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01/07

A Dissertation

on

Utilization of livestock keratin waste for sustainable and useful purposes

submitted in partial fulfillment of the requirement

for the award of a degree of

Masters in Technology

in

Environmental Science and Technology

Submitted by

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June, 2024

NAME OF THE ORGANISATION

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TITLE OF THE RESEARCH PROJECT:

Utilization of livestock keratin waste for sustainable and useful purposes

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ABSTRACT

This research investigates the waste generated by livestock farming that may have had potential value. Traditionally, waste is abandoned or burned, which can cause major environmental and economic consequences. Keratin is a by-product of livestock, and different keratin wastes such as buffalo and goat hair, sheep wool, and duck and hen feathers were considered and studied under various conditions. This study explores the scope of extracted protein from waste through the alkaline hydrolysis method and establishment at the commercial level that may provide employment opportunities and contribute to the rural agro-based industry.

KEYWORDS: livestock waste, extraction, keratin, alkaline hydrolysis, techno-economic analysis, sustainability, applications, challenges

DECLARATION CUM CERTIFICATE

DECLARATION CUM CERTIFICATE

I, at this moment, declare that the project work entitled (“**Utilization of livestock keratin waste for sustainable and useful purpose**”) is an authentic record of my work carried out at (Thapar Institute of Engineering and Technology, Patiala) as a requirement of a one-year project internship for the award of degree of MTech (Environmental science and technology), TIET, Patiala, under the guidance of (Dr. Dhamodharan. K) and (Dr. Gaurav Goel), during June 2023 to June 2024.

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Certified that the above statement made by the student is correct to the best of our knowledge and belief.

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LIST OF ABBREVIATIONS

S.NO	ABBREVIATION	FULL FORM
1.	α	Alpha
2.	β	Beta
3.	%	Percentage
4.	mg	milli-gram
5.	g	gram
6.	kg	Kilo-gram
7.	cm^{-1}	Centi-meter (or wavelength)
8.	h	Hour
9.	ºC	Degree Celsius
10.	mm	Milli-meter
11.	cm	Centi-meter
12.	µm	Micro-meter
13.	M	Molarity
14.	L	Litre
15.	ml	Milli-litre
16.	rpm	Revolutions per minute
17.	IW	Initial weight
18.	FW	Final weight
19.	Rs	Indian rupees
20.	UV	Ultra violet
21.	PPE	Personal protection equipment
22.	SDGs	Sustainable development goals
23.	R & D	Research and development
24.	RFID	Radio-frequency identification
25.	HVAC	Heating, ventilation, and air conditioning
26.	PVA	Polyvinyl alcohol
27.	N & P	Nitrogen and phosphorus
28.	GDP	Gross domestic product
29.	FT-IR	Fourier transform infrared spectroscopy
30.	DSC	Differential scanning calorimetry
31.	SEM	Scanning electron microscopy
32.	SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
33.	$Na_2S_2O_5$	Sodium metabisulfite
34.	HCL	Hydrochloric acid
35.	Na_2S	Sodium sulfide
36.	NaOH	Sodium hydroxide
37.	SDS	Sodium dodecyl sulfate
38.	MEC	mercaptoethanol
39.	KOH	Potassium hydroxide
40.	$NaHSO_3$	Sodium hydrogen sulfite
41.	SE	Steam explosion

CHAPTER 1: INTRODUCTION

1.1 Overview of livestock waste

Livestock waste, in other words, livestock excreta composed of organic by-products generated from animals raised for agricultural purposes. Livestock waste may contain urine, dung, residual milk, bedding materials, soil, hair, feathers, leftover feed, or other debris [1].

1.2 Livestock waste as a keratin source

Keratin is the natural polymer found in human and animal tissues. The vital sources of keratin in body parts are scales, wool, beaks, hooves, feathers, and wool [2], and around 5 million tons of keratin-based protein waste induced in butchery, dairy farms, slaughterhouses, and poultry industry in the form of feathers, hair, horns, waste wool, and nails [3,4]. Based on sulfur amount it can be sub-divided into hard and soft keratin. Hard keratin provides the structural and mechanical characteristics. Almost 85% of keratin is composed of proteins that provide insolubility in water and has the highest sulfur and cysteine content with 17% [5,6]. On a structure, keratin can be distinguished into α-keratin (alpha-keratin) or β-keratin (beta-keratin) where α-keratin contains α-helices while β-keratin contains β-sheets [7]. Alpha is found in the mammal's structures like hair, wool, horns, etc, and provides good mechanical strength. Beta keratin is available in the feathers and scales of reptiles and has good microbiological stability [8]. Disulfide bonds in cysteine with hydrogen bonds and hydrophobic interaction are so stable that it doesn't allow easy degradation by maintaining its rigidity and stability [6].

A α- keratin is rich in amino acids like alanine, phenylamine, valine, and methionine. The presence of both acidic (glutamic and aspartic acid), and alkaline groups (arginine, histidine, and lysine) make it an amphoteric molecule. Besides that, some free reactive groups like carboxyl, amide, aliphatic and phenolic hydroxyls, and sulfhydryl are also present in the keratin structure [9].

Thiol groups provide the formation and maintenance of disulfide covalent bonds. Thus, decomposing keratin waste requires high temperature and pressure [11]. Methods like Chemical and hydrothermal are frequently employed for the extraction due to their low cost and lower energy consumption. However, they provide deficiency in some amino acids like methionine, lysine, and tryptophan [10].

