molecular weight can reduce their ability to form strong continuous networks.

Feathers, containing approximately 90% keratin, are produced as a by-product of poultry slaughter and represent a large residual stream from the poultry industry (Kamarudin et al., 2017; Mitchell, 2021; Tesfaye et al., 2017). In Europe, this stream amounts to approximately 3.6 million tonnes of feathers per year (Mitchell, 2021). They are mostly treated as waste and disposed of through incineration or landfill, while only part of the total feather stream is recovered for higher-value applications (Mitchell, 2021; Tesfaye et al., 2017). At present, poultry feathers are mostly processed into feather meal, which is used as a protein source in animal feed or as an organic fertiliser (Tefaye et al., 2017). Processing feathers into keratin hydrolysates has created an alternative route to convert this residual stream into a resource for cosmetics or coatings (Holkar et al., 2018; Moktip et al., 2025).

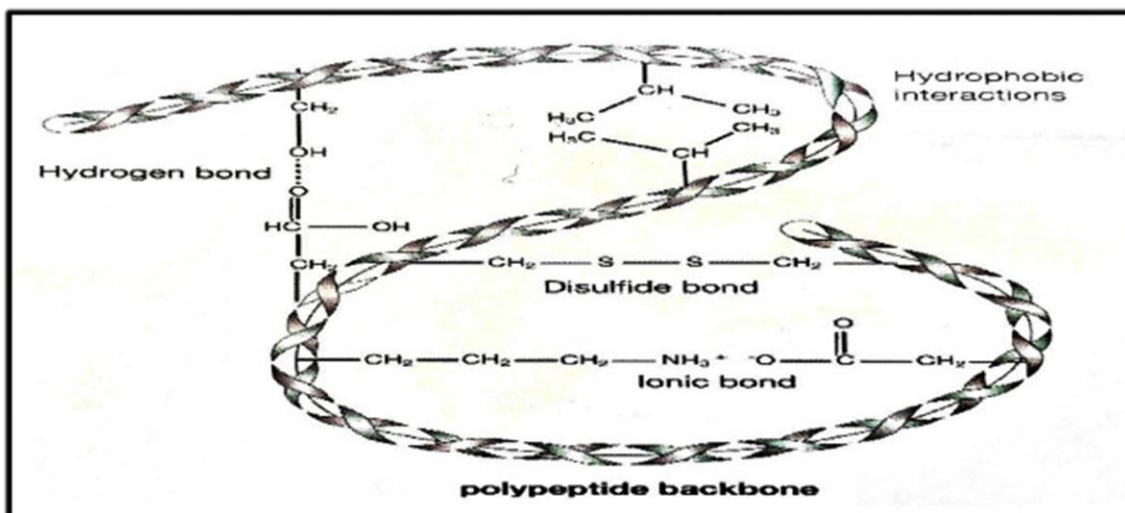
Hydrolysed keratin can be considered a relatively low-cost biopolymer, particularly when sourced as a cosmetic or bulk ingredient. Current B2B suppliers like Alibaba, Global Sources and ECHEMI place bulk hydrolysed keratin powder at approximately €30–45 kg⁻¹ (€0.03–0.05 g⁻¹) for cosmetic-grade material. No directly comparable highly purified research-grade hydrolysed keratin product was identified in the checked suppliers. Other research biopolymers are also substantially more expensive, especially collagen, with bovine type I collagen listed at approximately €185 per gram (Sigma-Aldrich, n.d.-a). Chitin is cheaper than collagen but still more expensive than bulk hydrolysed keratin, with shrimp-shell chitin listed at approximately €105 per 100 grams (€1.05 g⁻¹) (Sigma-Aldrich, n.d.-b). In practical terms, commercially available feather-derived hydrolysed keratin powder offers an accessible and relatively inexpensive starting material for material development.

1.3 Keratin classified into α - and β -keratin

Although keratin is often discussed as a single material, it refers to a group of related fibrous proteins that differ depending on their biological source and molecular organization (Banasaz & Ferraro, 2024). Like other proteins, keratin is composed of polypeptide chains built from amino acids. Its structure is characterized by relatively high amounts of cysteine and cystine, together with amino acids such as glycine, proline and serine (Feroz et al., 2020; Wang et al., 2016). These covalent bonds, together with hydrogen bonding, hydrophobic interactions and chain packing, contribute to the strength, stability and low solubility of keratin tissues (Moktip et al., 2025) (**Figure 1**).

Figure 1

Diagrammatic illustration of keratin structure



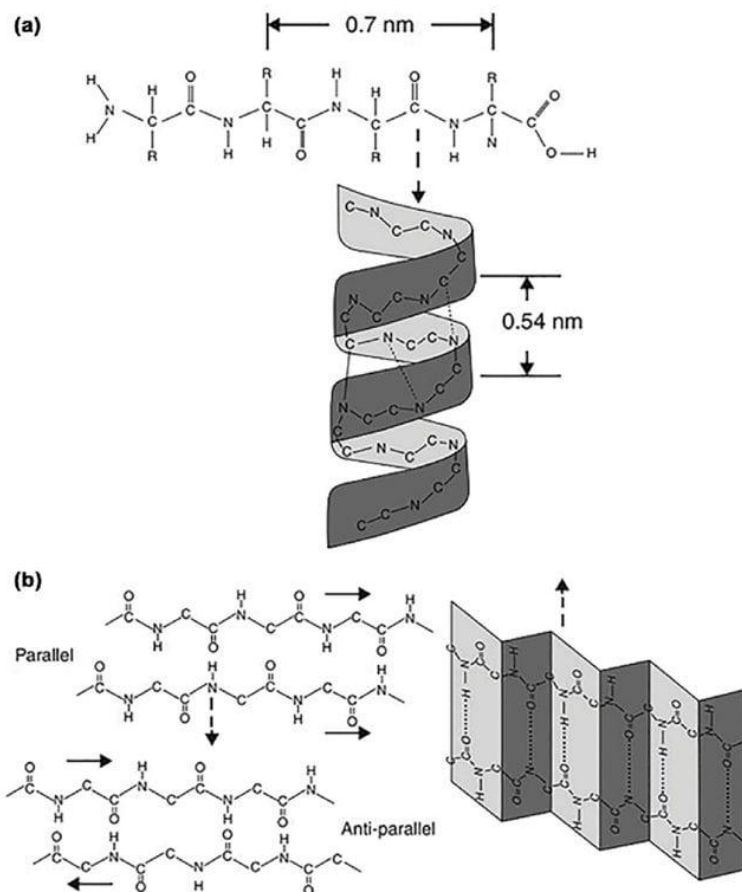
Note. Various inter and intramolecular bonding (hydrogen, ionic, disulphide bonds) increased the stability/strength of the keratin materials (Wang et al., 2016).

Keratins are commonly divided into α -keratin and β -keratin, based on their secondary structure (Moktip et al., 2025; Wang et al., 2016) (**Figure 2**). α -Keratin is mainly found in mammalian tissues such as wool, hair, horns and hooves (Feroz et al., 2020; Holkar et al., 2018). Its polypeptide chains are arranged in α -helical structures that assemble into intermediate filaments. β -Keratin is mainly associated with avian and reptilian structures such as feathers, beaks and claws (Banasaz & Ferraro, 2024; Feroz et al., 2020). Its sheet-like structure is strongly stabilized by intermolecular interactions and disulphide bonds, giving feather keratin a compact and resistant organization (Moktip et al., 2025; Wang et

al., 2016). This difference in molecular arrangement means that keratin from different biological sources cannot be treated as identical. However, once keratin is extracted or hydrolysed, the original hierarchical organization of the biological tissue is no longer fully preserved. At the same time, their practical use can be limited by insufficient mechanical strength, moisture sensitivity, poor stability, or processing difficulties (Mokrejš et al., 2011; Feroz et al., 2020; Rosenboom et al., 2022). Further modification or stabilisation is therefore often required before protein-based materials can be used in more demanding applications.

Figure 2

Molecule structure of keratin protein



Note. Two types of keratin structure with a: α-keratin (α-helix) and b: β-keratin (β-pleated sheet). (Chilakamarry et al., 2021)

1.4 Crosslinkers to improve performance of renewable keratin-based materials

One of the main strategies used to improve protein films is crosslinking. Crosslinking aims to increase the number of connections between polymer or protein chains, resulting in a denser and more stable network structure (Feroz et al., 2020; Wu et al., 2018). Depending on the crosslinker and processing conditions, these interactions may include hydrogen bonding, ionic interactions, disulphide bonds, or covalent bonds (Wu et al., 2018; Zhang, Luo, et al., 2024). By increasing intermolecular bonding, crosslinking can reduce chain mobility, improve resistance to swelling or dissolution, and increase mechanical stability (Iahnke et al., 2020; Wu et al., 2018; Zhang, Luo, et al., 2024). However, crosslinking should not be treated as a uniform modification strategy. More crosslinking may improve stability, but excessive or poorly controlled crosslinking can also reduce chain mobility to such an extent that the material becomes brittle (Feroz et al., 2020; Wu et al., 2018).

