Removal of Pigments from Human Hair to Obtain Purer Keratin Fractions for Downstream Biofabrication

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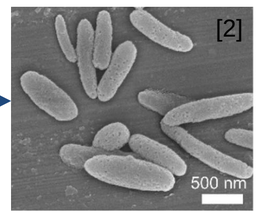
Introduction

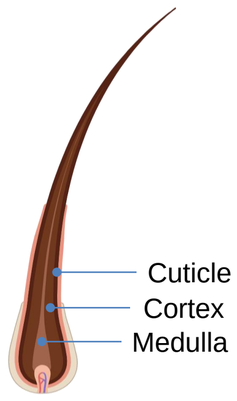
There are many practical sources of keratins. One of them is human hair. The abundance of human hair waste from salons makes it an accessible source of keratins.

Human Hair:

Mostly made up of
- **Keratin-associated proteins (KAPs)**
- **Keratins**

In the hair cortex region,
- **Melanosomes** containing melanin pigments – colour & **UV barrier properties**





Keratins and **melanosomes** have numerous properties, making them promising biomaterials. Their conversion into high-value products raises the sustainability index and value of human hair as a resource.

However, a common shortfall for keratins extracted from human hair is **contaminants** → melanosomes. They cause high pigmentation in fabricated templates, making them unsuitable for applications where transparency is key.

Proposed Solutions

To reduce the level of pigmentation within human hair and keratin extracts, and obtain purer keratin fractions for downstream biofabrication:

1. **Chemical Bleaching of Human Hair**
2. **Enzymatic Extraction of Melanosomes from Matrix-free Hair**
   → try to obtain cleaner fractions of keratins and melanosomes.

Objectives

I. To enzymatically extract functional melanosomes from matrix-free human hair.
II. To optimise yields of melanosome and keratin extraction.
III. To verify the UV-filtering ability of extracted melanosomes.
IV. To evaluate the quality of keratins extracted from bleached and melanosome-extracted matrix-free hair.

Methodology

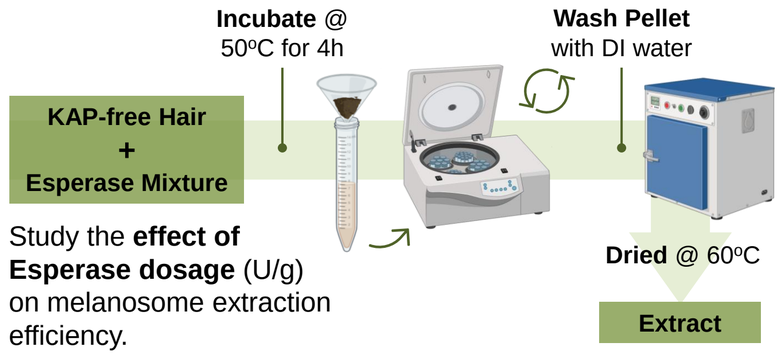
2 Approaches:

Human Hair → **Delipidisation** → **KAP Extraction** [1] → **Keratin Extraction** [1] → **Keratin**

Chemical Bleaching of Hair (1 cycle) ①

Enzymatic Extraction of Melanosomes from Matrix-free Hair (Green Method) ②

Extraction from matrix-free hair allows melanosomes to be extracted **before** the extraction of keratins from human hair.



Study the **effect of Esperase dosage** (U/g) on melanosome extraction efficiency.

Control (Hair Residue [2])

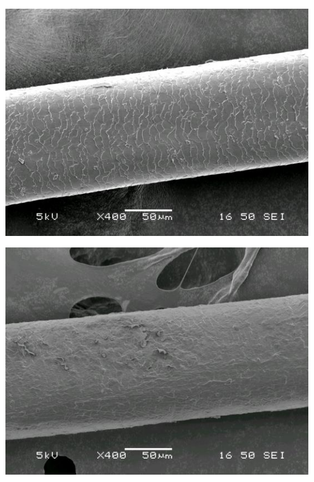
① Chemical Bleaching of Hair

For **bleached hair shafts**,

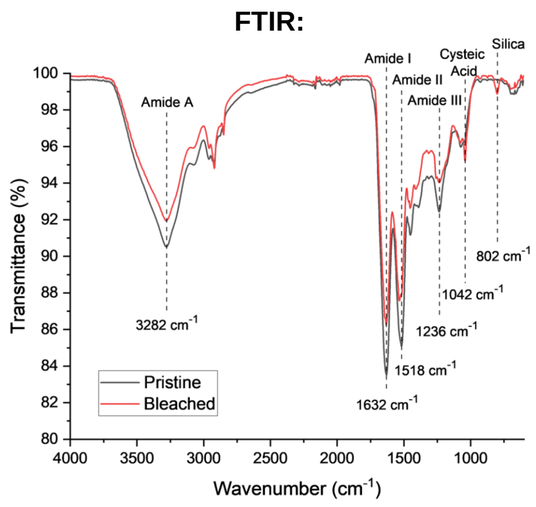
Even though surface cuticle damage was observed under the SEM, FTIR analysis showed that characteristic IR peaks for proteins are present and **secondary structure was retained**.