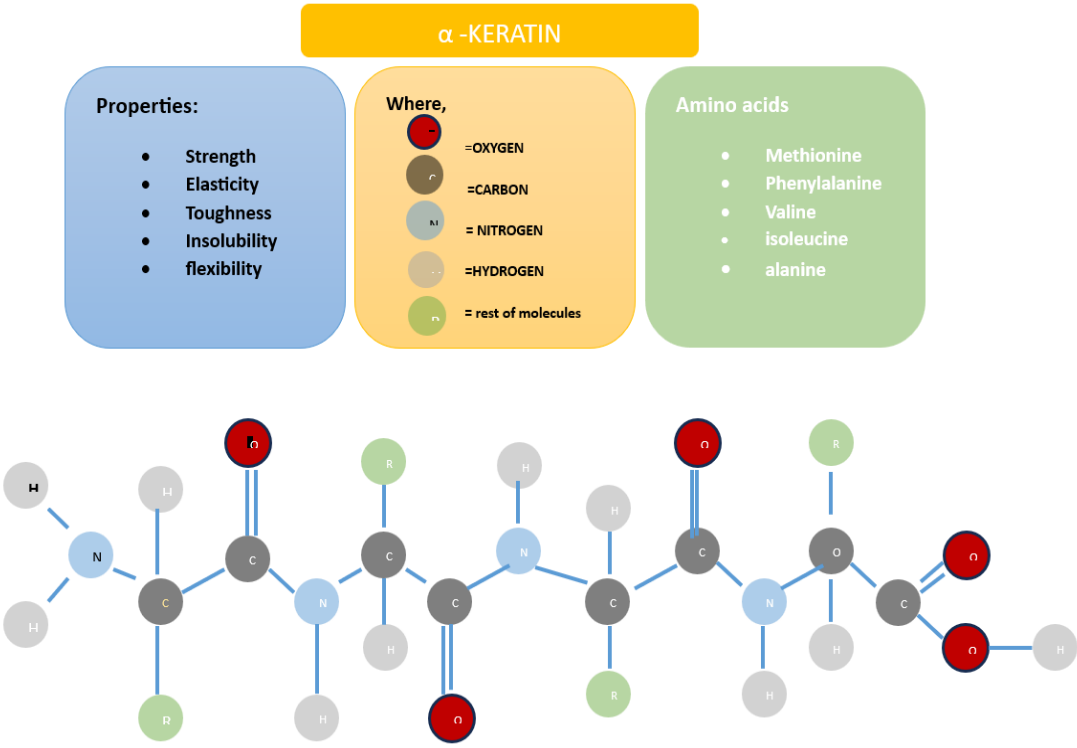

Figure 1: Alpha-keratin structure

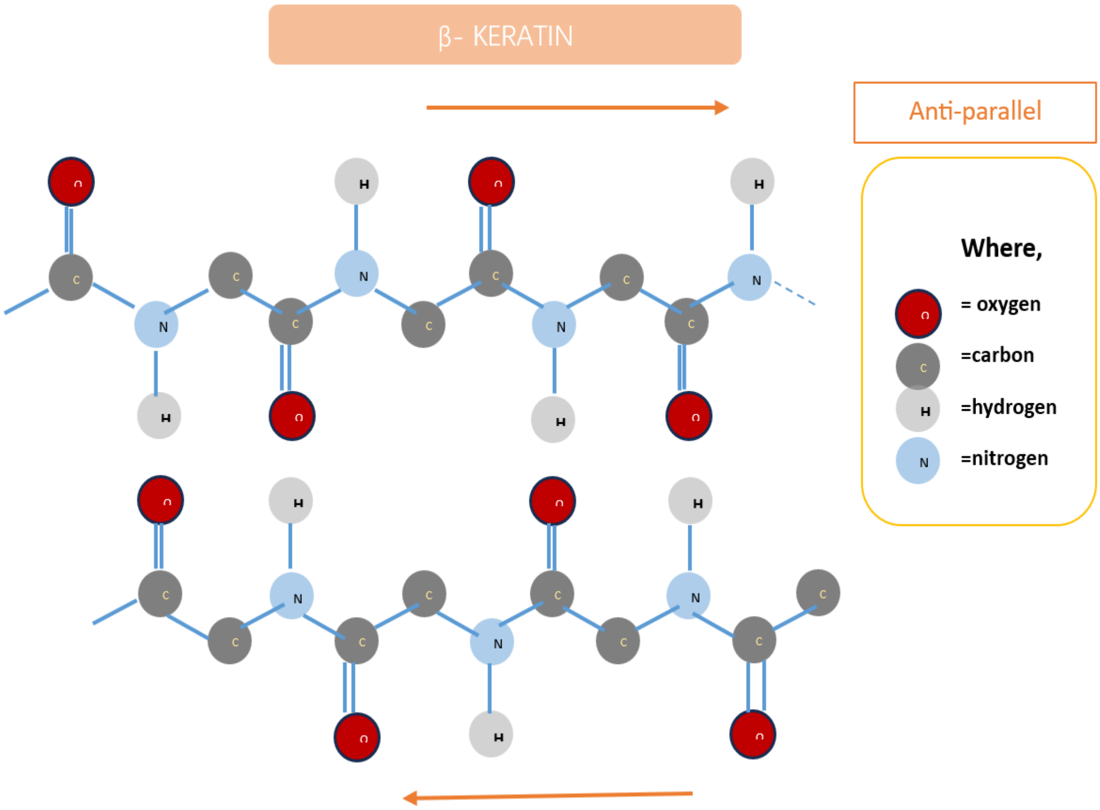

Figure 2: Beta-keratin structure

1.3 Sources of keratin waste

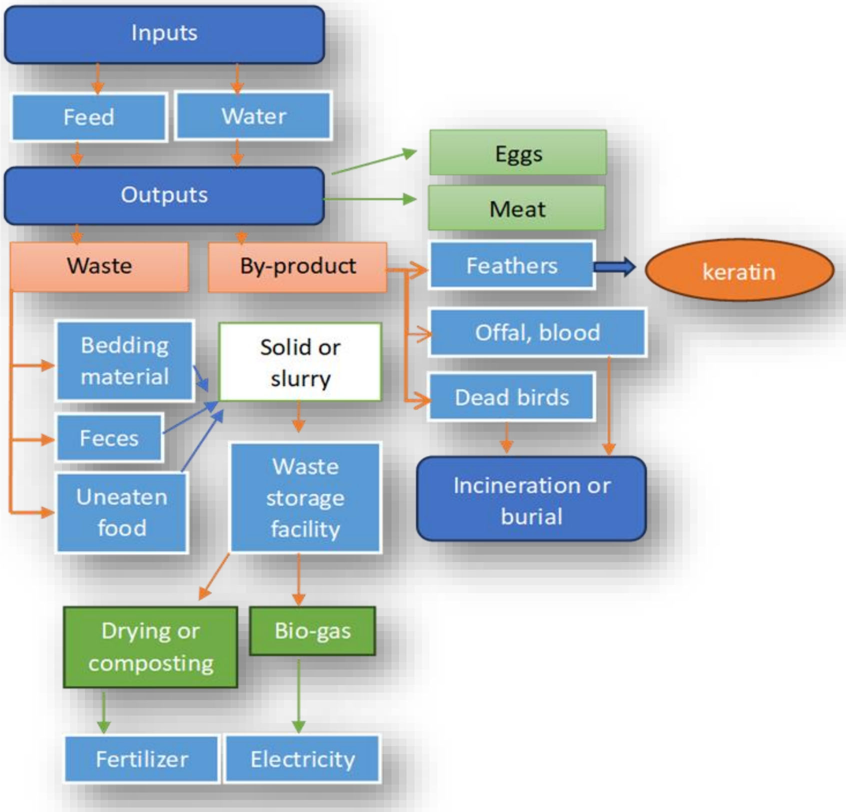

Figure 3: Input/output of poultry industry

The poultry industry is one of the fastest-growing agricultural businesses globally due to the increasing demand for meat and eggs [11]. Both solid and liquid state wastes such as feces, bedding material, feathers, empty shells, infertile eggs, dead embryos, sludge, offal, and blood are generated in this sector [12].

Chicken feather

- A hen's body is about 125mg composed of feathers, a billion got killed to meet the demand [13]. Feather consists of highly insoluble and stable fibrous protein known as keratin [10]. Feather contains around 90% of keratin with a molecular weight of around 10kDa nearly 41-47% is α-keratin, 33-38% is β-keratin, and amorphous keratin components [14].

Duck feather

- The rearing of ducks primarily focuses on meat production while causing many by-products like bone, skin, fat, internal organs, and feathers. Duck Feather has around 91% keratin, nearly 8% water, and lipid at 1% [11,14].

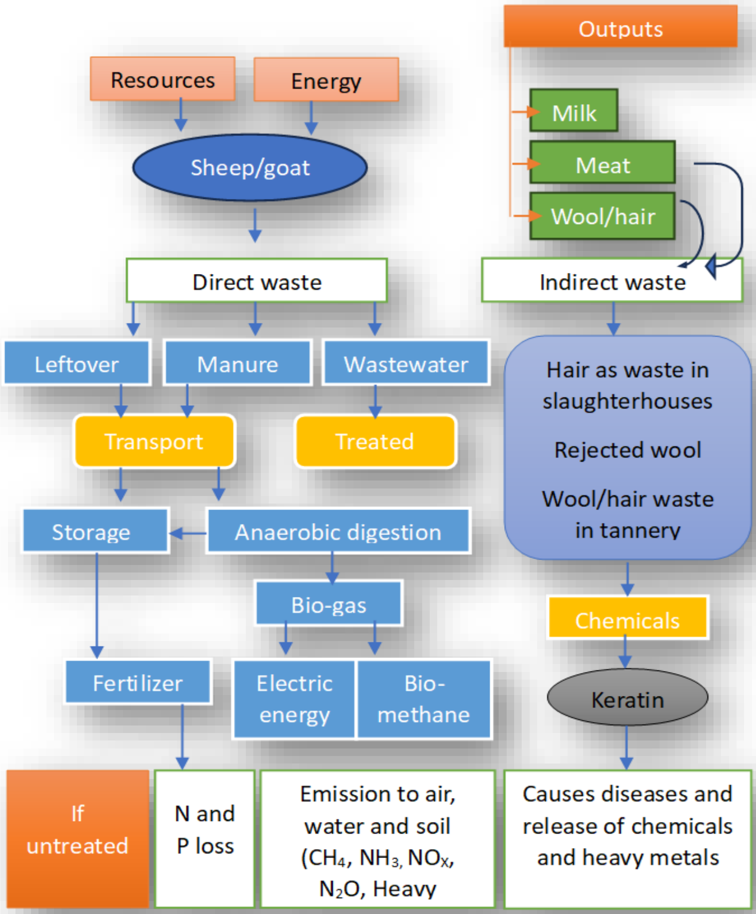

Figure 4: Input/output of Sheep and goat farming

Rejected wool

- Sheep are generally farmed for wool production, one of the most preferable natural fibers used in the textile industry, and the largest class of animal hair consists of α - helices [15,16]. Every year 2.5 million tonnes of wool is generated throughout the world, New Zealand, China, Australia, Iran, and Argentina are the largest suppliers of sheep wool [17].
- Wool proteins have around 19 amino acids composed of hydrogen, carbon, nitrogen, and sulfur linked together by peptide bonds to form a steps-like structure [18].
- In a 2020 survey report yarn share in the textile industry has been shrined to 1% due to increasing demand for synthetic polymers. Another reason for wool waste is the growing reserve of sheep wool resulting in a surplus quantity getting discarded. As a result, in total production, most get rejected due to quality standards and specifications and culminates in a landfill or incineration. [19].