Different crosslinking approaches have been used to improve protein-based materials, and they differ in the type of interaction they introduce into the system. Commonly investigated crosslinkers or stabilising agents include; transglutaminase, calcium chloride (CaCl_2), genipin, glutaraldehyde, and carbodiimide-based systems such as EDC/NHS (Farris et al., 2010; Wahba, 2024; Wu et al., 2018). Transglutaminase is an enzymatic crosslinker that can form bonds between glutamine and lysine residues in proteins, whereas CaCl_2 is not a covalent crosslinker but can contribute to ionic interactions and network stabilisation through charged groups (Wu et al., 2018). Aldehyde-based crosslinkers, such as glutaraldehyde, are widely used in protein materials because they react with free amino groups and can form stable networks (Farris et al., 2010; Wahba, 2023). Genipin has also been studied as a natural crosslinking agent for several biopolymers because it reacts with primary amines and can form covalent links in amine-containing polymers or proteins (Mi et al., 2005; Tacias-Pascacio et al., 2019; Wahba, 2024).

1.5 Glutaraldehyde, an effective but toxic crosslinker

Glutaraldehyde (GA) is one of the most established chemical crosslinkers used for protein-based materials (Farris et al., 2010; Wahba, 2023). It is highly reactive toward

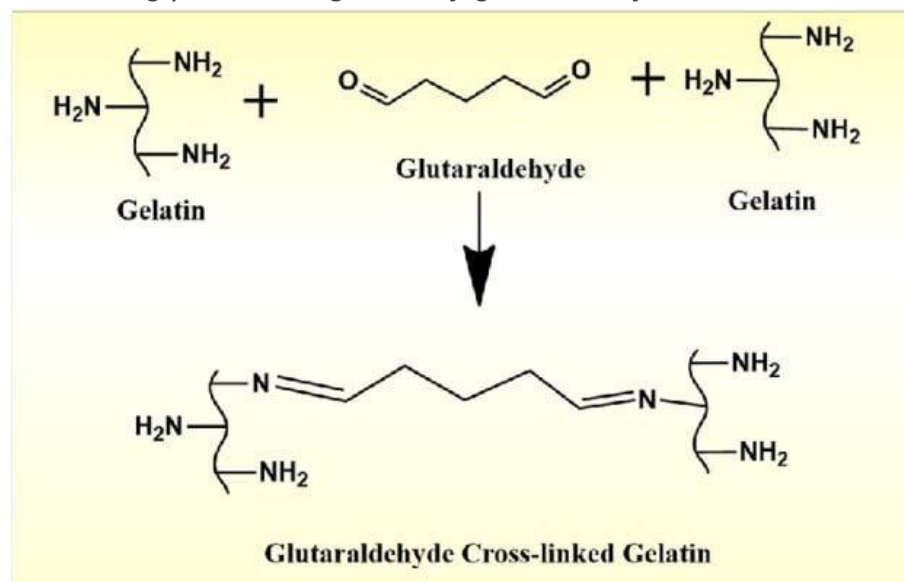
primary amines and is therefore effective in protein systems containing lysine residues, N-terminal amino groups, or other accessible amine-containing structures. GA reacts with primary amines mainly through Schiff base formation. In this reaction, the free, non-protonated amino group performs a nucleophilic attack on the aldehyde carbonyl carbon, forming an unstable carbinolamine intermediate. Subsequent proton transfer and dehydration result in the formation of an imine bond (Farris et al., 2010). Because GA contains two aldehyde groups, it can react with two amino groups and thereby form covalent crosslinks between polymer or protein chains (Farris et al., 2010; Wahba, 2023).

Figure 3 illustrates this reaction in gelatin, a protein containing many primary amines.

The effectiveness of GA makes it useful as a reference crosslinker. However, the same high reactivity that makes GA effective also creates important limitations. GA is associated with toxicity and can leave reactive residues in the material when crosslinking is incomplete or when washing is insufficient (Babb et al., 2001). Its aldehyde groups can react with nucleophilic functional groups in biological molecules, including proteins, enzymes, membrane components, and DNA-associated proteins (Farris et al., 2010; Speit et al., 2008). These reactions may disrupt biological function and can cause irritation upon contact with skin, eyes, or the respiratory tract (Babb et al., 2001).

Figure 3

Crosslinking process of gelatin by glutaraldehyde



Note. The scheme represents the final crosslinked structure and does not show intermediate reaction steps. Adapted from Sharma et al. (2022).

1.6 Genipin, a naturally derived and environmentally friendly alternative

Genipin (GNP) is a naturally occurring iridoid derived from the fruits of *Gardenia jasminoides* J. Ellis and *Genipa americana* L (**Figure 4**) (Ahmed et al., 2024; Ramos-de-la-Peña et al., 2016; Wahba, 2024). The compound can be found directly in these plants, but it can also be obtained through the hydrolysis of geniposide, its glycosylated precursor (Ahmed et al., 2024; Yang et al., 2018). Historically, both botanical sources were used before GNP became relevant in modern research. *Gardenia jasminoides*, which is widely distributed across several Asian countries, has a long history in traditional Chinese medicine, where fruit extracts were used in relation to inflammation, hepatic disorders, and other therapeutic applications (Bryś et al., 2022; Ramos-de-la-Peña et al., 2016). *Genipa americana*, native to Central and South America, has also been used traditionally, where its fruit, known as genipap, became relevant as a source of natural blue pigments (Ahmed et al., 2024; Assis et al., 2023; Náthia-Neves et al., 2019). In addition to these traditional uses, GNP has attracted pharmaceutical interest because studies have associated it with several biological activities, including anti-inflammatory, anticancer, and gastrointestinal effects (Bryś et al., 2022; Neri-Numa et al., 2020; Wahba, 2024). Beyond its botanical and pharmaceutical relevance, GNP has gained attention in material research because of its crosslinking capacity.

Figure 4

Fruit of Genipa americana, genipap and Gardenia jasminoides

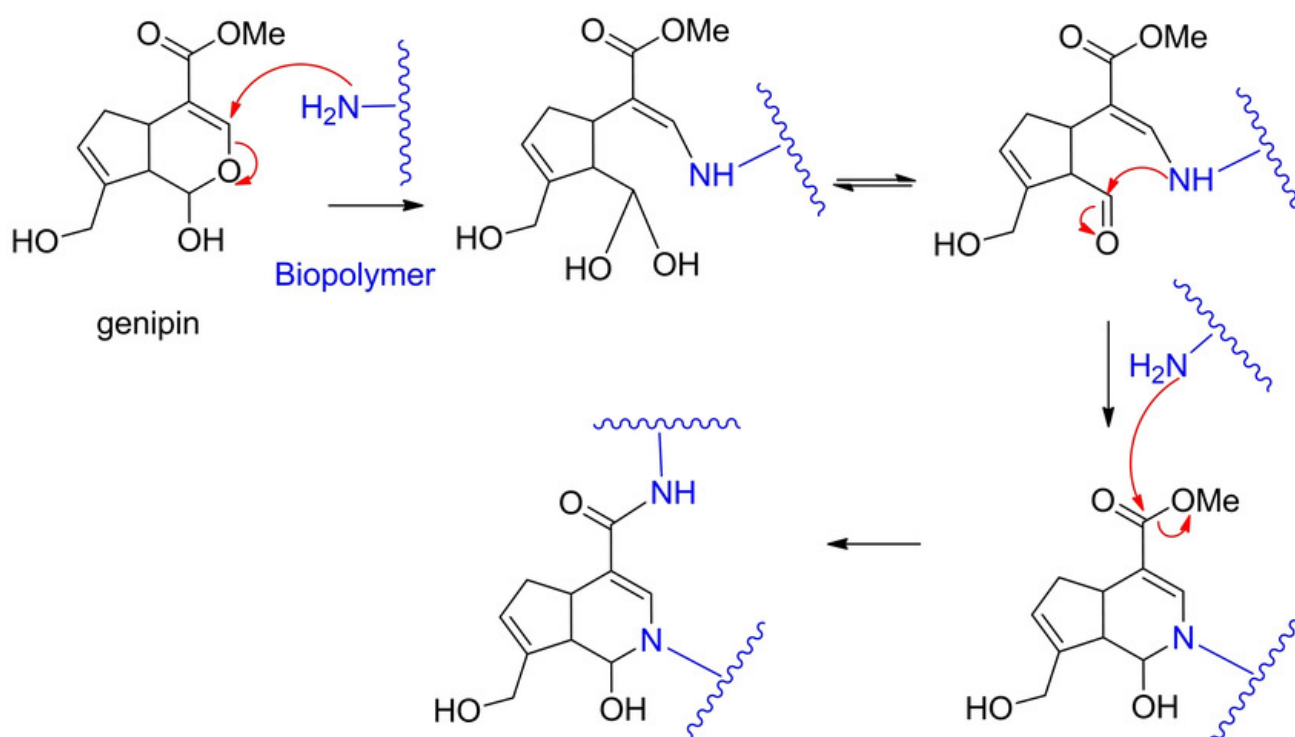


Note. Left image from *Genipa americana* by Trade Winds Fruit. Right image from *Gardenia jasminoides* fruit by Alpsdake, 2011, Wikimedia Commons, licensed under CC BY-SA 3.0.

