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Extract value from wool waste: keratin and the circular economy

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Spinning a yarn: explore the chemistry of wool and use it as a raw material for biobased products through simple hand-on activities.

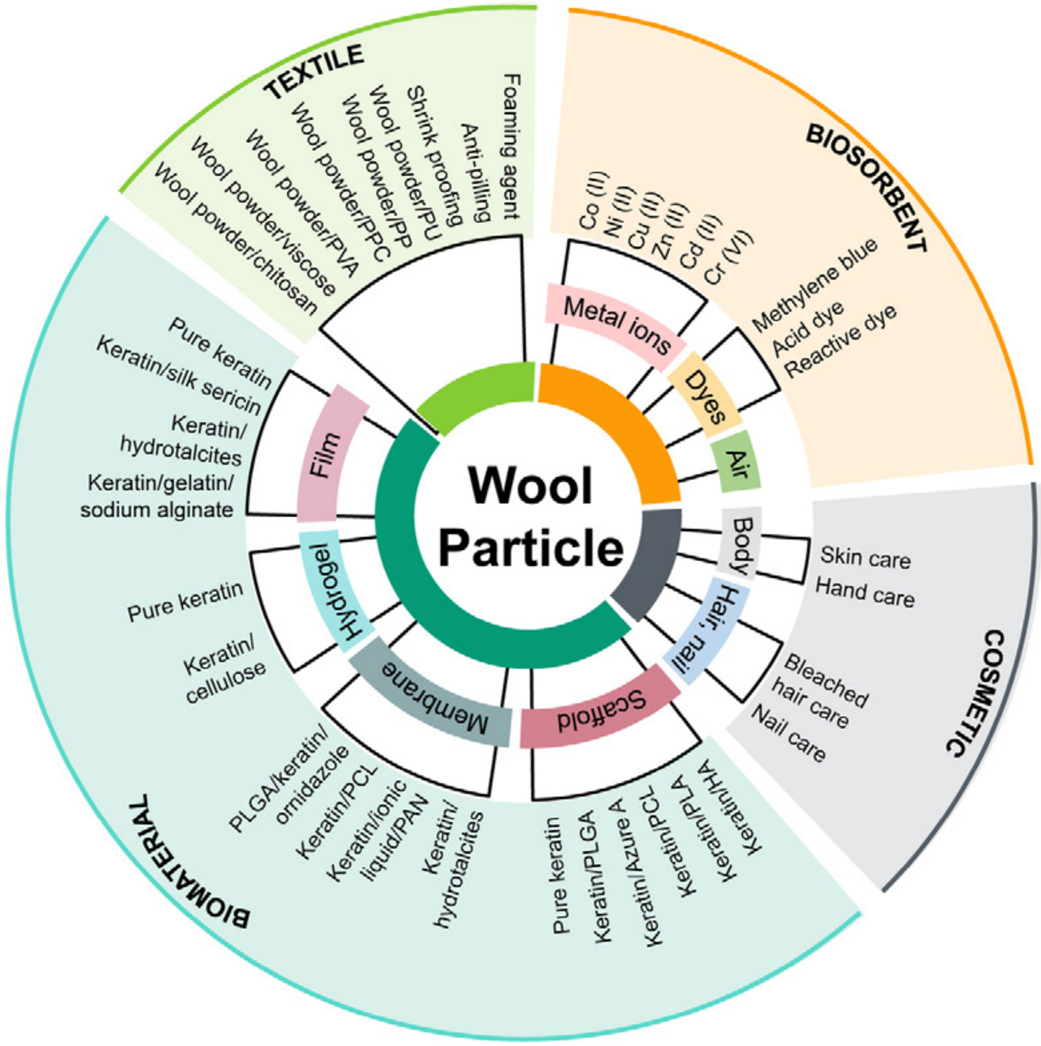
Introduction

Wool has always been a product of animal husbandry, especially of sheep and goats, and has been used for various purposes, including clothing, mattress stuffing, and thermal insulation.

Since 2002, as a result of European legislation, Regulation EC 1774/2002, later revised in 2009 (EC1069/2009), wool went from being classified as an agricultural product to being classified as processing waste, so it has to be disposed of through very expensive procedures.[1]

According to statistics reported by the International Wool Textile Organisation (IWTO),[2] the number of sheep farms is increasing as a result of interest in meat, and wool production is also on the rise, since, for their welfare, the animals must be sheared.