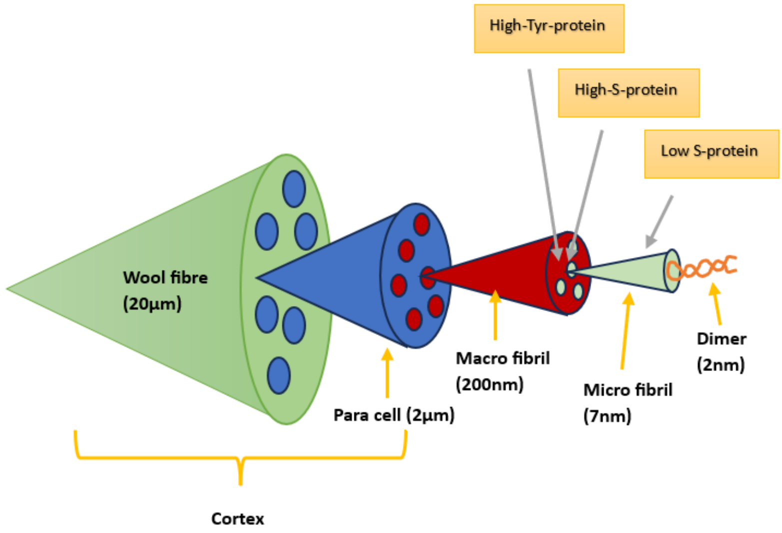

Figure 5: Wool fibre structure [20,21]

Goat hair

- Around 1 million goats are butchered each year for various livestock products globally. Hair is composed of fibrous protein with 50-60% α- helix and 20-30% β-matrix protein. The presence of amino acids and carboxyl groups provides properties like strength, compatibility, and degradability making it a potential product for biomedical, agriculture, and many other industries [22,23]. Yet, due to its coarse diameter and hardness abandoned in the meat processing industry. Hazards like sewage clogging, and the emission of toxic gases can cause respiratory problems, and become a breeding ground for many pathogens and bacteria [22,24]. In India, an enormous number of goats are sacrificed in religious celebrations that may produce waste like bones, blood, and hair [24].

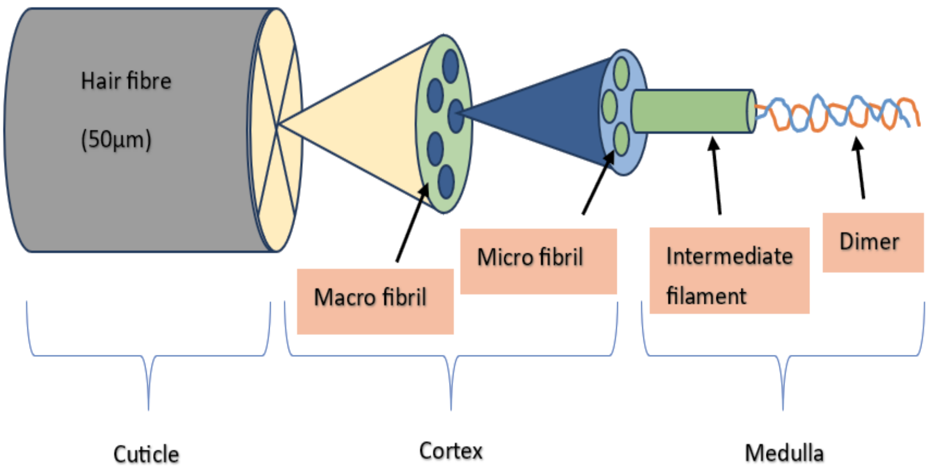

Figure 6: Hair fibre structure [25,26]

- Keratin can be sub-divided into sections: a cuticle- The outermost layer that protects from chemical and physical damage, a cortex at the middle, and the medulla- The centermost part that contains cells that provide a spindle-like shape [27,28].

Buffalo hair

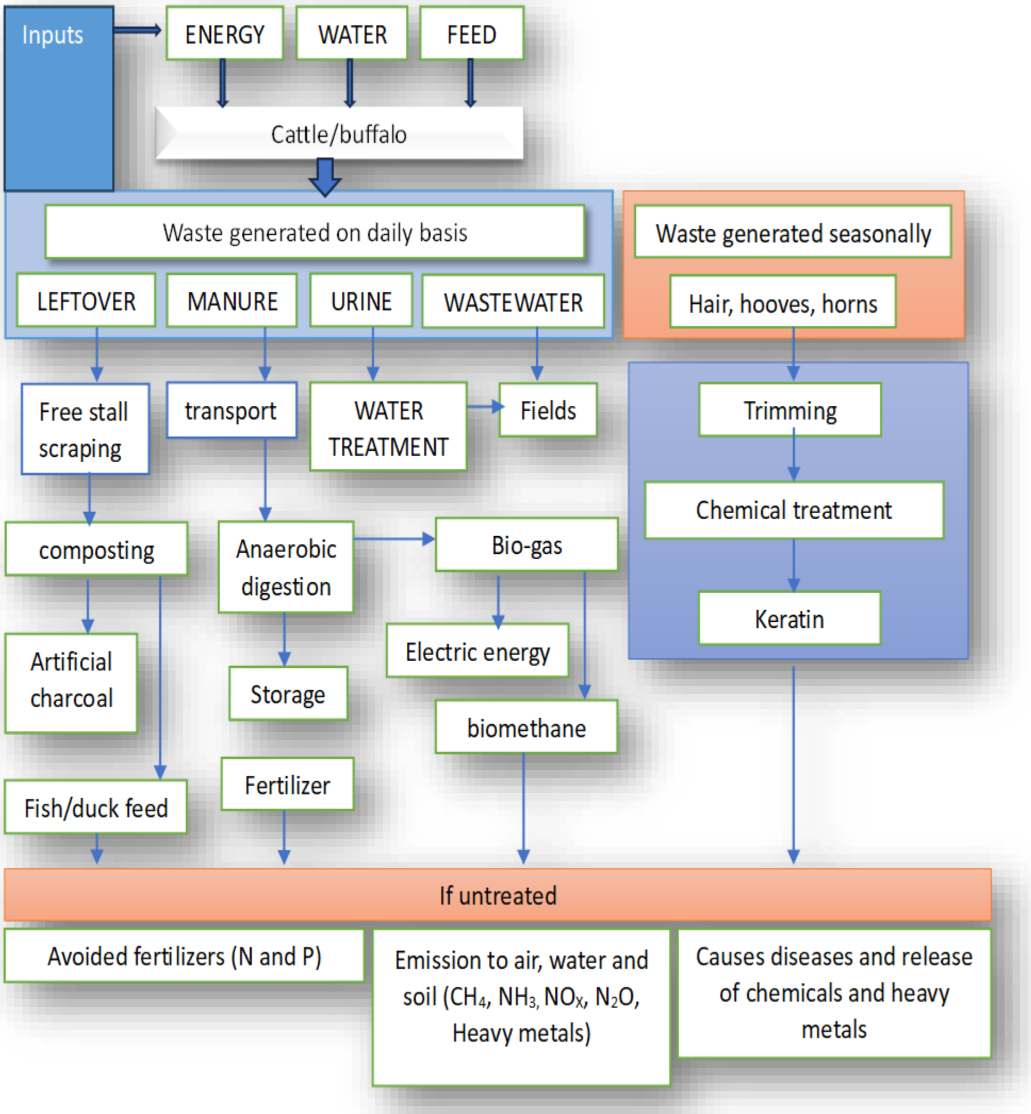

Figure 7: Input/outputs of dairy farming

- Waste produced in the dairy industry mostly happens from wasted milk, leftover food, manure, and urine. Additionally, in the processing industry, by-products like Bones, blood, skin, and hair waste also get generated [29]. Farmers often treat waste as a burden and neglect the potential it possesses [30,31]. Although, a small portion of wastewater and manure is cast for irrigation and processing crop fertilizers.

- The leather industry produces around 10 million tonnes per annum of skin and hides, processing lead generation of about 1.5 million tonnes of waste containing protein waste. India is one of the largest leather manufacturers after China [32]. The leather industry produces various types of proteinase biomass like hair, flashing, trimmings, splitting, and shaving that have high amounts of protein available [33,34].

Waste type	% (per 1000kg of product)
Meat residue (flesh waste)	56-60%
Fat or oils	18-20%
Tanning agents and dye chemicals	15-20%
Trimmings	5-7%
Hairs	2.5%

Table 1: Waste generated during meat processing [34]

1.4 Environmental impacts

Open disposal of keratin waste has the potential to affect both surface and groundwater due to the concentration of heavy metals like zinc and chromium. During ignition, various toxic gases like ammonia, carbonyl, and hydrogen sulfides get released causing air pollution and offensive Odors. Due to the presence of strong intermolecular bonding, keratin waste has resistance to degradation and occupies a large volume of space while burning contributes to environmental contamination [18,23].

CHAPTER 2: STUDY OBJECTIVE

The Livestock and agriculture sectors serve as the backbone of economic livelihood in society. Contribute between 13-16% of the GDP share of the country by providing a variety of products like meat, milk, eggs, and other useful household and industrial raw materials and generate income for small and marginal farmers [37].

The Indian poultry industry, valued at USD 18.5 billion in 2019-20, shows a 10.19% increase in egg production from the previous year (2018-19). The sector collectively generated USD 20.06 billion in products, with poultry meat (75.32%) and eggs (24.67%).

S.no	Category	Total population (in 2019 census)	Increase (from 2012 census)
1	Rural livestock	514.11 million	4.8%
2	Urban Livestock	22.65 million	4.8%
3	Bovine population (cattle, buffalo, Mithun, yak)	303.76 million	1.3%
4	Total cattle	193.46 million	1.3%
5	Exotic/crossbred cattle	51.36 million	29.3%
6	Indigenous/ non-descript cattle	142.11 million	10%
7	Buffalo	109.85 million	1.1%
8	Sheep	74.26 million	14.1%
9	Goat	148.89 million	10.1%
10	Poultry	851.81 million	16.8%
11	Backyard poultry	317.07 million	45.8%
12	Commercial poultry	534.74 million	4.5%

Table 2 : Livestock population in India [38]

Annual demand for high-protein food has shown an increment. Likewise, the livestock population and amount of waste produced through the life cycle [39]. Livestock waste has created several problems around the world. For example- global warming. However, due to the advantages over traditional sources of being an odorless fuel and helpful in minimizing soil, water, and air pollution. Livestock waste can be employed in many useful products or alternative energy sources like biogas and fertilizer [39-42]. Consequently, promoting a healthy environment, reducing toxic gas emissions, and sustainability.