Like GA, GNP reacts with primary amines and can form crosslinking bridges between amine-containing proteins or polymers (Mi et al., 2005; Pizzolitto et al., 2020; Wahba, 2024). During this reaction, it produces a characteristic blue pigment through a series of polymerization and oxidative coupling steps. It reacts with primary amines through two main reaction routes (**Figure 5**). In the first route, the nucleophilic amine attacks the electrophilic carbon of GNP's unsaturated dihydropyran ring, causing ring opening and formation of an aldehyde-containing intermediate. This intermediate can undergo intramolecular attack and rearrangement, producing a stable nitrogen-containing heterocycle (Mi et al., 2005; Pizzolitto et al., 2020; Wahba, 2024). In the second and slower route, a primary amine attacks the methyl ester carbonyl of GNP. This forms a tetrahedral intermediate, followed by elimination of the methoxy group and release of methanol, resulting in an amide bond (Mi et al., 2005; Pizzolitto et al., 2020; Wahba, 2024). Together, these reactions allow GNP to bind amine-containing polymers and form covalently crosslinked networks.

Figure 5

Mechanism for genipin crosslinking.



Note. The two nucleophilic reactions on genipin, reproduced from Shalayel et al. (2023)

1.7 Research gap

Previous research shows that keratin can be processed into films and that crosslinking is a common strategy to improve the stability and performance of protein-based materials (Ramakrishnan et al., 2018; Chen et al., 2018). However, much of the available literature focuses either on keratin blended with other polymers or on extracted keratin systems that differ from commercially available feather-derived hydrolysed keratin (Azeredo & Waldron, 2016; Ramakrishnan et al., 2018; Chen et al., 2018; Wu et al., 2018; Nuutinen et al., 2021). The specific challenge of using low-molecular-weight hydrolysed feather keratin as the main material component remains. This creates a gap between the availability of feather-derived keratin hydrolysates and their use in coherent, mechanically competent materials.

Within this gap, the comparison between GA and GNP is essential. GA can be used as an effective reference crosslinker because of its strong amine-reactive chemistry, but its toxicity limits its value as a sustainable long-term option (Farris et al., 2010; Wahba, 2023). GNP offers a less toxic and naturally derived alternative with a distinct reaction mechanism and visible blue pigment formation (Mi et al., 2005; Pizzolitto et al., 2020; Wahba, 2024), but its performance in hydrolysed keratin films depends on whether it can create a sufficiently effective network. The resulting material question is therefore whether commercially available feather-derived hydrolysed keratin can be processed into coherent films, and whether crosslinking with GA or GNP can improve their material behaviour.

2. Objectives

2.1 Research objectives

The aim of this thesis is to investigate whether commercially available and inexpensive hydrolysed keratin can be processed into a coherent film and whether the mechanical properties can be enhanced using different crosslinkers. Hydrolysed keratin is more readily processable than native keratin, but its lower molecular weight and reduced structural integrity may limit its ability to form stable materials on its own (Eslahi et al., 2013; Mokrejš et al., 2011; Sharma et al., 2022). Crosslinking is therefore explored as a strategy to improve network formation and mechanical performance. In this thesis, GA is a commonly used and effective, yet toxic reference crosslinker, while GNP is investigated as an environmentally friendly alternative (Farris et al., 2010; Tacias-Pascacio et al., 2019; Wahba, 2024). The resulting films are compared in terms of appearance, handling behaviour, and mechanical properties. Correspondingly, the research objectives (RO) of this thesis can be summarized as follows:

(RO1) To investigate whether stable keratin films can be obtained with and without crosslinkers (GA and GNP) using the commercially available feather-derived hydrolysed keratin.

(RO2) To compare the effect of GA and GNP on the appearance, flexibility, brittleness, and mechanical behaviour of the resulting films.

(RO3) To explore the influence of GNP concentration using an exploratory range of concentrations, with attention to the formulation that shows the most promising behaviour.

(RO4) To evaluate selected films quantitatively through material testing, focusing mainly on tensile strength, elongation and toughness.

2.2 Hypotheses

(H1) It is expected that hydrolysed keratin without a crosslinker will show limited film stability and material performance. Because commercially available hydrolysed keratin consists of relatively short protein fragments, these fragments are expected to have a reduced ability to form a strong and continuous network on their own (Eslahi et al., 2013; Mokrejš et al., 2011).

(H2) It is expected that crosslinking with GA or GNP will improve the coherence and mechanical stability of the keratin films compared with the un-crosslinked control. This is because crosslinking can increase intermolecular bonding and restrict chain mobility in protein-based materials (Feroz et al., 2020; Wu et al., 2018; Zhang, Luo, et al., 2024).

(H3) Lower GNP concentrations are expected to be insufficient to create a strong and coherent network, while higher concentrations may result in excessive crosslinking and increased brittleness. Therefore, the most useful GNP concentration is expected to be an intermediate concentration that balances film formation, handling, flexibility, and mechanical performance.

(H4) In the quantitative material tests, selected crosslinked films are expected to show improved mechanical behaviour compared with the un-crosslinked control, particularly in terms of tensile strength, elongation, and toughness. However, the improvement is not expected to be equal across all properties, as stronger crosslinking may increase stiffness or strength while reducing flexibility.

3. Materials and methods

3.1 Materials

Keratin derived from duck feathers through enzymatic hydrolysis was obtained from Yangzhou Joyvo Weikem Biology Co., Ltd. Genipin (GNP) (powder 98%) was obtained from Xi'an Finest Nutra Co., Ltd. Glutaraldehyde, glycerol and sodium hydroxide (NaOH) were supplied by Sigma-Aldrich. Deionized water and ethanol (ChemLab) were used for solution preparation.

3.2 Preparation of films

The film preparation procedure was adapted from Nuutinen et al. (2021). Keratin powder was first dissolved in deionized water. Glycerol was then added to the keratin solution at 30 wt% relative to the keratin mass, and the mixture was stirred homogeneously. The pH of the solution was adjusted to pH 8 using 5 M NaOH solution to increase the availability of nucleophilic amino groups (Nuutinen et al., 2021; Wahba, 2024). Afterwards, deionized water was added until a 20 wt% keratin solution of the ultimate mixture was obtained. For GA-crosslinked samples, the GA solution (25 wt%) was added to obtain a final GA concentration of 10 wt% relative to the dry keratin mass. For GNP-crosslinked samples, the required amount of GNP was first dissolved in a 20:80 ethanol/water solvent mixture to improve dispersion (Wahba, 2024), after which it was added to the keratin-glycerol solution at the selected concentration. The combinations considered during the preliminary screening are shown in **Table 1**. After crosslinker addition, the mixtures were gently mixed to obtain visually uniform solutions while avoiding bubble formation. The samples were incubated for 48 h at 50 °C under gentle stirring. After incubation, aliquots were cast into silicone molds and dried under a fume hood at room temperature until complete solvent evaporation.

Table 1.

Overview of the compositions included in the preliminary screening and final mechanical testing.

Test	Code	Crosslinker	Concentration relative to keratin	Performance
0_1	K0	None	0 wt%	Control
1_1	GA	Glutaraldehyde	10 wt%	Reference
2_1	GNP 0.5	Genipin	0.5 wt%	Preliminary
2_2	GNP 1	Genipin	1 wt%	Preliminary
2_3	GNP 2	Genipin	2 wt%	Preliminary
2_4	GNP 4	Genipin	4 wt%	Mechanical test
2_5	GNP 6	Genipin	6 wt%	Preliminary
2_6	GNP 8	Genipin	8 wt%	Preliminary
2_7	GNP 10	Genipin	10 wt%	Mechanical test
2_8	GNP 20	Genipin	20 wt%	Preliminary
2_9	GNP 30	Genipin	30 wt%	Preliminary

3.3 Preliminary screening

A preliminary screening was performed to evaluate the effect of crosslinker concentration on film formation and basic mechanical integrity. The purpose of this screening was to identify concentrations that could form coherent and manageable films suitable for mechanical testing. Manual tensile deformation was used only as a practical indication of whether the films had sufficient integrity for further mechanical testing. Based on this screening, the non-crosslinked keratin control was excluded from mechanical testing because it did not form coherent films (**Figure 6f**). GNP concentrations below 4 wt% resulted in films that formed but were too soft and difficult to handle reliably (**Figure 6g**). By contrast, concentrations above 10 wt% did not result in a clear additional improvement in film quality. Therefore, GNP-crosslinked samples containing 4 and 10 wt% GNP, referred to as GNP 4 and GNP 10, were selected for the final mechanical comparison.