To avoid having to send wool for disposal, industries and research centres have been developing several strands of study over the years, investigating alternative uses for wool. Material derived from wool can be used as adsorbent or insulating elements, and as starting materials for filters or other biomaterials.[3,4]



Applications for wool particles
Image from Ref [4]

Wool is a natural fibre that consists of proteins (about 95–98%, of which about 85% is keratin), lipids (about 2%), and mineral salts (1%). Keratin is a very abundant protein in nature that is found in hair, nails, horns, and bird feathers, in addition to wool.[5,6]

It is reported in some works that the first use of keratin for medical purposes dates back to China in 1650, but the term was first reported in the scientific literature in 1850 to explain the composition of the horns of some animals.[7]

The activities can be incorporated into different curricular tracks:
- biochemical characterization of wool;
- circular economy and waste reuse.

The in-depth interdisciplinary laboratory activities can be offered to students aged 14–16 and 16–19.

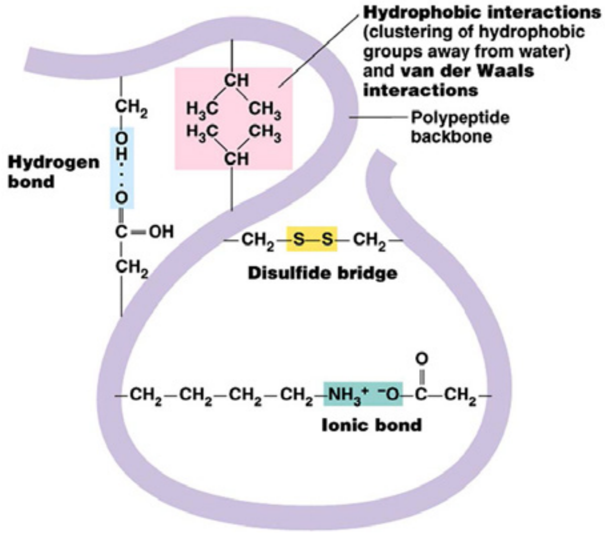
The goals are to engage students in active learning and stimulate their critical thinking.

Activity 1: Wool composition and keratin extraction

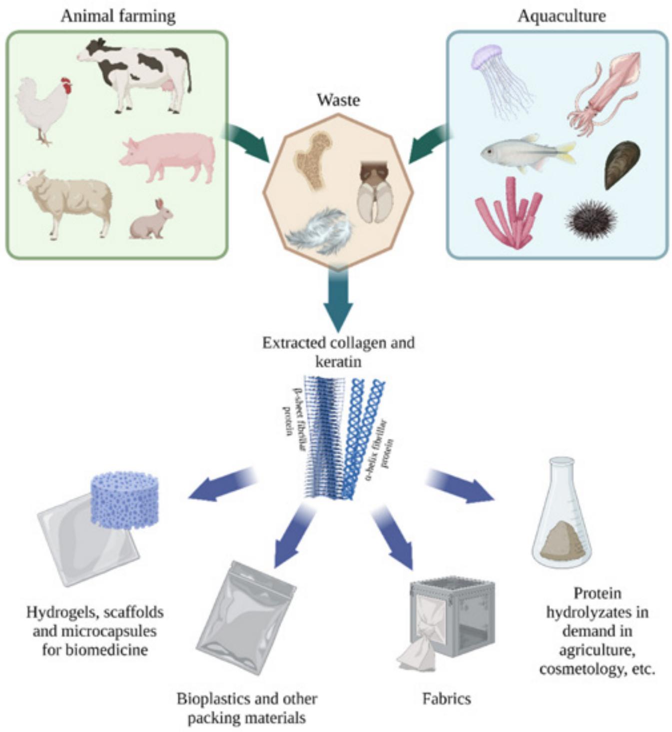
This activity involves the extraction of keratin from wool using a suitable extraction solution. It can be completed in one or two lessons; the extraction can be carried out for an hour with 1 M NaOH or overnight with 0.1 M NaOH.

The chemistry of keratin

Keratin is a fibrous protein that, in terms of secondary structure, can be organized as an alpha helix or beta sheet. The high content of cysteine residues promotes the formation of disulfide bridges that add to hydrogen bonds, and ionic and hydrophobic interactions contribute to the three-dimensional (3D) organization of the protein. Depending on the origin of keratin, the 3D structure and specific amino acid content changes, but the main inter- and intramolecular bonds remain the cystine disulfide bridges.