Keratin is the 3rd most common protein available in nature after cellulose and chitin [30]. Traditional Methods like burial, incineration, and composting are usually employed to eliminate waste generated due to a lack of awareness, farmers' attitudes, minimal government support, unavailability of dumping sites, and the high cost of modern solutions and technologies [10,44].

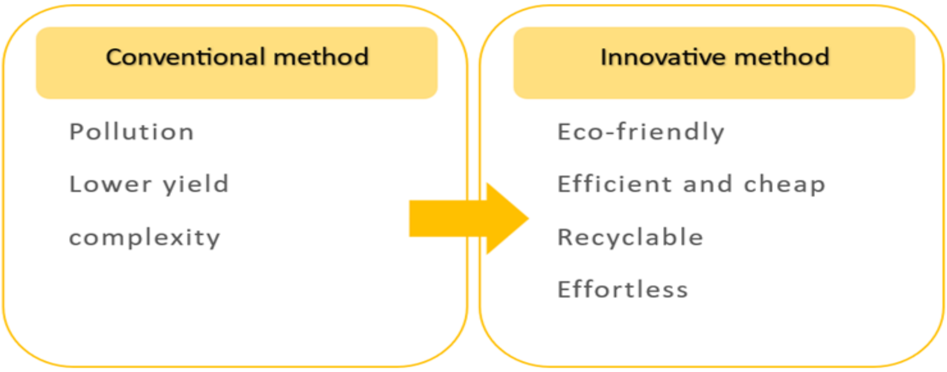

Figure 8: Conventional vs advanced management methods

Current disposal methods

- Incineration
- Burial
- Chemical treatment

Advances in Keratin Waste Management

- Bio-degradation
- Bio-conversion

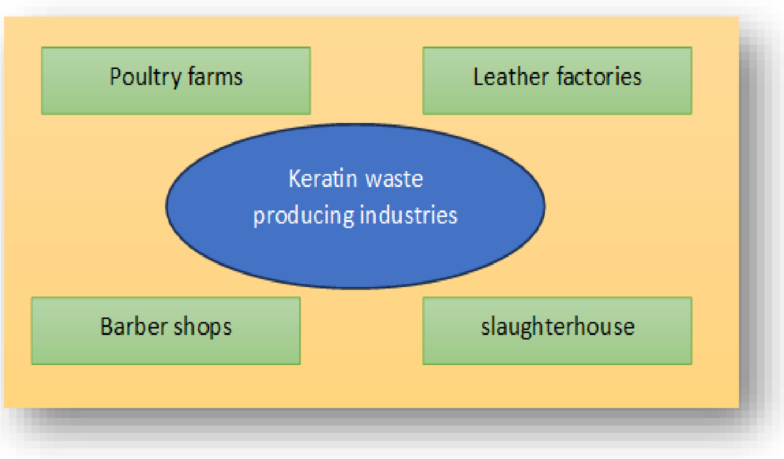

Figure 9: Keratin waste-producing industries

Millions of tonnes of biomass consisting of protein found in animal byproducts like hair and feathers are abundantly available in nature known as keratin reused for various applications like drug delivery, cosmetics, and bioplastics. It is an affordable and sustainable source for the manufacture of bioproducts. The practice of byproducts can minimize impact, and promote a safer and cleaner environment.

2.1 Motivation of the study

General trends of the increase in the consumption of goods generate the by-products with more complexity and toxicity. Procurement of the raw material, segregation of the waste collected, transportation of the waste, during the extraction processes, and final disposal after the recovery of valuables produce environmental pollution, and management for waste disposal is still a challenge.

The motivation behind this study is to utilize the waste generated in the processing units of the textile industry, slaughterhouses, poultry, and dairy farms as raw materials for other industries. The existing methods for waste management are inappropriate and unplanned. This led to various forms of pollution and health-related issues. Development of the advancement and sophistication of waste management techniques can potentially tackle the challenges provide an additional source of income to the rural sector, and arrange employment opportunities in the Indian economy.

2.2 Key objectives

- Extract the keratin waste from hen and duck feather, sheep wool, goat and buffalo hair from the waste
- Study the techno-economic feasibility of the alkaline hydrolysis method

To achieve the aforementioned objectives, the following activities were carried out: -

- Extraction of keratin from waste
- Comparison between various extraction methods
- Comparison between collected keratin waste

- FT-IR, DSC, and SEM analysis of various waste forms: To identify the composition of keratin waste
- The optimal method for extraction by varying concentration and amount of chemical employed, temperature, and filtration techniques.
- Resource estimation and costing for the establishment of plant
- Potential application of keratin waste and target markets
- Technical, economic, and regulatory challenges and solution

2.3 Outcomes of study

- By-product utilization
- Reducing the solid waste management load of municipalities and gram panchayats in the country
- Reducing the burden of farmers and industrialists
- Value-added product generation for economic gain
- Employment generation
- Sustainability and cleaner technology

CHAPTER 3: LITERATURE REVIEW

Rahmi et al.,2023 stated that Fauna species may carry either of two: hard or soft types of Keratins in their body parts like nails, horns, and skin. Human nails contain about 80% hard, and 20% soft keratin providing strong chemical bonding between them. Alkaline hydrolysis methods were employed for extraction at 70 degrees Celsius with NaOH and acetic acid. Analysis methods like FT-IR analysis of the presence of Amide1 at 1600cm^{-1}, Amide-II at 1551cm^{-1}, C-H and N-H bending at 1247cm^{-1}, CH bending 1395cm^{-1}, COC bond at 1124cm^{-1} where C-S stretch vibration nearby 825 cm^{-1}. Other parameters like intrinsic viscosity and melting point at high temperatures were examined to ensure stability, robustness, and solubility.

Alshehhi et al.,2024 research on gluten and keratin for printing 3D bio-plastic. They develop a new method of making bioplastic from wheat gluten and keratin from sheep wool. Films have a structure with both hydrophobic and hydrophilic regions that is a biodegradable and renewable product that lessens the environmental impact of by-products. FT-IR result reveals the presence of beta-sheets, random coils, and alpha helices. The film possesses a low temperature of degradation of 108.1 degrees with keratin and gluten. Films were able to 3d printing and absorb more water due to keratin containing hydrophilic groups. However, breakage of structure is directly proportional to keratin % in film.

Anurag Singh and Shyam d Maurya 2024 examine the production of bioplastic film by blending keratin and polyvinyl alcohol that possesses good properties for implementation in biomedical applications. Sodium sulfate, hydrochloric acid, and sodium hydroxide were employed for extraction purposes, and extracted keratin blended with PVA at various proportions, and concluded that 15% of keratin in PVA is stable, smoother, and biodegradable whereas, keratin with 5% has thermal stability. Analysis like FT-IR, SEM, and DSC were performed on keratin structure.

- Yield (%) = $\frac{Total\ weight\ of\ keratin\ extracted}{total\ weight\ of\ raw\ material} * 100$

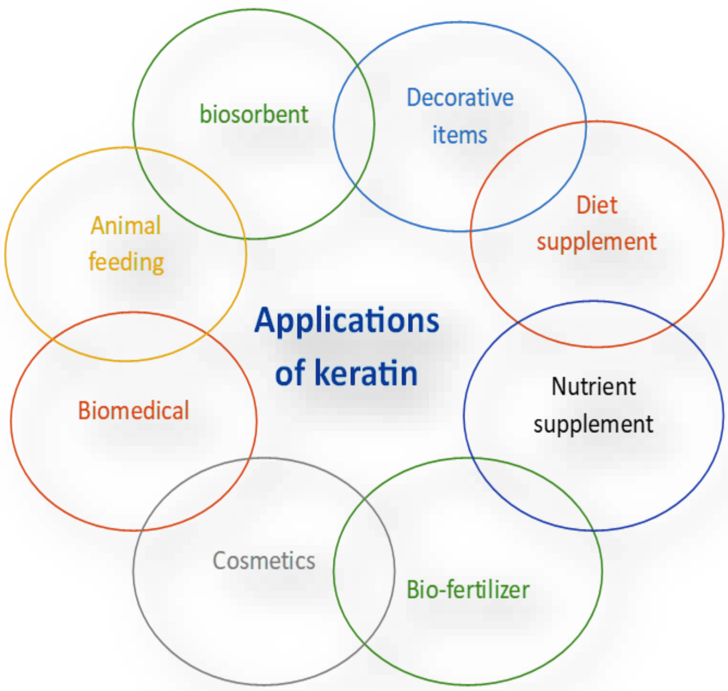

Figure 10: Application of keratin

A study conducted by *Cao et al. 2024* investigated the keratinase-based degradation of chicken feathers through Stenotrophomonas Maltophilia microorganism culture. Feather keratin monomer was prepared with the help of chemicals like $Na_2S_2O_5$ as a reducing agent for sulfitolysis for disulfide bond reduction, and then transferred into the culture of microorganism that was grown through feather meal medium at required conditions. Qualification tests like TEM and SDS-PAGE at different time frames and chemical analyses like fluorescence value, western blot, and colloidal gold labelling were performed. The requirement of special equipment, high energy, and reaction time provides some limitations while having potential for biomedical and other applications.