3.4 Mechanical testing

Based on the preliminary screening, three formulations were selected for mechanical testing: GA-crosslinked keratin, 4 wt% GNP-crosslinked keratin (GNP 4), and 10 wt% GNP-crosslinked keratin (GNP 10). The same general preparation procedure as described above was used for the final films. After drying, the films were carefully removed from the molds and cut into strips with a width of 3 mm and a thickness of 1 mm, corresponding to a cross-sectional area of 3 mm². Mechanical properties were evaluated in Tech Lane Ghent Science Park (Department of Materials, Textiles and Chemical Engineering) using a FAVIMAT Fiber Test system in a climate-controlled room maintained at 20 °C and 65% relative humidity. For each recipe, five replicates were tested. The FAVIMAT test settings included a gauge length of 10.0 mm, a test speed of 10.0 mm/min, a pretension of 0.10 cN/tex, a nominal linear density of 1.00 dtex, and a 1200 cN load cell. The mechanical parameters extracted from the tests were tensile strength, elongation at break, and toughness. Stress-strain curves were also generated to visualize the mechanical response of the different formulations. Tensile strength was calculated by dividing the force at break by the nominal cross-sectional area of the specimen:

$$\text{Tensile strength (MPa)} = \frac{\text{force at break (N)}}{A \text{ (mm}^2\text{)}}$$

Elongation at break was obtained from the deformation at failure and expressed as a percentage. Toughness was calculated by dividing the work at break by the tested specimen volume:

$$\text{Toughness (kJ/m}^3\text{)} = \frac{\text{work at break (cNm)}}{\text{volume (m}^3\text{)}} \times 10^{-5}$$

Young's modulus was not used as a primary outcome because several stress-strain curves did not show a clearly defined and consistent linear elastic region. As a result, the calculated modulus would depend on the selected strain interval and would therefore be less informative for comparing the formulations.

3.5 Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) was used in Tech Lane Ghent Science Park (Department of Materials, Textiles and Chemical Engineering) as a complementary analysis to characterize the chemical profiles of the keratin powder and the crosslinked keratin-based films. FTIR spectra were obtained following a similar approach to Maudens et al. (2025). Spectra of keratin powder and of the GA-, GNP 4-, and GNP 10 films were recorded using a Nicolet™ iS50 FTIR spectrometer (Thermo Scientific) equipped with an attenuated total reflectance (ATR) module. The samples were excited using light with wavenumbers ranging from 4000 to 400 cm^{-1} , with a resolution of 4 cm^{-1} and 32 scans per measurement. Baselines were corrected using Origin 8.5 (Peak Analyzer) and spectra were normalized to their maximum peak.

3.6 Data analysis

Data analysis was performed in RStudio (version 4.5.2). The three recipes were compared for tensile strength, elongation at break, and toughness. Because each condition contained a small number of replicates, non-parametric statistical tests were used. For each mechanical parameter, a Kruskal-Wallis test was performed to assess whether there were overall differences between the three recipes. When the Kruskal-Wallis test indicated significant group differences, pairwise Wilcoxon rank-sum tests were conducted as post hoc comparisons. p -values from the pairwise comparisons were adjusted for multiple testing using the Benjamini-Hochberg correction. Statistical significance was evaluated at $\alpha = 0.05$.

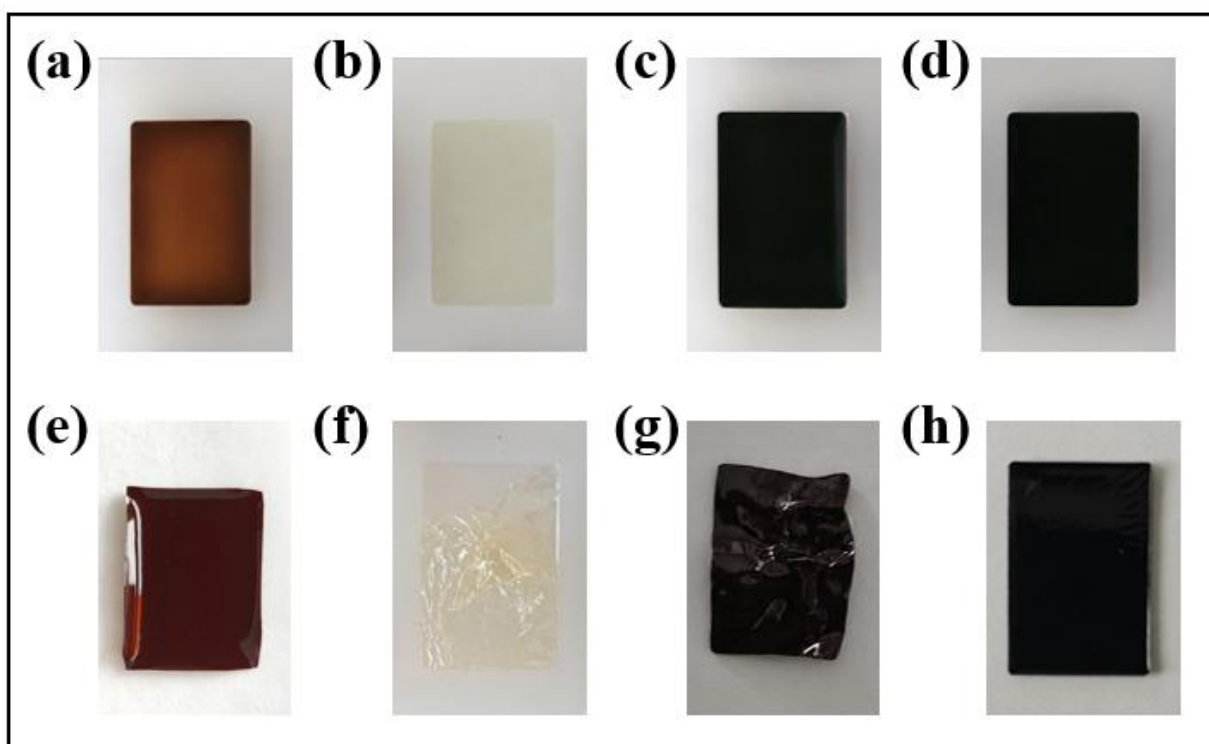
4. Results

4.1 Film formation and qualitative appearance

The tested compositions differed in film formation, appearance, handling behaviour, and water sensitivity. The non-crosslinked control did not form a continuous film (**Figure 6b, 6f**), whereas the crosslinked samples did produce films (**Figure 6e, 6g, 6h**). Clear visual differences were observed between the two crosslinking systems. GA-crosslinked films developed a brown colour after reaction (**Figure 6a**), while GNP-crosslinked films showed the dark blue colour typically associated with genipin crosslinking (**Figure 6c, 6d**). The samples also differed during handling: GA-crosslinked films appeared more brittle, whereas GNP-crosslinked films were more flexible but showed greater water sensitivity. Both GA- and GNP-crosslinked films dissolved in water, although dissolution occurred more slowly for the GA-crosslinked samples.

Figure 6

Keratin-based films: before, and after removal from the silicone mold.



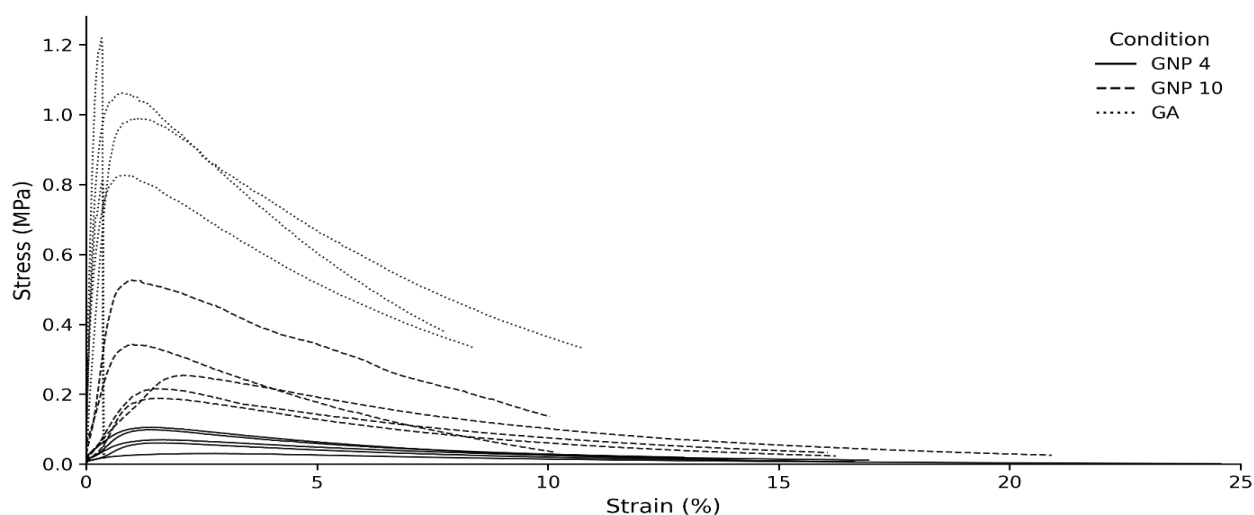
Note. Before: GA (a), K0 (b), GNP 2 (c) and GNP 4 (d), and after removal: GA (e), K0 (f), GNP 2 (g) and GNP 4 (h).

