DEVELOPMENT OF AN EFFICIENT ULTRASONIC-ASSISTED EXTRACTION METHOD FOR HUMAN HAIR KERATIN

A Sustainable Physical Disruption Strategy for Green Biomaterial Recovery

Student: Chuan-Yu Yang | Advisor: Dr. Yang Wei

National Taipei University of Technology | Department of Chemical Engineering and Biotechnology

Abstract

Traditional keratin extraction methods (e.g., reductive Shindai method) rely on chemical solvents that diffuse slowly, requiring reaction times up to 24–72 h and high concentrations of chaotropes. This creates environmental burdens and risks protein degradation. This project introduces an Ultrasound-Assisted Extraction (UAE) method to optimize extraction from human hair. Utilizing cavitation and mechanical shear forces, this method actively disrupts the dense cuticle to lower chemical dependence and shorten reaction times. We investigated ultrasonic power gradients (0W, 200W, 400W, 600W) over 1 h. high-quality keratin while preserving natural molecular distribution, offering an efficient, eco-friendly pathway for sustainable recovery.

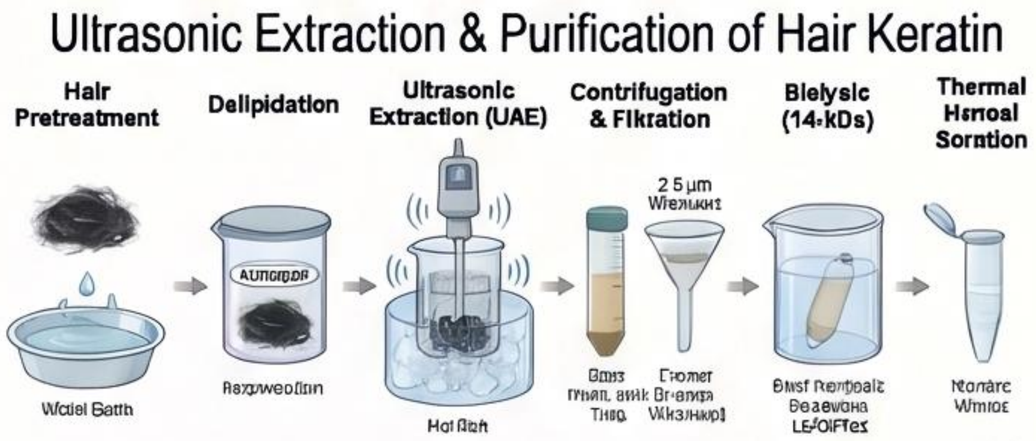
Methodology

1. Sample Pretreatment

- **Hair Washing:** Cleaned with DI water and crystal soap to remove surface dirt, repeated 2-3 times, dried at 37°C.
- **Delipidation:** Soaked in 95% n-hesane for 12 h to remove surface lipids, washed with DI water, and dried at 37°C.

2. Extraction Formulation & Experimental Groups

- **Extraction Buffer:** Tris-base (0.242g), DTT (2.57g), Urea (30g), adjusted to pH 9.8, made up to 100 mL with DI water.
- **Sample Setup:** Each beaker filled with 1.25g chopped hair and 25 mL buffer.
- **Groups** (Fixed 1 h):
  1. **Control:** Traditional Shindai method (50°C, 72 hr)
  2. **Low Power:** 200W (UAE, 1 hr)
  3. **Medium Power:** 400W (UAE, 1 hr)
  4. **High Power:** 600W (UAE, 1 hr)
- **Temp. & Safety:** Ice bath (15±5°C), pulse mode "5s ON / 5s OFF" to prevent thermal degradation.



3. Separation, Purification & Analysis Workflow

Filtration & Centrifugation: 2.5 µm Whatman paper filtration to remove debris, centrifuged at 1500xg for 30 min, dialysis bags (14 kDa MWCO) used to dialyze against DI water for 3 days.