The article in *2024 by Bhat et al.,* examines the potential of goat hair keratin for wound healing and tissue regeneration which can be beneficial material for manufacturing biomaterial due to their compatibility. For example: - coating material and testing on different parameters to check compatibility for culturing and differentiating stem cells like mesenchymal stem cells and primary fibroblast cells from goat umbilical cord. Goat hair is rich in cysteine and has good mechanical flexibility and biological properties that promote tissue growth and nerve generation and support cellular attachment and proliferation in vitro. These fibers are cost-effective and easy to use, low immunogenicity, anti-inflammatory, and resistant to proteolytic degradation promote waste-to-energy, and avoid issues like oxygen and solubility.

Meko et al.,2023 investigate the effect of parameters like concentration of the solution, retention period, and temperature on chicken feathers during the extraction of keratin through alkaline hydrolysis due to benefits like being easiest, economically and environment-friendly method and the extracted product can be used for various application range from animals feed, bio-compote, biomedical in the formation of gel or film can be employed. Different concentrations of sodium hydroxide (0.1, 1.0, 2.0, 3.0, and 6M) and retention periods of 1h, 3h,6h,12h, and 24 h were taken to test at the temperature range from 8c to 60c were examined. Through the results, 3M NaOH concentration at 37°c for 24 h gives the highest yield of keratin with 62%. Various analyses like SEM, FT-IR, and DSC were employed to know the structure and particle size of Keratin revealing the amide group at 1600cm^{-1} and 1200cm^{-1} of carboxylic acid, hydroxyl, and sulfhydryl and the highest melting point of 110°c and SEM depict the unsmooth surface and particle size of 0.56mm.

	SOURCES	**APPLICATION**	**References**
1	**Human Hair**	Medical usage	*Zheng et al.,2005*
		biomaterials	*De Masi et al.,2019*
2	**Sheep Wool**	To investigate the biological and structural characteristics of self-assembled keratin	*Reddy and Yang,2014*
3	**Horns and hoof**	Biomedical applications, tissue engineering, composites, and aerospace application	*Baillie and soutam,2000*
4	**Chicken feathers**	Delivery of drugs	*Church and Huson,2009*
		Newly grown fibres	*Yang et al.,2014*
		Nano and micro-particles	*Sun and liu,2009* *Aluigi et al.,2018* *Zhang and Liu, 2019*
		Pharmaceutical nano-particles	*Reddy and Yang,2014*
		Feeding supplements for ruminants	*Amieva and Ramirez 2015*
		Food packaging	*Reddy and Jiang, 2013*
		Textile	*Reddy et al.,2013*

		Fabrication of bio-composites	*Spiridon et al.,2012*
		Bio-fertilizer, thermoplastic films for food packaging, hydrogels	*Donato and Mija, 2019*
		Paper production	*Tesfaye et al.,2017*
		Making paper	*Tesfaye et al.,2017*
		Chicken feather and polypropylene are utilized as a non-woven insulator	*Reddy et al.,2021*
		Protein for ruminants	*Coward-Kelly et al.,2006*
		Fish feed	*Jumini 2017*
		Biosorbent	*Solis-Moreno et al.,2021* *Chen et al.,2021* *Cheng et al.,2023*
		Hydrogel	*Wang et al.,2017* *Esparza et al., 2018* *Cao et al.,2019*
		Films	*Dou et al.,2014* *Martelli et al.,2006*
		Scaffolds	*Nayak and Gupta 2015* *Esparza et al.,2017* *Saravanan et al.,2013*
		Nitrogen fertilizer	*Yang et al.,2013*
		Coating material for controlled-release fertilizer; water retention/ superabsorbent coating	*Yang et al.,2013* *Zhang and Chen 2021* *Faraon et al.,2020*
5	**Feather hydrolysate**	Supplementary protein	*Grazziotin et al.,2008*
		Biofertilizer	*Tamreihao et al.,2017* *Nafady et al.,2018*
6	**Hydrolyzed feather meal**	Fertilizer	*Thuries et al 2001*
7	**Wool**	Nanofibers, antipilling processing, porous foams	*Tonazzini et al.,2019*

8	**Sheep wool**	Carpet industry, apparel, regenerative medicines, and coating	*Gong et al.,2016*
		Nitrogen fertilizer	*Bhaysar et al.,2016*
		Nitrogen fertilizer	*Gaidau et al.,2019*
9	**Bovine hair**	Sprayable mulch, with a holding capacity (380%) and high mineral nutrient content	*Zhang and Chen 2021*
10	**Bovine hoof**	biomedical	*Kakkar et al.,2014*
		Aerospace application	*Baillie et al.,2000*

Table 3: Research conducted and their application

Maurya and Singh published research in 2023 about the extraction of keratin from chicken feathers as a valuable source of keratin with the treatment of sodium sulfide and precipitation with HCL. Broiler feathers are available to provide 81.1% extraction of protein that is essential for human survival and one of the required components for body cells. Yield of keratin depends on the proper concentration of Na2s, temperature, and time. The Ash content of chicken boiler is 2% of 2g and peaks at 3296.9cm^{-1}, 2925.4cm^{-1}, 1660 cm^{-1}, 1533.7 cm^{-1} and 1216.8 cm^{-1} for the O-H stretching, -H stretching, amide-I, The amide-II and amide-III. XRD peaks between 10.61-19.55 of b-sheet and degradation at 280^{o}c by TGA analysis extracted keratin has potential in many applications while minimizing the environmental challenges.

Anurag and Maurya's study in 2023 on the application and future perspective of keratin contains protein with 90%, nitrogen of nearly 17%, sulfur of up to 5%, and fat with 1.20%. form as a by-product in many industries like wool processing, meat markets, and slaughterhouses. Extraction through chemical and mechanical techniques like alkaline hydrolysis, reduction, oxidation, alkali, steam explosion, and microwave irradiation can be employed while considering their advantages and disadvantages for utilization in daily-use products. Keratin is composed of alpha keratin gives strength, toughness, and flexibility, and has many hydrophobic amino acids. On the other hand, beta keratin provides stiffness and resistance to degradation.

2021 research conducted by Yan et al. depicts keratin applications like biomedical engineering due to properties like bio-compatibility, bio-degradability, and mechanical and biological activity while mostly discarded into the environment or disposed of by incineration and contributing to environmental pollution. This waste may contain 90% keratin formed by the disulfide, hydrogen, and salt bonds, insoluble in water, and resistant to common enzymes, weak acids, and organic solvents. The primary structure has 19 amino acids and alpha-helices. Beta-sheet is a secondary structure. However, alpha contains hydrogen bonds within peptide chains and beta between peptide chains, cysteine content can be of soft and hard keratin. It contains around 400-600 amino acids and cell-binding sites essential for mechanical properties like strength and elastic modulus serve for bone tissue engineering.

S.NO	**FUNCTION IN PLANT GROWTH**	**AMINO ACID**
1	*Anti-stress agent chelating*	Proline
2	*Chelating agent*	Cystine, glutamic acid, glycine, histidine, lysine
3	*Cold weather resistance*	Alanine, arginine
4	*Development of plants and improvement of pollen fertility*	Proline
5	*Growth stimulator*	Glutamic acid
6	*Precursor of auxin precursor*	Serine, tryptophan, valine
7	*Precursor of chlorophyll*	Glycine
8	*Precursor to the formation of lignin and woody tissues*	Phenylalanine
9	*Regulation of the water balance*	Proline, serine
10	*Reserve nitrogen for the synthesis of amino acids and proteins*	Glutamine acid
11	*Stimulation of the chlorophyll synthesis*	Alanine, lysine, serine
12	*Stimulation of the germination*	Aspartic acid, glutamic methionine acid, lysine
13	*Stimulation of the hormone metabolism*	Alanine
14	*Stimulation of the resistance mechanism to viruses*	Alanine