4.2 Mechanical response under tensile loading

The stress-strain curves provided a first overview of the mechanical response of the three crosslinked samples (**Figure 7**). The curves show a clear trade-off between stress resistance and deformability. GA-crosslinked samples reached the highest stress values but failed at lower strain values than the GNP-crosslinked samples. GNP 4 showed the lowest stress values but extended further before failure. Increasing the GNP concentration from GNP 4 to GNP 10 shifted the response towards higher stress values, while elongation remained higher than in the GA-crosslinked samples. GNP 10 therefore showed an intermediate mechanical profile between the more deformable GNP 4 samples and the stronger GA samples.

Figure 7

Stress-strain curves of individual keratin film samples by crosslinking condition



Note. Each curve represents one sample (total samples $n = 15$).

4.3 Mechanical properties and statistical comparison

The calculated mechanical properties confirmed the pattern observed in the stress-strain curves (**Table 2; Figure 8**). Tensile strength increased from GNP 4 (0.073 ± 0.030 MPa) to GNP 10 (0.306 ± 0.140 MPa). The tensile strength in GA-crosslinked films (0.982 ± 0.170 MPa) was on average 13 times larger than that of GNP 4. Elongation at break followed the opposite trend, with the highest values in GNP 4 ($17.71 \pm 6.08\%$) and the lowest values in GA ($6.80 \pm 3.19\%$). GNP 10 showed intermediate elongation ($14.68 \pm 4.62\%$). Toughness also increased from GNP 4 (8.58 ± 1.59 kJ/m³) to GNP 10 ($27.35 \pm$

6.55 kJ/m³) and was highest on average for GA (51.03 ± 25.26 kJ/m³), although variability was larger in the GA samples.

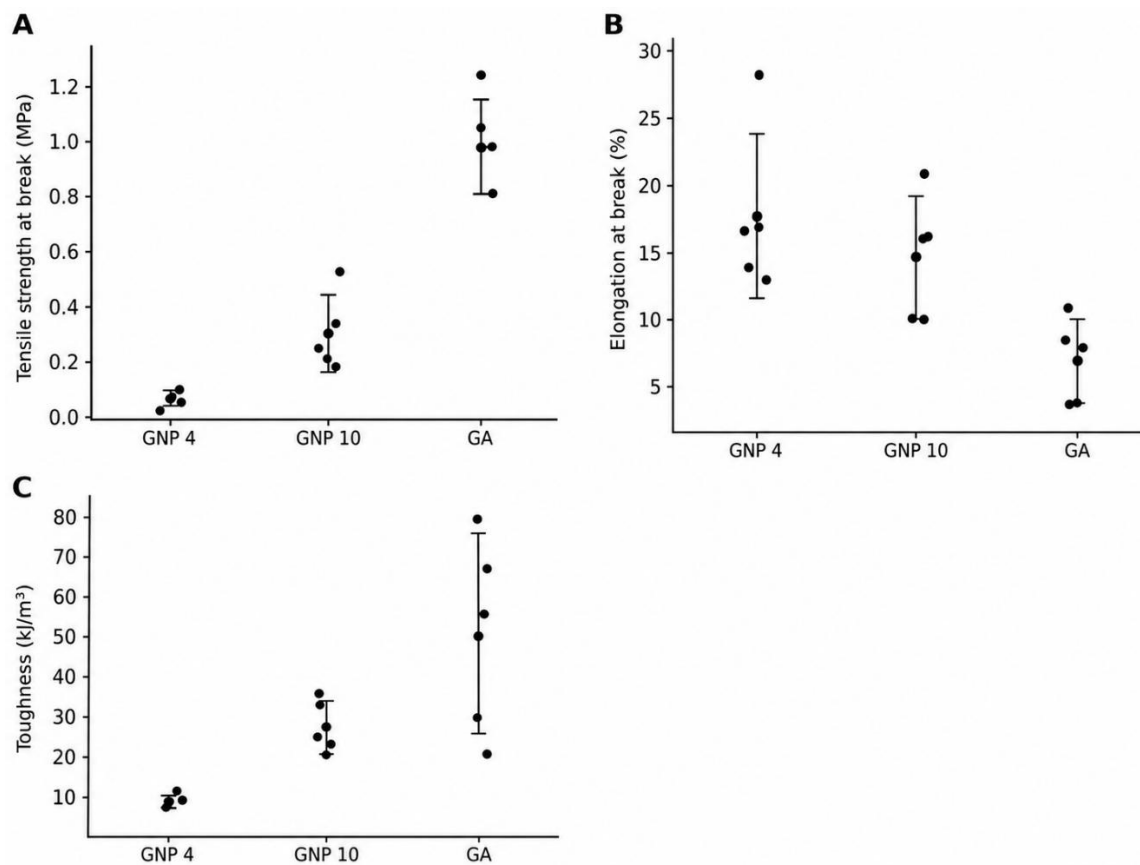
Table 2.

Mechanical properties of keratin films by crosslinking condition

Sample	Tensile strength (MPa)	Elongation at break (%)	Toughness (kJ/m ³)
GNP 4	0.073 ± 0.030	17.71 ± 6.08	8.58 ± 1.59
GNP 10	0.306 ± 0.140	14.68 ± 4.62	27.35 ± 6.55
GA	0.982 ± 0.170	6.80 ± 3.19	51.03 ± 25.26

Figure 8

Distribution of mechanical properties of keratin films by crosslinking condition.



Note. Individual points represent replicate samples. A = tensile strength, B = elongation at break, and C = toughness.

The statistical comparisons confirmed that crosslinking condition had a significant effect on all measured mechanical properties, including tensile strength, elongation at break, and toughness (**Table 3**). Tensile strength was the most clearly differentiated parameter, with significant pairwise differences between all samples after BH correction (**Table 4**). This supports the observed increase in strength from GNP 4 to GNP 10 and the substantially higher strength of GA-crosslinked films. For elongation at break, the statistical separation was weaker: GA differed significantly from GNP 4, but not from GNP 10 after correction, indicating that the reduction in deformability was mainly evident when comparing GA with the most extensible GNP sample. Toughness increased significantly from GNP 4 to both GNP 10 and GA, while GNP 10 and GA did not differ significantly from each other.

Table 3

Kruskal-Wallis tests comparing mechanical properties between crosslinking conditions.

Mechanical property	<i>p</i> -value	Significance
Tensile strength	0.0019	**
Elongation at break	0.0140	*
Toughness	0.0060	*

Table 4

Pairwise Wilcoxon rank-sum comparisons of mechanical properties between samples.

Mechanical property	Comparison	Adjusted <i>p</i> -value	Significance
Tensile strength	GNP 10 vs GNP 4	0.012	*
Tensile strength	GA vs GNP 4	0.012	*
Tensile strength	GA vs GNP 10	0.012	*
Elongation at break	GNP 10 vs GNP 4	0.403	<i>ns</i>
Elongation at break	GA vs GNP 4	0.037	*
Elongation at break	GA vs GNP 10	0.055	.
Toughness	GNP 10 vs GNP 4	0.018	*
Toughness	GA vs GNP 4	0.018	*
Toughness	GA vs GNP 10	0.210	<i>ns</i>