Pristine Hair

Bleached Hair



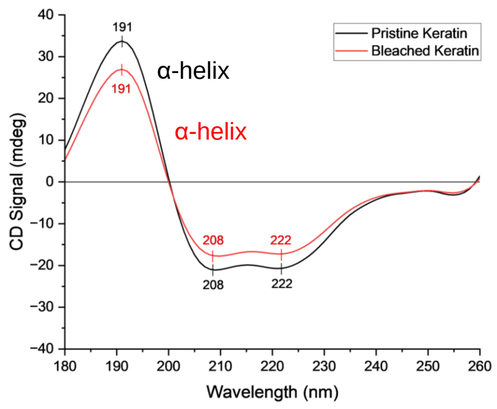
FTIR:



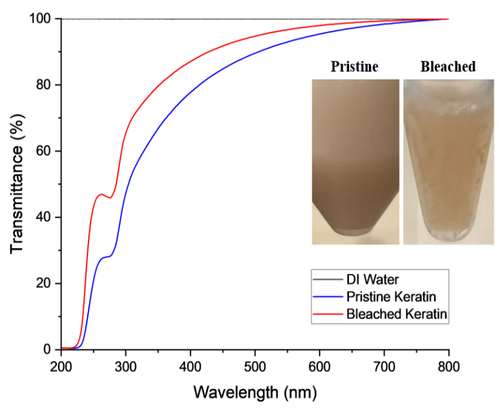
For **keratins extracted from bleached hair shafts**,

Protein extraction yield (45 ± 1.06%) is comparable to pristine keratins (49 ± 3.99%).

Circular Dichroism:



UV-vis:



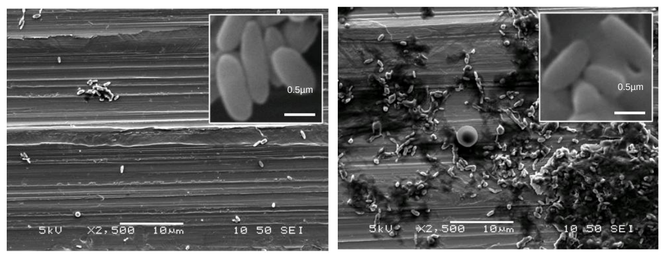
② Enzymatic Extraction of Melanosomes

Structurally intact and functional melanosomes were successfully extracted from KAP-free hair instead of hair residues.

Highest yield:
Esperase dosage of **1.00 U/g** of KAP-free hair, although keratins were also extracted.

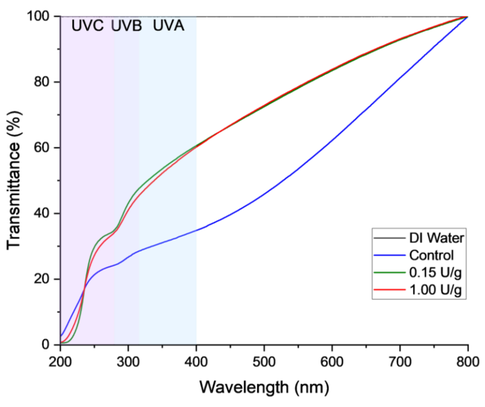
From 0.15 U/g Hair Residue

From 1.00 U/g KAP-free Hair



High-magnification SEM images from Dr Marin Yee.

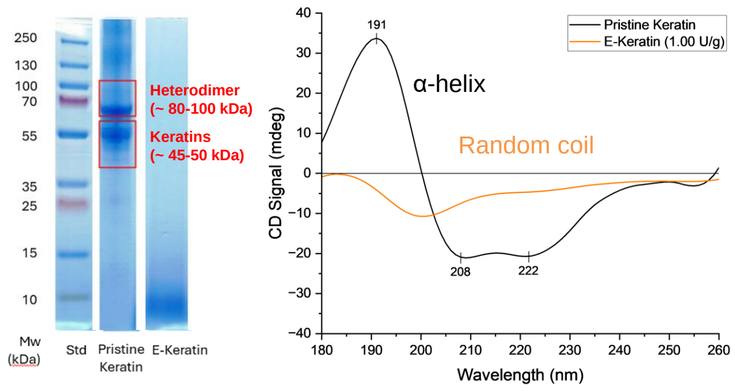
UV-vis:



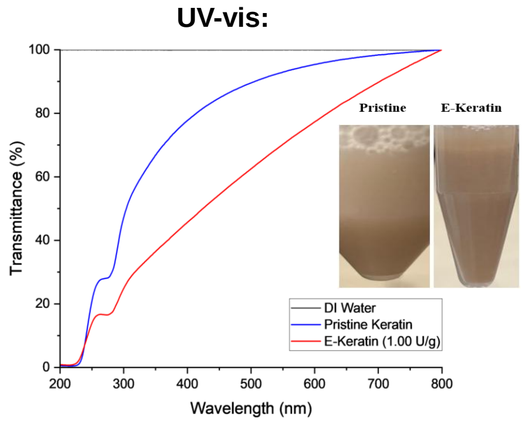
For **melanosome-extracted (E-) keratins**,

Precipitation observed post-dialysis of E-keratin extracts led to a decrease in the transmittance of the sample, which impeded accurate assessment of lower pigmentation levels.

Circular Dichroism:



UV-vis:



Conclusion

Chemical bleaching effectively **lowers pigmentation** in hair and, ultimately, its keratin extracts. The bleaching process did not affect the yield and quality of bleached keratins.

Functional **melanosomes** were **successfully extracted** from KAP-free hair via **enzymatic extraction** albeit they were embedded within a protein network. The enzymatic extraction process resulted in the degradation of E-keratins extracted from E-KAP-free hair.

References

[1] T. Fujii, S. Takayama, and Y. Ito, "A novel purification procedure for keratin-associated proteins and keratin from human hair," *J. Biol. Macromol.,* vol. 13, no. 3, pp. 92–106, 2013, doi: 10.14533/jbm.13.92.
[2] N. Zhang *et al*., "An Enzymatic Method for Harvesting Functional Melanosomes after Keratin Extraction: Maximizing Resource Recovery from Human Hair," *J Polym Environ*, vol. 30, no. 3, pp. 1045–1054, Mar. 2022, doi: 10.1007/s10924-021-02246-8.