Intramolecular bonding in keratin
Image from Ref [8]



Sources and applications of collagen and keratin
Image: Polymer 2022, 14(8), 1601

Keratin extraction depends precisely on breaking of the above-mentioned interactions. Depending on the extracting agent, different amino acid compositions of the extracted keratin and different chemical and physical characteristics will be obtained. For this reason, considering the possibility of employing keratin in the production of new biomaterials, different fields of research have been developed. In recent years, with the aim of developing increasingly effective extraction techniques and production methods for hydrolyzed keratin, which vary according to the characteristics of the product required and its use, several new keratin-based products have been developed: films, adsorbent sponges, polymeric fibres, particular applications in the field of biomaterials.[7,9–12]

Safety notes

Dilute 0.1 M solutions of sodium hydroxide are not classified as hazardous, but 1 M sodium hydroxide is corrosive. Gloves and eye protection should be worn to avoid eye and skin contact. Working under a fume hood is ideal.

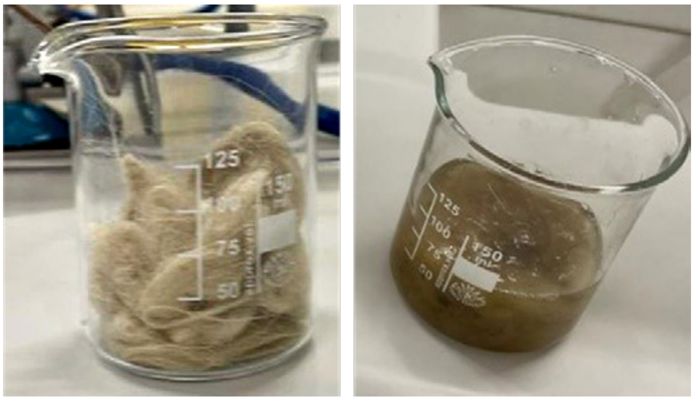
Teachers should make up the solutions so that students don't use the more concentrated forms.

Materials

- Raw wool fibres (Wool must be untreated, raw, and undyed to prevent treatments from altering results. Teachers can find this wool online, from farmers, by consulting the IWTO https://iwto.org/.)
- NaOH (as 1.0 or 0.1 M solutions)
- Beaker
- Glass rod
- Gloves
- Lab coat
- Safety glasses
- Activity 1 Worksheet

Procedure

1. Weigh 5 g of raw wool into a beaker.
2. Cover the wool with the 1 M sodium hydroxide solution (150 ml) and stir the mixture with a glass rod. Make sure the wool is completely covered by the extraction solution.
3. Allow it to sit for 1 hour for keratin extraction. If it is not possible to wait, a period of 30 minutes allows for a sufficient result. Alternatively, a less concentrated solution (0.1 M) can be used and allowed to sit overnight (at least 12 h).
4. After the extraction period has elapsed, extract the fibres from the solution using a glass funnel and gauze filter or a strainer. Observe the appearance of the wool and solution.



Wool before (left) and after (right) extraction
Image courtesy of the author

Results/discussion

During the experiments, students should record their observations on the worksheet and afterwards the answers should be discussed with the class.

The wool generally takes on a darker and gelatinous appearance during extraction and the clear solution becomes dark and cloudy. These changes are due to chemical reactions. As described above, the 3D shape of proteins depends on the numerous bonds formed between different peptide chains and within the same protein chain. These are generally weak bonds (hydrogen bonds, hydrophobic interactions, dipolar bonds), which are strongly influenced by external factors, such as pH, temperature, the presence of ionic salts, emulsifiers, the activity of certain enzymes, and the action of certain microorganisms.

Alkaline hydrolysis of wool produces 75–80% water-soluble materials, including amino acids and peptides.[11] Upon treatment with alkali solutions, different chemical reactions occur to denature the proteins and disrupt the 3D structure through breakdown of the hydrogen and disulfide bonds, ionization of the carboxylic groups of amino acids, and changes in solubility. The use of alkali solutions can also lead to the formation of a sulfide odour during the process. [9]

Activity 2: Keratin flocculation

In this activity, the effects of different chemicals on the flocculation of keratin are investigated. The activity takes about 30 minutes to complete.

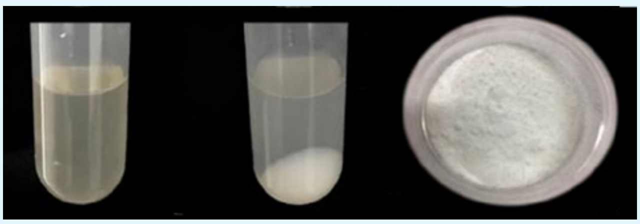
Keratin flocculation

Once the solution containing the extracted protein is obtained, it can be precipitated by using several methods: addition of salts to change the ionic strength or changing the solvent polarity, temperature, or pH.