Table 4: Function of amino acid in plant growth

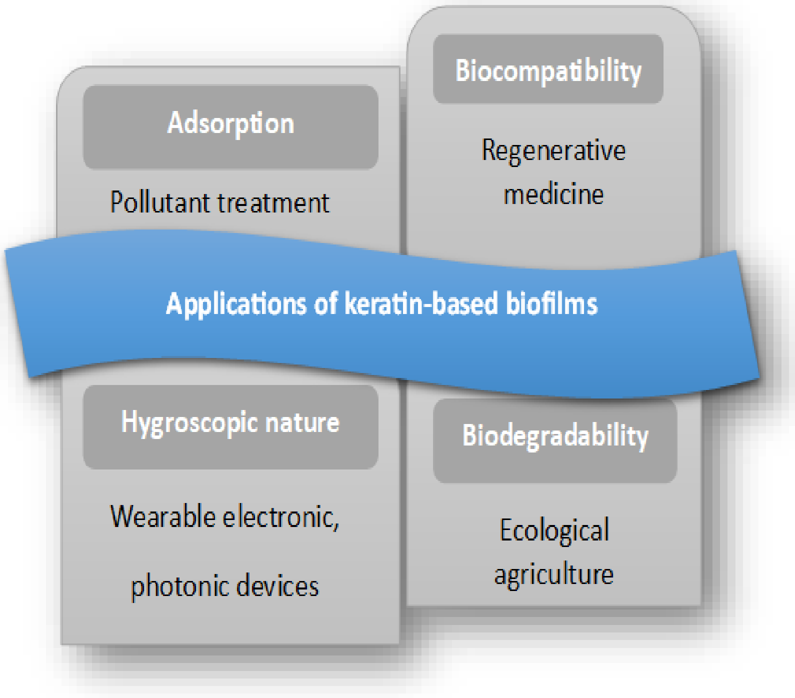

Figure 11 : Properties of keratin bio-film

Research conducted by *Yilma et al., in June 2022* for optimizing the keratin extraction from cattle hoofs using CCD (central composite design) and fat content was examined by soaking the hoof into diethyl ether and left for 2 hours for sedimentation and later on, dried for 24 hours at 50c.

- Fat content (%) $=\frac{weight\ of\ fat * 100}{weight\ of\ sample}$
- Moisture content (%) = $\frac{(W1-W2)* 100}{W1}$
- Where, W1=weight before drying and W2= weight after drying
- Ash content (%) = $\frac{weight\ of\ ash* 100}{weight\ of\ sample}$
- Protein content (%) = Nitrogen content in sample * 6.25 (according to Kjeldahl method)

CHAPTER 4: METHODS OF EXTRACTION

4.1 Methods for Drying

FREEZE DRYING	SPRAY DRYING	VACUUM OVEN DRYING
To remove water from the frozen sample using a sublimation and desorption process. Removing water from the material is essential as it prevents reactions like disulfide interchange that can lead to inactivating proteins [43].	The mixture is sprayed into the hot dry chamber where the water evaporates and leaves behind the dry powder. Work on the concept of the micro-encapsulation process by maintaining a low temperature. During the process, rapid water evaporation is performed.	Under this method, heat waves are supplied to the sample to remove the moisture, and the high-energy water molecules settle down to the surface below and then evaporate at low pressure. This process is frequently used in the food industry [43].

Table 5: Methods of drying

4.2 Methods for Extraction

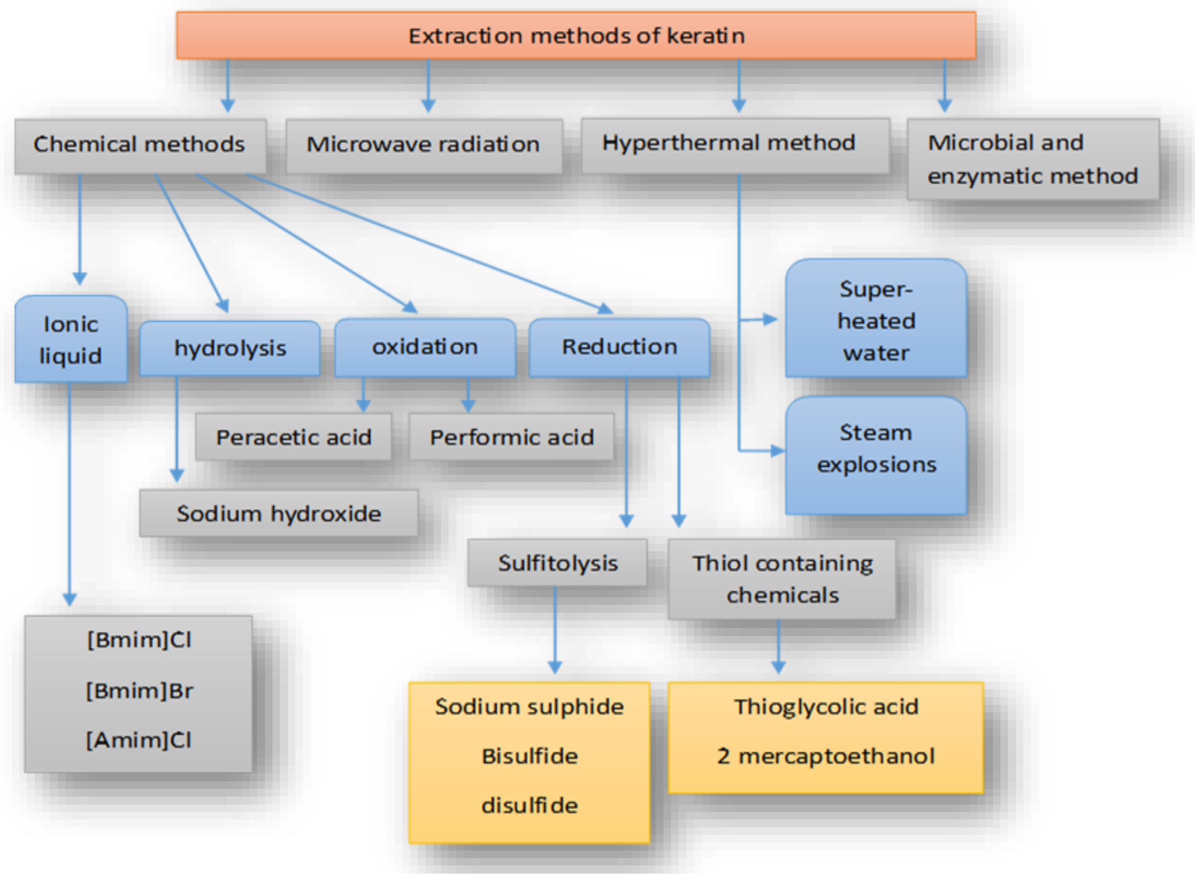

Figure 12: Methods for extraction of keratin

4.2.1 Alkaline Hydrolysis Method

The process uses NaOH as a solvent at a temperature range between 60-70 degrees Celsius for a retention period of 2 to 24 hours. Na_2s with NaOH can accelerate the process and solubility by degrading the keratin structure. The extracted solution has a high amount of lysine, methionine, and glutamic acid but low levels of serine, arginine, and cysteine. Some of the amino acids get destroyed in the extraction process. But the damage is lower than acid hydrolysis. Feather and wool have around 7% to 17% cysteine which is more stable than cystine and dissolves in an alkali solution and forms oxalic and pyruvic acids. The amount of alkali used during the extraction splits the sulfurs and damages the polypeptide chain. Speeding the process with sodium sulfide to the NaOH solution may result in high sulfur content. Alteration can be achieved by adding Na_2So_3, SDS, and Urea in the NaOH solvent. Cysteine gets affected easily and causes amino profile and yield while the secondary structure remains preserved [45].

Advantages

- It's a relatively simple and cost-effective method [46].

Disadvantages

- Chemicals used in this process can't be reused or recycled.
- It is a time-consuming process due to the retention period and provides a lower yield.
- By-products like oxalic and pyruvic acids formed
- Harsh conditions can lead to keratin degradation

Research conducted

Solution	**Conditions**	**Yield**	**References**
Wool (10g) with 0.2M NaOH, HCL, Ph =7	T=80 ºC t=3h	25	*Shavandi et al., 2017*
Wool with KOH: NaOH (14:1)	Ultrasonic radiation t=30min	100	*Holkar et al.,2016*
Sheep Wool- KOH; CaO 5%,10% 15%	T=140-170 ºC t=1h	53.5	*Bhavsar et al.,2016*
Chicken feather-NaOH=0.5M (1:20)	T=60 ºC t=3h	-	*Thangam and Madhan, 2020*
Chicken feather-NaOH=0.75M	T=60 ºC t=45min	82	*Abebe, 2017*

Table 6: Extraction through Alkaline hydrolysis method

4.2.2 Reduction Method

Thiol-containing chemicals like sodium thioglycolate and thioglycolic acid of different amounts or concentrations are used for the reduction process by reducing disulfide linkage, factors like pH, time, and concentration can affect the yield of keratin. Provided the keratin with high molecular weight and structure remains undamaged. The method mostly takes place at the pH range of 10.5 or more. This method has the efficiency to provide around 47% protein content but also increase the level of lanthionine that may result in unrequired nutritional effects like a reduction in protein digestibility and toxic side effects [17]. Reducing agents like mercaptoethanol (MEC) are more effective than potassium thioglycolate due to their high concentration and ionic strength [7]. Chemicals used in this process are harmful and toxic to humans and nature. These processes can provide around 75% yield.

There is an interrelation between the amount of surfactants utilized and the yield of keratin. [47]. Yield output depends upon the temperature, concentration of sodium bisulfite, and the SDS reaction taking place [39].