- **BCA Assay**: 595 nm ELISA vs BSA std.
- **SDS-PAGE**: 12% polyacrylamide gel (60V/80V) for molecular weight (40-60 kDa).
- **FE-SEM**: Sputter gold-coated, 15.0 kV, 9-10 mm WD for morphology (1000x-5000x).
- **FTIR**: ATR-FTIR (600-3500 cm^{-1}) for secondary structure.
- **LC-MS/MS**: Trypsin digestion (18 hr, 37°C), searched against UniProt.

Results & Discussion

1. BCA Protein Quantification & Recovery Yield

Sufficient ultrasonic power is highly effective for keratin dissolution. 600W (1 h) yielded 32.19 mg/mL with n recovery yield of 74-82%, a ~3-fold increase over traditional methods.

Group	Conditions	Vol. (mL)	BCA (mg/mL)	Yield (%)
Shindai (72 hr)	50°C, 72 hr	19-22	15.34	22-26%
UAE 200W	Iec, 1 hr	20-23	11.36	17-20%
UAE 400W	Iec, 1 hr	27-30	22.30	46-32%
UAE 600W	Iec, 1 hr	30-33	32.19	74-62%

2. Molecular Weight (SDS-PAGE) & Micro-Morphology (FE-SEM)

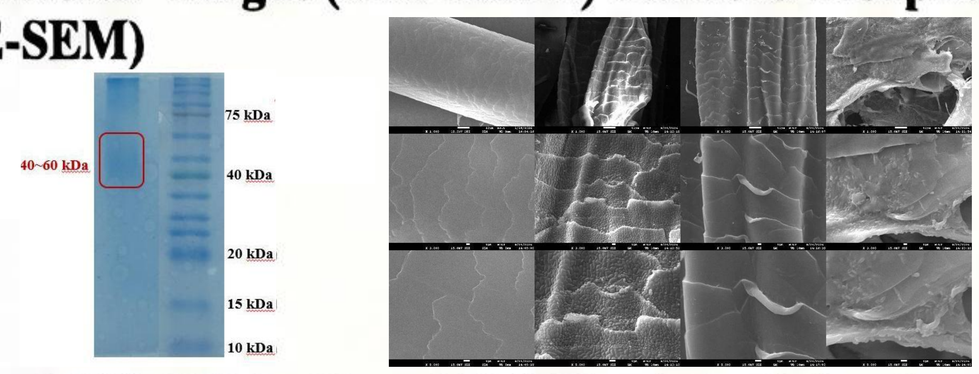


Figure 1. SDS-PAGE profiles and FE-SEM analysis of hair surfaces.

- **SDS-PAGE**: 400W and 600W groups showed clear hands at 40-60 kDa (KIFs), proving that ultrasonic treatment does not cause excessive degradation.
- **FE-SEM**: Raw hair scale remained tight. Shindai displayed mild surface erosion; 600W UAE showed extensive exfoliation and micro-porosity, facilitating solute diffusion.

3. Secondary Structure (FTIR) & Proteomics (LC-MS/MS)

- **FTIR**: UAE bands matching Shindai (Amide I, II, III & A), confirming n-helix backbone conservation.
- **LC-MS/MS**: 600W UAE significantly increased deep-cortex Type II keratins (KRT86: 38%, KRT85: 38%) and uniquely extracted the ultra-high sulfur matrix protein KRA52_HUMAN (KAP5-2, 25%).

Conclusions

- UAE reduces extraction time from 72 h to 1 h, improving yield to 74-82% (~3-fold improvement).
- High-intensity ultrasound preserves native α-helix structures and 40-60 kDa KIFs without excessive shearing.
- Demonstrated efficacy in disrupting deep cortical macrofibril networks, extracting valuable KAP proteins undetected by chemical methods.
- **Future Work:** Apply bio-compatible green reducers (e.g., L-cysteine) & continuous-flow systems.