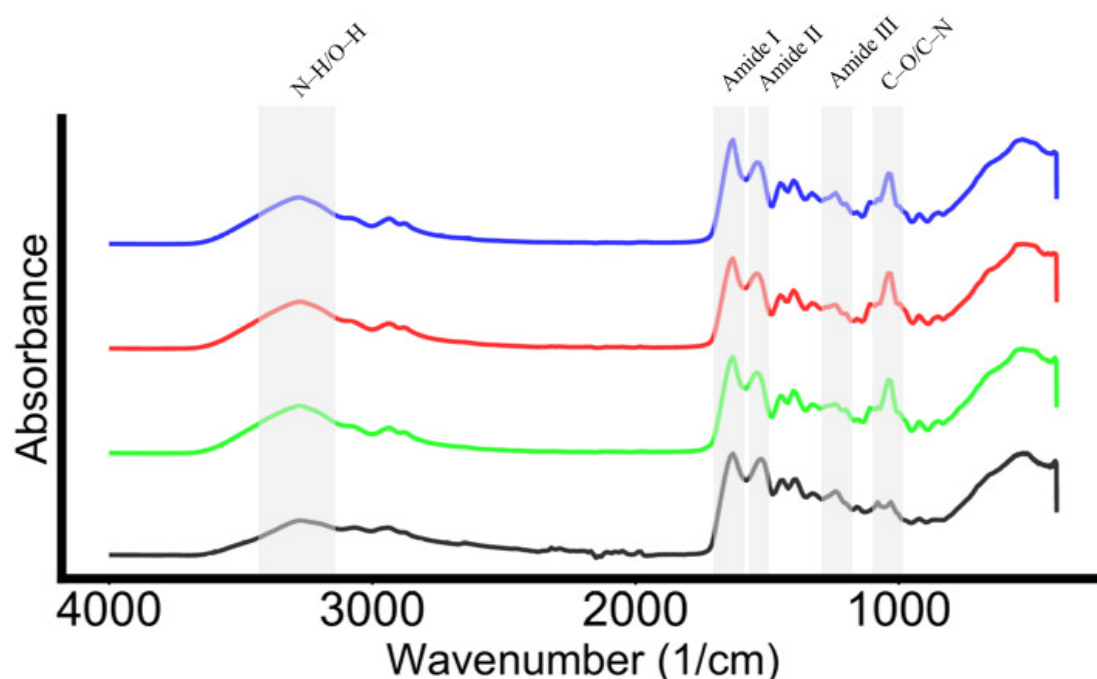
4.4 FTIR analysis

FTIR spectroscopy was used as a complementary analysis to compare the chemical profiles of keratin powder and the crosslinked keratin-based films. The spectra are shown in **Figure 9**. All samples showed characteristic protein- and keratin-related absorption bands. The broad absorption band around $3200\text{--}3500\text{ cm}^{-1}$ was assigned to N–H and O–H stretching vibrations, while the bands around $1600\text{--}1700\text{ cm}^{-1}$, $1520\text{--}1540\text{ cm}^{-1}$, and $1230\text{--}1240\text{ cm}^{-1}$ corresponded to the typical amide I, amide II, and amide III vibrations of protein-based materials (Barth, 2007; Movasaghi et al., 2008; Maudens et al., 2025). The region around $1000\text{--}1150\text{ cm}^{-1}$ was interpreted as part of the fingerprint region, where C–O and C–N related vibrations can contribute to the observed absorption pattern (Coates, 2006; Movasaghi et al., 2008).

The crosslinked samples showed spectral changes mainly in the amide II and C–N-related regions compared with keratin powder. Because these regions are associated with amino groups and peptide structures involved in GA- and GNP-mediated reactions, the observed changes support that the crosslinkers chemically modified the keratin system and altered intermolecular interactions within the films. The spectra of GNP 4 and GNP 10 were highly similar, with no clearly distinguishable new absorption bands separating the two genipin concentrations. FTIR indicated that keratin remained the dominant chemical component in all samples.

Figure 9

FTIR spectra of keratin powder and the crosslinked keratin-based samples



Note. Keratin powder (black) GNP 4 (green), GNP 10 (red), GA (blue). Grey shaded regions indicate the main absorption regions: N–H/O–H, amide I, amide II, amide III, and C–O/C–N-related vibrations.

4.5 Summary

In summary, the results showed that the non-crosslinked keratin control did not form a continuous film, while the GA- and GNP-crosslinked samples did. GA-crosslinked films were brown and appeared more brittle, whereas GNP-crosslinked films were dark blue and more flexible depending on the concentration. Mechanical testing confirmed this pattern quantitatively: tensile strength increased significantly from GNP 4 to GNP 10 and was highest in the GA-crosslinked samples. Elongation at break followed the opposite pattern, but only with a significant difference between GNP 4 and GA. Toughness was lowest for GNP 4, higher for GNP 10, and highest on average for GA, although this difference was not statistically significant, partly reflecting the higher variability observed in the GA samples. FTIR analysis showed characteristic keratin-related absorption bands in all samples and crosslinking-related chemical modifications in the crosslinked samples compared to the keratin powder.

5. Discussion

5.1 Film formation from hydrolysed feather keratin

This thesis investigated whether commercially available hydrolysed feather keratin can be processed into coherent films and whether crosslinking can compensate for the expected limitations of this low-molecular-weight material. The results show that, under the tested conditions, the non-crosslinked keratin control did not form a coherent film, whereas crosslinked samples did. This supports the assumption that hydrolysed keratin is processable but structurally limited. The FTIR spectra confirmed that the crosslinked films remained dominated by characteristic keratin amide bands, indicating that film formation did not replace the keratin-based character, but crosslinking-related chemical modification did occur. Native feather keratin derives its mechanical stability from hierarchical organization, disulphide bonds, hydrogen bonding, hydrophobic interactions, and chain packing (Feroz et al., 2020; Wang et al., 2016). Hydrolysed keratin no longer retains this native structure in the same way. Nuutinen et al. (2021) showed that higher-molecular-weight keratin fractions produced films with better mechanical properties and moisture stability than lower-molecular-weight fractions, supporting the idea that molecular weight and network formation are critical for keratin film performance.

5.2 Crosslinker network formation

The comparison between GA and GNP indicates that crosslinking did not simply “increase strength,” but produced different network structures. GA generated the strongest films, which is consistent with its established role as a highly effective protein crosslinker. However, GA is not a clean bifunctional linker that only connects two primary amines. In aqueous solution, GA can exist in multiple hydrated, cyclic, oligomeric, and polymeric forms (Migneault et al., 2004). This complex chemistry may have produced a dense and heterogeneous network, possibly including multi-point anchoring or GA-polymeric segments. Such a network could explain the high tensile strength of GA films, but also their lower deformability. Similar behaviour was reported by Bigi et al. (2001), who found that increasing glutaraldehyde crosslinking in gelatin films increased stress at break and Young’s modulus while reducing deformability. GA’s performance is best interpreted as a strong but restrictive reinforcement. In protein-based materials, plasticization generally

increases elasticity (Lamp et al., 2022). The stronger GA network probably limited molecular mobility and reduced the ability of glycerol to act as an effective plasticizer and may therefore have shifted the material too far towards rigidity. Its strength confirms that hydrolysed keratin fragments can be mechanically reinforced.

The toughness results suggest that energy absorption can arise from different mechanical strategies. In the GA films, toughness was mainly driven by high stress resistance, whereas the increased toughness of GNP 10 resulted from a more balanced combination of moderate strength and higher elongation. This indicates that the GNP network may have retained more molecular mobility before failure, allowing mechanisms such as chain sliding, fragment rearrangement, local stress redistribution, or delayed crack propagation. For flexible film development, toughness is therefore more informative than tensile strength alone, because it captures how much force the material can resist and how much deformation it can tolerate before breaking.

5.3 Influence of GNP concentration

The recipes selected for mechanical testing had relatively high GNP concentrations. Bigi et al. (2002), Wu et al. (2018), Rao et al. (2022) and Hackenhaar et al. (2022) used much lower genipin concentrations, ranging from 0.05 – 2.0 wt% relative to the protein source. These studies use longer protein chains and additional polymer matrices. In the present study, hydrolysed keratin had to function as the main structural phase. Since hydrolysis shortens peptide chains and may increase the relative number of terminal amino groups, a higher amount of GNP may be needed before enough intermolecular bridges form. However, because free amine content was not quantified, this remains a plausible explanation rather than a confirmed mechanism. The blue colour of the GNP films supports that genipin reacted with amine-containing compounds, but it should not be treated as direct evidence of mechanically effective crosslinking. Genipin binding and interchain reticulation are not necessarily the same event (Pizzolitto et al., 2020). Therefore, the visible pigment formation confirms chemical activity, but not whether those reactions produced load-bearing bridges.

Genipin produced a mechanical profile that depended strongly on concentration. At the lower tested concentration, film formation was possible, but the resulting network remained mechanically weak, as reflected by limited strength and toughness. Increasing

the GNP concentration improved both properties while preserving more deformability than GA. In polymer networks, mechanical behaviour depends not only on crosslinking chemistry, but also on topology, connectivity, heterogeneity, and stress transfer through the network (Creton, 2017; Rajawasam et al., 2023). This suggests that the lower concentration may have generated mainly local reactions or isolated crosslinked clusters, without sufficient network-spanning connectivity to carry load efficiently. At the higher concentration, more keratin fragments may have become incorporated into a continuous load-bearing network. The observed improvement can be interpreted as a concentration-dependent increase in network effectiveness, rather than simply as the formation of more chemical bonds. The FTIR analysis did not clearly distinguish GNP 4 from GNP 10. However, this does not contradict the mechanical results: FTIR detects functional groups and molecular environments but does not directly measure load-bearing network architecture, crosslink effectiveness, or stress distribution (Rajawasam et al., 2023).

Overall, GNP 10 appears to be the most promising condition in this exploratory study: not as an optimized material, but as a more balanced and less toxic starting point for further development of hydrolysed keratin-based films.

5.4 Research limitations

This thesis provides an exploratory first step in the development of hydrolysed feather keratin films, but several limitations restrict the strength of the conclusions. First, the study used one commercially available hydrolysed keratin source. The free amine content of this material was not experimentally determined. The interpretation of film formation and GNP concentration effects relies strongly on chain length and terminal amines. Assays such as TNBS or ninhydrin-based free amine quantification could help determine whether GNP should be added based on wt% relative to keratin or based on a molar ratio between genipin and available amine groups.

Second, the preliminary screening of GNP concentrations was mainly qualitative. It was useful for identifying samples that could be handled and mechanically tested, but it did not provide quantitative information on crosslinking degree, film homogeneity, solubility, or network formation. As a result, the selection of GNP 4 and GNP 10 should be interpreted as a practical selection based on observed film quality rather than as evidence that these concentrations define the true lower and upper limits of useful GNP crosslinking. The

apparent lack of improvement above 10 wt% also cannot be interpreted as proof of chemical saturation, because no direct crosslink-density measurements were performed.

