**Application:** Keratin extraction can generate revenue and mitigate the environmental and health problems caused by the disposal of feathers in landfills. This research provides evidence that high keratin yields can be achieved using cheap non-toxic solvents in relatively short durations.

**Introduction:** Every week ~22 million chickens are slaughtered in the UK with an average weight at slaughter of 2.3 kg (*Department for Environment Food & Rural Affairs*, 2023). Feathers represent ~5.5% of chicken weight and they are currently underutilized (McGauran et al., 2021). Keratin accounts for >90% of feathers dry weight (Zhang et al., 2021). It has several applications in the biomedical and cosmetic industries, and it can be used to produce a biodegradable polymer for packaging, wound dressing, and tissue regeneration (Senthilkumar et al., 2022). Keratin is insoluble in polar and non-polar solvents due to the cross-linking by disulphide bonds, which must be broken to solubilize keratin (Dąbrowska et al., 2022). This is usually achieved through chemical or enzymatic treatment. The study aims to optimize keratin extraction, using sulphites as reduction agents, by investigating the effects of varying the extraction parameters on the dissolution rate.

**Materials and methods:** Feathers were obtained from Moy Park, Armagh, UK. They were milled, washed with water and detergents, and dried in an oven at 37.5 °C before extraction. The composition of raw feathers was analysed using proximate analysis. All chemicals used were of analytical grade.

Ground feathers (m1) were mixed with a solution containing reduction agent, urea, and sodium dodecyl sulphate and heated in a water bath with frequent agitation using a vortex mixer. The mixture was then centrifuged, and the undissolved feathers were washed thoroughly and dried in an oven at 105 °C until a constant mass was achieved (m2). The soluble keratin was dialyzed against distilled water for two days using a dialysis tubing (molecular weight cut-off of 3.5 kilo Dalton) and the water was changed twice a day. Keratin was then dried for three days at 60 °C and weighed (m3). Thedissolution rate and yield were calculated using Equations [1 & 2].

[1]

[2]

The parameters investigated and their ranges were, temperature (40-100 °C), time (1-10 hours), urea concentration (2-8 M), reduction agent (sodium sulphite, sodium bisulphite, and sodium metabisulphite), reduction agent concentration (0-0.7 M), sodium dodecyl sulphate concentration (0-1 g/g feathers), and solid-to-liquid ratio (1:25-1:10).

Keratin secondary structure, thermal stability, crystallinity, and surface morphology were characterised using Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), X-ray diffraction analysis (XRD), and scanning electron microscopy (SEM).

All analyses were carried out in triplicate and results are expressed as means with standard deviations (± SD). Differences among means, using the t-Test statistical method, were considered significant at p-value < 0.05.

**Results:** The parameters with the most significant influence on the dissolution rate were temperature, urea concentration, and time. The dissolution rate increased linearly from 61.2% to 98.3% when temperature increased from 40 °C to 100 °C and from 72.5% to 92.3% when urea concentration increased from 2 M to 8 M. It also increased with time up to 6 hours (92.3%) with no significant increase at longer durations. The conditions resulting in the highest dissolution rate were:

* Temperature: 100 °C.
* Time: six hours.
* Urea concentration: 8 M.
* Reduction agent: sodium sulphite.
* Reduction agent concentration: 0.5 M.
* sodium dodecyl sulphate concentration: 1 g/g feathers.
* Solid-to-liquid ratio: 1:15.

The dissolution rate and yield were 98.3±0.3% and 73.3±5.4% respectively.

FTIR spectra of keratin show all the distinct protein absorption peaks (amide A, amide I, amide II, and amide III) which confirms the preservation of protein secondary structure. The disulphide bridge peak in the spectra of raw feathers at 531 cm-1 disappeared in the keratin spectra which confirms the breakdown of disulphide bonds. TGA results show that the highest mass loss for keratin occurred at a temperature of 250 °C compared to 325 °C for raw feathers, indicating a reduction in the thermal stability because of the breakdown of disulphide bonds. Nevertheless, keratin has a relatively high thermal stability that allows for thermal processing. XRD results show that keratin has a higher degree of crystallinity evident by the sharp peak at 22.6 ° compared to the broad peak at 18 ° in raw feathers. While the shift to a higher angle indicates a reduction of β-sheet structure, because of disulphide bond breakdown, and an increase of random coil structure. SEM images show that keratin had a smooth flake-like structure while raw feathers have hollow fibrous structure.

**Conclusion:** Keratin can be extracted efficiently using sulphites. Under the optimal extraction conditions > 98% of the feathers were dissolved and the yield was 73.3±5.4%. The secondary structure of keratin was preserved during extraction. The thermal stability of keratin was lower than raw feathers, but high enough for thermal processing.

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