Advantages

- Can be implemented for food and medical application

Dis-advantages

- It's an expensive and complicated process that can't be recycled and reused.
- It can't be implemented for industrial purposes.
- Removal of MEC and SDS in dialysis may oxidize the cysteine amino acid.

Research conducted

Solution	**Condition**	**Yield**	**References**
Chicken Feather- Urea=8M; SDS=0.26; tris-HCL=0.2M; 2-mercaptoethanol=1.66M	T=50ºC t=2h	83.80	*Sinkiewicz et al.,2016*
Chicken Feather- NaHSO3=0.5M, urea=8M; SDS=0.08M	T=50 ºC t=2h	82.4	*Sinkiewicz et al.,2016*
Chicken Feather- NaHSO3=0.5M, urea=8M; SDS=0.2M	T=50 ºC t=2h	62.9	*Sinkiewicz et al.,2016*
Sheep Wool- Na2s2o5= 0.5M; Urea=8M; SDS=0.1M	T=65 ºC t=12h	28.1	*Ramya et al.,2020*

Table 7: Extraction through Reduction method

4.2.3 Oxidation Method

This process extracts keratin from hair and wool with HCL, ammonia, and peracetic acid 2% [39]. During this process, disulfide linkage in the structure gets converted into sulfonate groups when treated with peracetic acid (2%). Amino acids get converted to cysteine monoxide, dioxide, and sulfonate groups. Extracted keratin has lower molecular weight and yield but good solubility and can be performed at normal temperature and pressure conditions.

The output of oxidated wool can be subdivided into alpha (60%) beta (10%) and gamma (30%) keratose, and feathers with alpha (31%), beta (18%), and gamma (35%) keratose can be achieved. Gamma keratose has a high content of cysteic acid, proline, serine, and threonine but low in the value of aspartic acid, alanine, glutamic acid, leucine, lysine, phenylalanine, and tyrosine. While, beta keratose has high phenylalanine, glycine, lysine, valine, and histidine [17].

Dis-advantages

- Cystine gets converted into cysteic acid and loss of amino acids like tryptophan, methionine, cysteine, serine, threonine, tyrosine, histidine, and phenylalanine. De-structure may depend on the conditions. Hydrogen peroxide can minimize the impact but can't fully recover tyrosine and phenylalanine.
- Loss in the values of threonine, serine, tyrosine, histidine, and phenylalanine
- Requires large quantity of agents and extraction period [3].

Research conducted

Solution	**Condition**	**Yield**	**Reference**
Sheep Wool-sodium percarbonate 4.5%, NaOH (1:35)	37 ºC t=3-4h	-	*Brown et al.,2016*
Sheep wool- Peracetic acid 24% (1:60)	T=37ºC t=48h	57	*Shavandi and Bekhit, 2017*
Human Hair -Performic acid, h_2o_2, 98% formic acid	T=40ºC, t=18h	-	*Buchanan, 1977*
Human Hair- Peracetic acid 2% (1:20)	T=37ºC t=10h	-	*Merrill et al.,2011*
Human hair- Thioglycolic acid 0.5M	T=37ºC t=15h	-	*Indrakumar et al., 2020*

Table 8: Extraction through oxidation method

4.2.4 Steam Explosion

The conventional steam explosion method was developed by Manson in 1928 and used for the bioconversion of biomass like barley, wood, and wheat straw. During the process, thermal energy gets converted into mechanical energy, followed by an explosion that creates physical tearing and dissociation of the sample. [17]. The modern steam explosion method contains a mechanical system with a piston and cylinder that drive the steam energy, the conventional method uses high-temperature steam and takes place in two phases - boiling followed by adiabatic expansion. High temperatures cause keratin solubility, pepsin digestibility, and degradation-led color change. Disulfide bonds break under mechanical and thermal effects. Results vary by temperature, time, resistance, particle size, and moisture.

Advantages

- It is an eco-friendly method and requires less consumption of chemicals.

Dis-advantages

- The large amounts of cystine content and sulfur factors get diminished and lanthionine is available which is an undesirable product. This process can be optimized by using the alkaline method with SE.

4.2.5 Microwave Radiations

Uniform heat distribution for 7 minutes and a temperature of 180 degrees Celsius with a power range between 150-570kW are supplied which can provide a yield of up to 60% and a reduced retention period compared to other conventional methods [3]. However, Multiple bonds like electrostatic, hydrophobic, hydrogen, and disulfide bonds and structural components like alpha-helix and beta-sheet make the microwave process complicated causing the loss of amino acids like cysteine.

Advantages

- Fastest process for extraction of keratin
- Yield of extraction is nearly 60%
- Heating of solution makes the short processing time.

Disadvantages

- Cysteine gets lost during the process
- Large amount of energy consumption
- A Large Investment is required

4.2.6 Sulfitolysis

Sulfitolysis is less toxic and cost-effective than other methods to convert the cysteine residues into thiol and s-sulfonated residues [3].

Meta sulfite - Urea(8M) with Meta sulfite (0.5M) can be used for 2 hours at 65 degrees Celsius with pH-6.5. It is highly efficient, provides low molecular weight keratin, and takes a shorter time for extraction. At the pH range of 9, it reacts faster with cystine than bisulfide ions, hydrosulfate, and hydroxyl ions formed in reaction with water that break disulfide bonds and form de-hydroplaning with cysteine and lysine damaging protein structure and form perthiocysteine, lanthionine, and lysinoalanine. However, the extraction solution has low solubility and higher temperatures. It is efficient, economical, provides high yield, and requires low temperature for extraction but provides low solubility and a time-consuming process. It has moderate molecular keratin, good solubility, and is non-toxic.

8M urea and 0.165M L-cysteine with pH adjusted with NaOH to 10.5 is the green solution and requires less initial cost. It does not damage the keratin structure due to minimal temperature. However, the output is too low. This process can be more optimized by the reaction temperature, bisulfide concentration, and adding urea and SDS.

Research conducted

Solution	**Condition**	**Yield**	**References**
Chicken feather-Urea=8M, $Na_2S_2O_5$ =0.5M, NaOH to pH=6.5 (1:30)	T=60 ºC t=5h	22	*Zhang et al.,2019*
Chicken feather-Urea=8M, LiBr=0.1M, SDS=0.02, NaOH to pH=12	T=90 ºC t=4h	50	*Rajabinejad et al., 2020*
Chicken feather-sodium sulphide=0.125M	T=40 ºC T=4.5h	-	*Indrakumar et al., 2020*
Sheep Wool- Urea=8M $Na_2S_2O_5$ =0.5M (1:25)	T=65 ºC t=2.5h	41	*Rajabinejad et al., 2020*
Sheep wool- Urea=8M $Na_2S_2O_5$ = 0.5M (1:20)	T=65 ºC t=2h	-	*Ramya et al.,2020*

Table 9: Extraction through sulphitolysis

4.2.7 Thermal Hydrolysis

Processing without chemicals during extraction makes the process safe and eco-friendly [48] providing keratin has lower molecular weight but the presence of amino acids and polypeptides. The two-step process of initially heating at a temperature above the denaturing temperature disrupts the keratin molecular structure and bonds. In the 2^{nd} step, the temperature is set between 100-200 degrees Celsius which diffuses water molecules into the keratin fibers [5] It replicates the chemical action of 2-mercaptoethanol.

4.2.8 Ultrasonic Technology

In this process, mechanical vibration sound cavitation is used for the disruption of the molecular structure of keratin but performed at high temperatures. Some modifications with the ionic liquid with the ultrasonic have given better-proven results in the extraction time and temperature. It is an eco-friendly and promising method.

4.2.9 Ionic Liquid (ILs)

Extraction with the help of ions of salts forms various organic and inorganic anions and cations under a temperature of 100 degrees [39]. Material can be used for biomass, catalysts, electrochemistry, and blended with other materials for thermal stability and mechanical properties [17].

- Ionic liquids like [Bmim]cl, and [Amim]cl with $Na_2S_2O_5$ help disruption of hydrogen bonds [3].
- It is eco-friendly, recyclable, non-volatile, non-flammable, chemical and thermal stable.
- Ions have high melting and boiling points but low vapor pressure.
- Modified with the addition of solvents like $NaHSo_3$, $Na_2S_2O_5$, SDS, and urea.
- Ionic liquid with chloride is better as it helps break down hydrogen bonds.
- Loss of water-soluble proteins affects the yield and quality of keratin.

4.2.10 Enzymatic Method

Similar to the microbial method but uses the cell-free extracts of keratinases produced by microorganisms [39]. Microbes like Bacillus licheniformis can provide sulfhydryl compounds, and kocuria rosea provides good amino acid and carotenoid pigments. pH maintained between 5-9, and temperature at 40-60 degrees [49] at aerobic conditions. The internal structure of disulfide, hydrophobic, and hydrogen bonds are very stable and don't easily degrade thus, enzymes like keratinases can help in hydrolyzing. [50].