Third, the mechanical testing was limited by the small number of replicates and by the practical difficulty of preparing uniform film strips. Although all samples included in the mechanical analysis were intact, the strip width was targeted at 3 mm but had slight deviations for every specimen. Because tensile strength and toughness depend directly on specimen dimensions, small deviations in width may have affected the calculated values. In addition, Young's modulus was not used as a primary outcome because the stress-strain curves did not show a consistent linear elastic region. This means that stiffness cannot be compared reliably between conditions.

Fourth, the chemical interpretation remains partly speculative. FTIR confirmed the keratin-based character of the films and supported that processing and crosslinking changed the molecular environment, but it did not quantify crosslink density or distinguish clearly between GNP 4 and GNP 10. FTIR is therefore useful as supporting evidence, but it cannot prove whether reactions formed local or network-spanning crosslinks. More direct chemical and network characterization would be needed to support this mechanism.

Finally, the observed water sensitivity was not systematically tested. The dissolution behaviour provides a relevant practical indication that the films are not yet suitable for humid or aqueous applications, but it should not be treated as a formal water-stability result. Similarly, the sustainability relevance of GNP compared with GA was discussed from the perspective of toxicity and material development, but no life-cycle assessment, residual crosslinker analysis, or biodegradation testing was performed. The present study therefore supports the feasibility of crosslinked hydrolysed keratin films, but not their application readiness.

5.5 Future research

Future research should not only optimize the amount of GNP but also explore alternative strategies for building network connectivity in hydrolysed keratin films. One direction would be to test hybrid crosslinking systems, such as low-dose GA combined with GNP, to determine whether different crosslinking chemistries create additive or complementary effects on strength, deformability, and toughness. This would be useful mainly as a mechanistic comparison, because GA remains less suitable from a sustainability and toxicity perspective.

A second direction is to target functional groups other than primary amines. Since hydrolysed keratin contains multiple reactive chemical groups, future studies could evaluate more sustainable crosslinkers that act through different mechanisms, such as enzymatic crosslinking, oxidized sugar derivatives, polyphenol-based interactions, or disulphide-restoration strategies. This would test whether a more effective network can be created by combining different types of molecular interactions.

Another promising route is to improve the network-forming capacity of hydrolysed keratin before crosslinking. Future studies could compare the present commercial hydrolysate with higher-molecular-weight keratin fractions, or explore approaches such as enzymatic chain extension, controlled self-assembly, or disulphide-restoration strategies before film casting. Such strategies would shift the focus toward partially rebuilding the structural features that make keratin mechanically useful. However, this route would only be valuable if the added processing steps do not substantially increase production cost or complexity, because the accessibility and low cost of hydrolysed keratin are central to its material-development potential.

Finally, once the pure hydrolysed keratin system is better understood, future research can explore broader material-development routes. These may include combining crosslinked keratin with reinforcing biopolymers, hydrophobic modifications, or layered film structures.

6. Conclusion

This thesis investigated whether commercially available feather-derived hydrolysed keratin can be processed into coherent films and whether crosslinking with glutaraldehyde (GA) or genipin (GNP) can improve their material behaviour. Based on the obtained results, the following conclusions can be drawn:

- Commercially available hydrolysed feather keratin used in this study showed limited film-forming ability without crosslinking. Coherent films can be obtained after hydrolysed keratin is crosslinked with glutaraldehyde or genipin.
- Glutaraldehyde- and genipin-crosslinked keratin films showed different mechanical properties: where glutaraldehyde/keratin films can be strongly reinforced, but the resulting films became relatively rigid, whereas genipin/keratin films appeared to improve film stability without shifting the material as strongly towards rigidity.
- Genipin concentration also significantly influenced the mechanical performance of hydrolysed keratin films, with higher concentrations producing stronger and tougher films while preserving deformability. The preliminary screening suggests that genipin concentration must be sufficiently high to produce films that are coherent enough to handle.
- In this study, glutaraldehyde-treated films showed the highest tensile strength, while genipin samples showed the greatest elongation.

Overall, the results show that commercially available feather-derived hydrolysed keratin can be processed into coherent films when crosslinking is applied, although further optimization is needed before the materials can meet the requirements for practical use.

7. Summary

Conventional petroleum-based materials remain widely used because they are inexpensive, available at large scale, and technically versatile. However, their full life cycle is associated with environmental burdens, including dependence on non-renewable resources, greenhouse gas emissions, and persistent waste. These concerns have increased interest in materials that are functional during use, but also better aligned with sustainable production, processing, and end-of-life strategies. Within this context, protein-based materials may provide great potential, because they can be derived from renewable or residual biological streams and contain functional groups that allow network formation, biodegradability, and chemical modification, to better fit the target functions.

Keratin is one such protein. It occurs in biological structures such as feathers, hair, wool, and claws, where it provides mechanical support and protection. Feathers contain a high keratin content and are produced in large quantities as a by-product of the poultry industry. This residual stream could therefore be used as a renewable resource for material development. Hydrolysed feather keratin is especially accessible because it is commercially available, relatively inexpensive, and easier to dissolve and process than native keratin. However, this processability comes with a structural limitation. Hydrolysis breaks keratin into shorter peptide fragments, which reduces the ability of the material to form strong and continuous networks on its own.

Although keratin films and crosslinking strategies have been studied before, most research focuses on keratin blends or extracted keratin systems that differ from commercially available feather-derived hydrolysed keratin. This leaves uncertainty about whether this accessible hydrolysate can function as the main structural phase in coherent, mechanically competent films. Within this gap, the comparison between glutaraldehyde (GA) and genipin (GNP) is relevant. GA is an effective and widely used protein crosslinker, but its toxicity limits its value as a sustainable long-term option. GNP is a naturally derived and less toxic alternative that reacts with primary amines and forms a characteristic blue pigment, but its ability to create mechanically effective networks in hydrolysed keratin films remains less clear.

The aim of this thesis was therefore to investigate whether commercially available feather-derived hydrolysed keratin can be processed into coherent films and whether crosslinking with GA or GNP can improve their material performance. The study focused on film formation, qualitative appearance, handling behaviour, tensile strength, elongation at break, toughness, and chemical characterization using Fourier-transform infrared spectroscopy. The central expectation was that crosslinking would compensate for the limited network-forming ability of hydrolysed keratin, but that GA and GNP would produce different balances between strength and deformability.

Films were prepared from duck feather-derived hydrolysed keratin and crosslinked with either GA or GNP. GA was used at 10 wt% relative to keratin as a reference crosslinker, while GNP concentrations were first screened to identify handleable films. Based on this screening, GNP 4 and GNP 10 were selected for mechanical comparison with GA. Five replicates per condition were tested, and tensile strength, elongation at break, and toughness were calculated from stress-strain data. FTIR spectroscopy was used as a complementary method to compare the chemical profiles of the keratin powder and crosslinked films.

The results showed that the non-crosslinked keratin control did not form a continuous film, whereas the crosslinked samples did. GA-crosslinked films developed a brown colour and appeared more brittle, whereas GNP-crosslinked films became dark blue and were generally more flexible. Both crosslinked systems remained water-sensitive. The stress-strain curves showed a clear trade-off between stress resistance and deformability. GA-crosslinked films reached the highest stress values but failed at lower strain values. GNP 4 showed the lowest stress resistance but the highest elongation. Increasing the GNP concentration from 4 to 10 wt% improved tensile strength and toughness while preserving more deformability than GA.

Quantitatively, tensile strength increased from 0.073 ± 0.030 MPa for GNP 4 to 0.306 ± 0.140 MPa for GNP 10, and was highest for GA at 0.982 ± 0.170 MPa. Elongation followed the opposite pattern, with GNP 4 reaching $17.71 \pm 6.08\%$, GNP 10 reaching $14.68 \pm 4.62\%$, and GA reaching $6.80 \pm 3.19\%$. Toughness was lowest for GNP 4, higher for GNP 10, and highest on average for GA, although GA also showed greater variability.

Statistical comparisons confirmed significant differences between crosslinking conditions, especially for tensile strength and toughness.

These results indicate that crosslinking can partly compensate for the structural limitations of hydrolysed keratin, with GA and GNP reinforcing the material through different mechanical profiles. GA produced the strongest films, likely due to its high reactivity and ability to create a dense network, but this also restricted deformability. GNP produced a more concentration-dependent response. At 4 wt%, the network was too weak to provide substantial strength or toughness. At 10 wt%, GNP appeared to create a more effective load-bearing network, resulting in improved toughness while still maintaining relatively high elongation. The blue colour of the GNP films confirmed chemical activity, while FTIR showed that keratin remained the dominant chemical component.

Several limitations still exist in this thesis. First, only one commercial hydrolysed keratin source was used, and its free amine content were not determined. The GNP screening was qualitative, the mechanical sample size was limited, and small variations in specimen dimensions may have influenced the calculated values. In addition, water stability, biodegradation, residual crosslinker content, and life-cycle impacts were not systematically assessed. Future research should therefore quantify free amine content, optimize GNP concentration based on available reactive groups, evaluate water stability, and explore additional strategies to improve network connectivity.