The moment the protein is denatured, it 'opens up' and interactions that were previously intramolecular become intermolecular, aggregating the protein and increasing its hydrophobic character. This decreased affinity to water can evolve toward aggregation and polymerization phenomena, which can be
- disordered, giving rise to precipitation, flocculation, and coagulation
- ordered, generating more or less stable gels.

Precipitation is characterized by the loss of solubility alone, due to association phenomena and consequent separation of proteins from solution.

Flocculation occurs when floccules, that is, large micellar aggregates, are formed, without denaturation of the protein structures. This occurs when protein micelles of colloidal size no longer exhibit electrostatic repulsions, and thus, aggregate.



After precipitation from the extracted keratin solution (left), keratin sinks to the bottom of the tube (centre), and this can then be collected and dried to give keratin powder (right).
Image: Adapted from Materials 2021, 14(16), 4696

Flocculation of proteins can be achieved 1) by changing the pH by adding weak acid solutions, and 2) through treatment with organic solvents miscible with the aqueous phase (such as ethanol or acetone) in which the proteins are dissolved.

Safety notes

Dilute 0.1 M solutions of sodium hydroxide are not classified as hazardous, but 1 M sodium hydroxide is corrosive. Gloves and eye protection should be worn to avoid eye and skin contact with the solutions. Working under a fume hood is ideal.

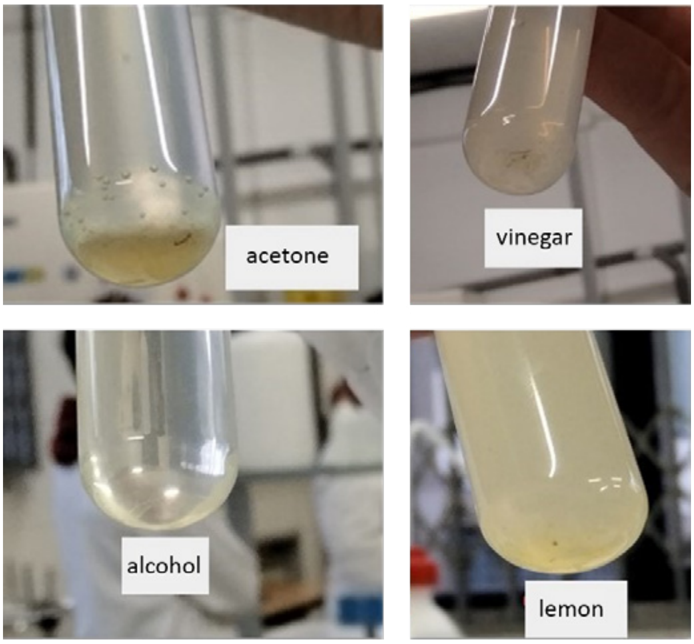
Teachers should make up the solutions so that students don't use the more concentrated forms.

Materials

- Keratin solution in NaOH
- Test tubes
- Strainer
- Pipettes
- Petri dishes
- Test tube rack
- Protective goggles, Gloves
- Ethanol (CH_3CH_2OH)
- Acetone (propanone, $H_3C–CO–CH_3$)
- Citric acid (6% (m/m), 2-hydroxypropane-1,2,3-tricarboxylic acid, $C_6H_8O_7$) or lemon juice
- White wine vinegar
- Lemon juice
- Filter paper
- Glass funnel
- Activity 2 Worksheet

Procedure

1. Arrange four identical test tubes in a test tube rack.
2. Pour 10 ml acetone into the first test tube, 10 ml ethanol into the second, 10 ml citric acid into the third, and finally 10 ml vinegar into the fourth. Citric acid (6%) solution has a pH value similar to that of lemon, so lemon juice can be used instead. It is necessary to filter the lemon juice, using a glass funnel and paper filter, before use to reduce turbidity.
3. Use the marker to label the respective test tube with the name of the substance.
4. Then, determine the pH of the different solutions using litmus paper.
5. Add about 2 ml of the extraction solution dropwise to each test tube.
6. Students should record their observations in the results table in the worksheet.
7. Pour the contents of each test tube into a Petri dish and allow the solutions to evaporate until completely dry; solutions may need to be left overnight.
8. After observing the effects of different types of solvents, the rest of the extracted solution can be flocculated by adding acetic acid to the extract under a fume hood until crystals are observed to precipitate (keratin flocculation).
9. Separate keratin crystals from the solution in which they are contained by filtration through a strainer. Transfer the keratin to a watch glass and allow to dry. Again record observations in the table.



Flocculation of keratin

Images courtesy of the author

Results/discussion

Ask students what differences they notice when flocculation is induced by pH or solvent changes. Can they think of explanations for why pH or solvent changes might cause flocculation?

- Proteins can be positively or negatively charged, but at certain pH values the net charge is zero; this is called the isoelectric point of the protein. These conditions are best for proteins to form aggregates with each other. The isoelectric point is specific for each protein. The use of weak acids, such as vinegar (acetic acid) or lemon juice (citric acid), brings the solution close to the isoelectric point of keratin, which has a pH value of 4.2–4.5, and promotes precipitation.
- The solubility of proteins in aqueous solutions containing water-miscible organic solvents (ethanol, acetone) changes. The solvent lowers the dielectric constant and removes water molecules from the surface of the protein; thus, protein–protein interactions are promoted (electrostatic rather than hydrophobic).

Activity 3: Preparation of hair conditioner with keratin

Hydrolysed keratin produced by wool is mainly used in cosmetics as an ingredient in hair-specific products.

The final proposed activity is to make hair conditioner using keratin. For better success in the classroom, it is best to buy keratin as a 25% (m/V) solution glycerine. It is difficult to use the extracted keratin because it is not sufficiently soluble in water and needs to be purified.

This activity takes 30 minutes to complete.

Safety notes

The solutions used are not particularly hazardous, but ideally gloves and eye protection should be worn.

Materials

For 50 ml of product:

- Keratin - 7.5 g of a solution in glycerine with concentration 25 % m/V
- Cetyl alcohol (1-hexadecanol, $CH_3(CH_2)_{15}OH$) 3 g
- Shea butter 7.5 g
- Almond oil and citric acid 7 g
- Optional: essential oils

Method

1. Weigh all ingredients into a beaker.
2. Heat the beaker in a water bath until all ingredients are melted (around 40°C).
3. Add 1–2 drops of essential oil and transfer the resulting product to a container for use.

Results/discussion

Students should understand that it is possible to use materials produced from waste to produce cosmetics, which is better more sustainable using virgin materials. As an extension activity, the different aspects of the circular economy of wool could be researched in more detail.

Conclusions

The proposed activities provide serval useful aspects for teaching:

- a simple path for teaching biochemistry;
- a broader project related to the valorization of waste; and
- an interdisciplinary study of ancient and modern technology and the use of wool.

These are simple experiments that only barely touch the extensive literature related to the use of biomass from waste

Hair conditioner produced with keratin

Image courtesy of the author

material, but they start from a material that is known to all and easy to find. The reagents and materials are also not difficult to find, even in a poorly equipped chemistry laboratory.

Other interesting keratin activities can be found on the Science on Stage website.

Acknowledgements

This activity was presented at the Science on Stage Festival 2022.

The project presented in this paper grew out of a collaboration with researcher Annalisa Aluigi and CNR Biella. Subsequently, some activities were developed with Science on Stage coordination and are part of the unit 'The 3 Rs and the Products of the Future' created within the project https://www.science-on-stage.eu/act-now-sdg

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Resources

- Watch a video on flocculation.
- Find resources to bring the science of sustainability into the classroom: Philippsen M (2024) Sustainability in the classroom: teaching materials from Science on Stage. *Science in School* **66**.
- Discover simple adaptations for experiments to make chemistry accessible to students with vision impairments: Chataway-Green R, Schnepp Z (2023) Making chemistry accessible for students with vision impairment. *Science in School* **64**.
- Take a virtual tour of The World of Molecular Biology: Reiriz Martinez E, Hall SL (2024) Explore the world of molecular biology without leaving the classroom. *Science in School* **68**.
- Discover some useful materials to explore the science behind some of the SDGs: Pisano MP, Godinho T (2022) Sustainable Science: Articles for European Sustainable Development Week. *Science in School* **59**.
- Try a classroom activity to extract essential oils from fragrant plants: Allan A, Worley B, Owen M (2018) Perfumes with a pop: aroma chemistry with essential oils. *Science in School* **44**: 40–46.

- Discover the science of limonene: Butturini F, Fernández JJ (2022) Citrus science: learn with limonene. *Science in School* **58**.
- Learn about a variety of biochemical aspects of honey through a series of simple experiments: Scheuber T (2023) To bee or not to bee: the chemistry of honey. *Science in School* **65**.
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- Explore the beauty of proteins with the PDB Art Project: Gupta D, Armstrong D (2021) Bringing the beauty of proteins to the classroom: the PDB Art Project. *Science in School* **54**.
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- Discover how mealworms could offer a sustainable alternative source of animal protein: Bonin L, Jeran M (2024) Towards sustainable nutrition: could mealworms provide a solution? *Science in School* **66**.

AUTHOR BIOGRAPHY

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With Science on Stage Deutschland, she has developed STEM activities related to the UN's Sustainable Development Goals.