Overall, this thesis shows that commercially available feather-derived hydrolysed keratin can form coherent films when crosslinked. GA produced the strongest but least deformable films, whereas GNP, especially at 10 wt%, offered a less toxic route with a more balanced mechanical profile. Although the films are not yet application-ready, GNP-crosslinked hydrolysed keratin paves a new pathway for yielding low-cost, residue-derived protein-based materials.

8. Nederlandse samenvatting

Conventionele aardoliegebaseerde materialen worden nog steeds op grote schaal gebruikt omdat ze goedkoop, beschikbaar en technisch veelzijdig zijn. Hun levenscyclus gaat echter gepaard met milieubelasting, waaronder het gebruik van niet-hernieuwbare grondstoffen, broeikasgasemissies en aanhoudende afvalvorming. Daardoor groeit de belangstelling voor materialen die niet alleen functioneel zijn tijdens gebruik, maar ook beter aansluiten bij duurzame productie, verwerking en eindelevensstrategieën. In deze context vormen eiwitgebaseerde materialen een relevante piste, omdat ze afkomstig kunnen zijn uit hernieuwbare of reststromen en functionele groepen bevatten die netwerkvorming, biologische afbreekbaarheid en chemische modificatie mogelijk maken.

Keratine is zo'n eiwit. Het komt voor in biologische structuren zoals veren, haar, wol en klauwen, waar het mechanische ondersteuning en bescherming biedt. Veren zijn bijzonder relevant omdat ze een hoog keratinegehalte hebben en in grote hoeveelheden ontstaan als bijproduct van de pluimvee-industrie. Deze reststroom kan daarom worden beschouwd als een potentiële hernieuwbare grondstof voor materiaalontwikkeling. Gehydrolyseerde verenkeratine is daarbij interessant omdat het commercieel verkrijgbaar, relatief goedkoop en gemakkelijker te verwerken is dan natieve keratine. Die betere verwerkbaarheid gaat echter gepaard met een structurele beperking: hydrolyse breekt keratine af tot kortere peptidefragmenten, waardoor het materiaal minder goed in staat is om zelfstandig sterke en continue netwerken te vormen.

Hoewel keratinefilms en crosslinkingstrategieën eerder onderzocht werden, richt veel literatuur zich op keratine gemengd met andere polymeren of op geëxtraheerde keratinesystemen die verschillen van commercieel verkrijgbare gehydrolyseerde verenkeratine. Daardoor blijft onduidelijk of deze toegankelijke hydrolysaatvorm kan functioneren als belangrijkste structurele fase in samenhangende en mechanisch competente films. Binnen deze leemte is de vergelijking tussen glutaaraldehyde (GA) en genipine (GNP) relevant. GA is een effectieve en veelgebruikte eiwitcrosslinker, maar de toxiciteit beperkt de waarde ervan als duurzame langetermijnoptie. GNP is een natuurlijk afgeleid en minder toxisch alternatief dat reageert met primaire aminen en een

karacteristiek blauw pigment vormt, maar het vermogen ervan om mechanisch effectieve netwerken te vormen in gehydrolyseerde keratinefilms is minder duidelijk.

Het doel van deze thesis was daarom te onderzoeken of commercieel verkrijgbare, uit veren afkomstige gehydrolyseerde keratine kan worden verwerkt tot samenhangende films en of crosslinking met GA of GNP het materiaalgedrag kan verbeteren. Het onderzoek richtte zich op filmvorming, uiterlijk, hanteerbaarheid, treksterkte, rek bij breuk, taaheid en chemische karakterisering via FTIR-spectroscopie. De centrale verwachting was dat crosslinking de beperkte netwerkvormende capaciteit van gehydrolyseerde keratine gedeeltelijk zou compenseren, maar dat GA en GNP een verschillend evenwicht tussen sterkte en vervormbaarheid zouden opleveren.

De films werden bereid uit gehydrolyseerde eendenverenkeratine en gecrosslinkt met GA of GNP. GA werd gebruikt aan 10 wt% ten opzichte van keratine als referentiecrosslinker. Voor GNP werd eerst een concentratiereeks gescreend om hanteerbare films te identificeren. Op basis daarvan werden GNP 4 en GNP 10 geselecteerd voor mechanische vergelijking met GA. Per conditie werden vijf replicaten getest. Treksterkte, rek bij breuk en taaheid werden berekend op basis van spanning-rekgegevens. FTIR-spectroscopie werd gebruikt als aanvullende methode om het chemische profiel van keratinepoeder en de gecrosslinkte films te vergelijken.

De resultaten toonden aan dat de niet-gecrosslinkte keratinecontrole geen continue film vormde, terwijl de gecrosslinkte samples dat wel deden. GA-gecrosslinkte films kregen een bruine kleur en leken brozer, terwijl GNP-gecrosslinkte films donkerblauw werden en over het algemeen flexibeler waren. Beide gecrosslinkte systemen bleven wel watergevoelig. De spanning-rekcurves lieten een duidelijke afweging zien tussen spanningsweerstand en vervormbaarheid. GA-gecrosslinkte films bereikten de hoogste spanningswaarden, maar faalden bij lagere rekwaarden. GNP 4 vertoonde de laagste spanningsweerstand, maar de hoogste rek. Het verhogen van de GNP-concentratie van 4 naar 10 wt% verbeterde de treksterkte en taaheid, terwijl meer vervormbaarheid behouden bleef dan bij GA.

Kwantitatief nam de treksterkte toe van $0,073 \pm 0,030$ MPa voor GNP 4 naar $0,306 \pm 0,140$ MPa voor GNP 10, en was deze het hoogst voor GA met $0,982 \pm 0,170$ MPa. Rek bij breuk volgde het omgekeerde patroon, met $17,71 \pm 6,08\%$ voor GNP 4, $14,68 \pm 4,62\%$

voor GNP 10 en $6,80 \pm 3,19\%$ voor GA. Taaiheid was het laagst voor GNP 4, hoger voor GNP 10 en gemiddeld het hoogst voor GA, al vertoonde GA meer variabiliteit. Statistische vergelijkingen bevestigden significante verschillen tussen de crosslinkingscondities, vooral voor treksterkte en taaiheid.

Deze resultaten tonen aan dat crosslinking de structurele beperkingen van gehydrolyseerde keratine gedeeltelijk kan compenseren, maar dat GA en GNP het materiaal via verschillende mechanische profielen versterken. GA produceerde de sterkste films, waarschijnlijk door de hoge reactiviteit en de vorming van een dicht netwerk, maar dit beperkte ook de vervormbaarheid. GNP vertoonde een meer concentratieafhankelijke respons. Bij 4 wt% was het netwerk te zwak om substantiële sterkte of taaiheid te bieden. Bij 10 wt% leek GNP een effectiever dragend netwerk te vormen, met hogere taaiheid en behoud van relatief hoge rek. De blauwe kleur van de GNP-films bevestigde chemische activiteit, terwijl FTIR aantoonde dat keratine de dominante chemische component bleef.

Deze studie blijft verkennend en kent verschillende beperkingen. Er werd slechts één commerciële bron van gehydrolyseerde keratine gebruikt, waarvan het vrije-aminegehalte niet experimenteel werd bepaald. De GNP-screening was kwalitatief, het aantal mechanische replicaten was beperkt en kleine afwijkingen in sampledimensies kunnen de berekende waarden beïnvloeden. Daarnaast werden waterstabiliteit, biologische afbreekbaarheid, residuele crosslinkerinhoud en levenscyclusimpact niet systematisch onderzocht. Toekomstig onderzoek moet daarom het vrije-aminegehalte kwantificeren, GNP-concentraties optimaliseren op basis van beschikbare reactieve groepen, waterstabiliteit evalueren en bijkomende strategieën onderzoeken om de netwerkconnectiviteit te verbeteren.

Deze thesis toont aan dat commercieel verkrijgbare gehydrolyseerde verenkeratine kan worden verwerkt tot samenhangende films wanneer crosslinking wordt toegepast. GA leverde de sterkste maar minst vervormbare films op, terwijl GNP, vooral bij 10 wt%, een minder toxische route bood met een evenwichtiger mechanisch profiel. Hoewel de films nog niet klaar zijn voor toepassing, vormt GNP-gecrosslinkte gehydrolyseerde keratine een veelbelovend uitgangspunt voor de verdere ontwikkeling van goedkope, eiwitgebaseerde materialen.

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10. Disclaimer of GAI usage

During this master's thesis, DeepL was used to support translations into English. ChatGPT, model GPT-5, was used for an initial orientation on relevant literature and for rephrasing, refining, and improving the language of text fragments. The source suggestions obtained through ChatGPT were subsequently checked and further supplemented using scientific databases and the original publications. ChatGPT was not used for data analysis, statistical processing, or the substantive interpretation of the results. To ensure data protection and confidentiality, no raw research data was entered into AI tools.

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