Research conducted

Solution	Condition	Yield	References
Feather (Salicola Mara Sensis)	-	80	*Khoshnevis et al., 2019*
Turkey (B. licheniformis ALW1)	-	63	*Abdel-Fattah et al.,2018*
Wool (B. subtilis WB600)	-	68.1	*Su et al.,2020*
Feather (B. subtilis WB600)	-	66.4	*Gong et al.,2020*

Table 10: Extraction through enzymatic method

4.2.11 Microbial Method

Bacteria like Chryse bacterium, fervid bacterium, kocuria, lysobacter, Staphylococcus, Stenotrophomonas, Streptomyces, Thermoactinomyces, and Vibrio [39] degrade the keratin and convert it into peptides preferably utilized in biomedical and food applications [17]. It is a safe and simple process, creates no pollution to nature, and provides the keratin with a lower molecular weight due to the disturbance in peptide bonds. It can be time-consuming but solves problems like complex processing. Some other limitations include production costs and poor reproductivity. Keratin hydrolyzes organisms like bacillus are abundant in nature [51].

The keratin products are rich in carbon, nitrogen, and sulfur content and act as food sources for microorganisms like keratinolytic that degrade the keratin and release nitrogen and sulfur during the process. Some factors like pH, temperature, rate of agitation, and energy source influence the yield rate of keratin. The efficiency is dependent upon enzyme production and substrate type.

Research conducted

Solution - Feather	Condition	Yield	References
Bacillus subtilis	pH=9, t=50	-	*Villa and Aragao,2013*
Bacillus cereus Wu2	pH=7, t=30	-	*lo et al.,2012*
Brevibacillus brevis US575	pH=8, t=55	-	*Jaouadi et al.,2013*
Meiothermus sp. 140	pH=8, t=70	-	*Kuo et al.,2012*
Bacillus Pumilus	pH=8, t=35	-	*Kumar et al.,2008*
Aspergillus Niger	pH=5	-	*Mazotto et al.,2013*
Trichoderma asperellum	pH=7.5, t=26	-	*Raut et al.,2019*
fusarium sp. 1a	pH=7.5, t=27	-	*Alexandrescu et al.,2017*

Table 11: Extraction through microbial method

CHAPTER 5: EXTRACTION BY ALKALINE HYDROLYSIS METHOD

5.1 Raw material

Figure 13: Raw material

Chicken feathers are individually plucked from the leftovers generated in slaughterhouses in Patiala, Punjab, India where they are mostly burned or dumped into the landfills.

Buffalo hair and goat hair are collected from the dairy farmers in the village Hakamwala of district Mansa in Punjab, as animals are regularly shorn for protection from hot and humid climate conditions as excess hair can cause overheating and stress. Shearing helps stay refreshed and comfortable, especially in June and July.

Duck feathers are usually scattered along the shoreline, float on the water's surface, or are nested among the reeds, and were collected individually for extraction from the village of Gamiwala, Mansa, Punjab, India.

Sheep wool was procured from Mansa, Punjab, India as being rejected by farmers, often due to its quality and condition.

5.2 Chemicals used

- Hydrochloric acid (37% purity) (LOBA CHEMIE PVT. LTD, Mumbai, India)
- Sodium hydroxide pellets 98% (LOBA CHEMIE PVT. LTD, Mumbai, India)
- Distilled water of laboratory grade (SEE, TIET, Patiala, Punjab, India)
- Acetone
- Detergent

5.3 Equipment employed

- pH meter
- Weighing machine
- Hot air Oven
- Magnetic stirrer
- Vortex shaker
- Centrifuge machine

5.4 Pre-treatment of materials

In the first phase, to remove the dust, dirt, and blood from the rejected sheep wool, buffalo hair, goat hair, hen feather, and duck feather, each material was washed with the help of detergent for about 15-20 minutes, rinsed with tap water. This process was repeated thrice to remove impurities. The collected material was then sun-dried for about 3 to 4 hours.

Collected material got tangled after initially washing and dissolving of samples became difficult into chemicals in later stages and the size of raw materials was reduced to 2-3 cm with the help of the scissors.

After the size reduction, 20g of each was placed into the 250ml glassware. Acetone of about 200ml was poured into each glassware for 24 hours to remove grease and other impurities from waste. Aluminum foil to cover and eliminate the evaporation of acetone.

Oven-dried afterward at 50 degrees Celsius for 24 hours to remove the moisture from the sample. Store in a zip-lock bag until further use.

Figure 14: Size reduction

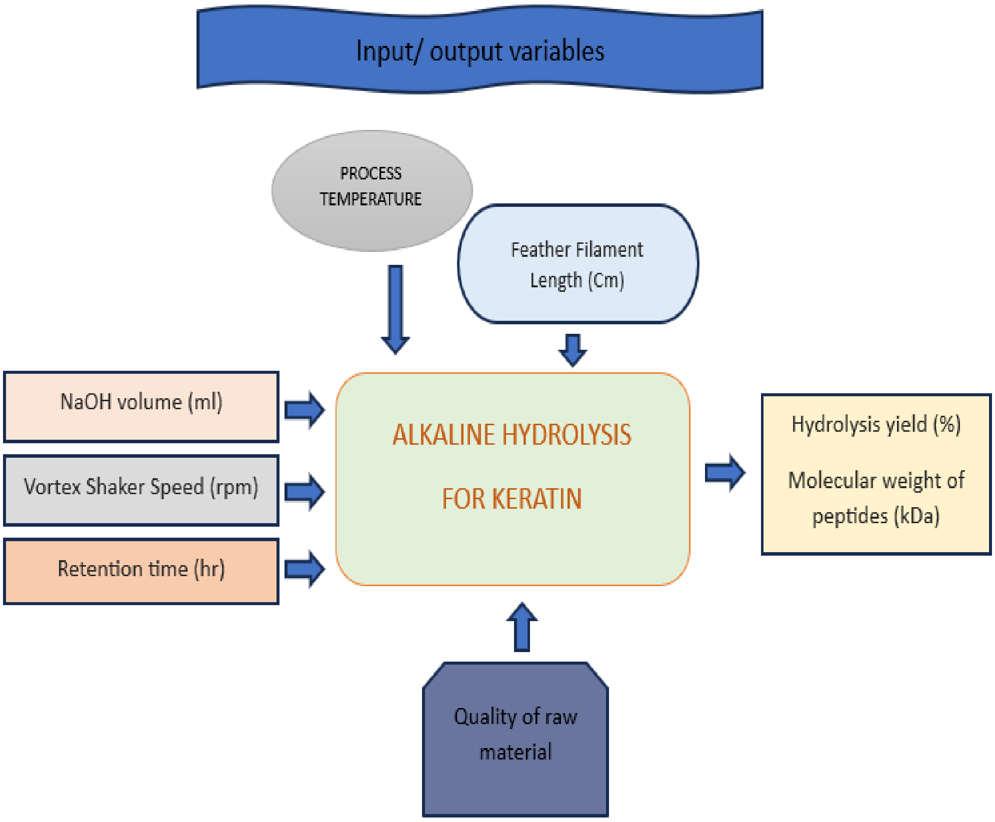

Figure 15: input/ output variables for the alkaline hydrolysis process [45]

5.5 Preparation of the sodium hydroxide solution

The alkaline hydrolysis method was used for the extraction of keratin.

For the preparation of 0.5M NaOH solution in 1L distilled water

1. Took the volumetric flask of 1L.
2. The initial weight of the volumetric flask was noted, and the balance was adjusted to zero disregarding the weight of the flask, so that the display should read 0.00 grams.
3. 20g of NaOH pellets were added into the flask.
4. Then the flask was filled with the distilled water until the 1000ml calibration mark was reached.
5. The solution was then placed on the magnetic stirrer for proper mixing of the solution.

The same procedure was repeated for the preparation of 0.75M, 1.0M, 1.25M, 1.5M, and 2.0M concentrations of NaOH

5.6 Extraction process

1. Took 100ml plastic oven-safe bottles.
2. Placed the beaker on the weighing platform and the weight was adjusted by pressing the 'tare' or 'zero' button on the weighing machine,
3. Around 1g of each sample was filled into the bottles.
4. About 30ml of NaOH was poured into the bottle to maintain (1:30) volume.
5. Repeated the step for samples with the (0.5, 0.75, 1.0, 1.25, 1.5, 2M) NaOH concentration while making the volume of NaOH of 30g.
6. The bottle cap was tightened and the bottle was placed on the vortex shaker to thoroughly mix the solution with the material at medium to high speed.
7. The cap of the bottle was removed, and covered with aluminum foil, poked small holes in the foil.
8. Samples were divided into two parts.
   For example: - Buffalo hair is divided into
   B1=0.5M, B2=0.75M, B3=1.0M, B4=1.25M, B5=1.5M, B6=2.0M were kept at 60^{o}c.
   Whereas,
   B7=0.5M, B8=0.75M, B9=1.0M, B10=1.25M, B11=1.5M, B12=2.0M were kept at 40^{o}c.
9. Place the bottles in the hot air oven at 60 and 40^{o}c for 24 hours.

Figure 16: Extraction at oven-temp at 60 and 40

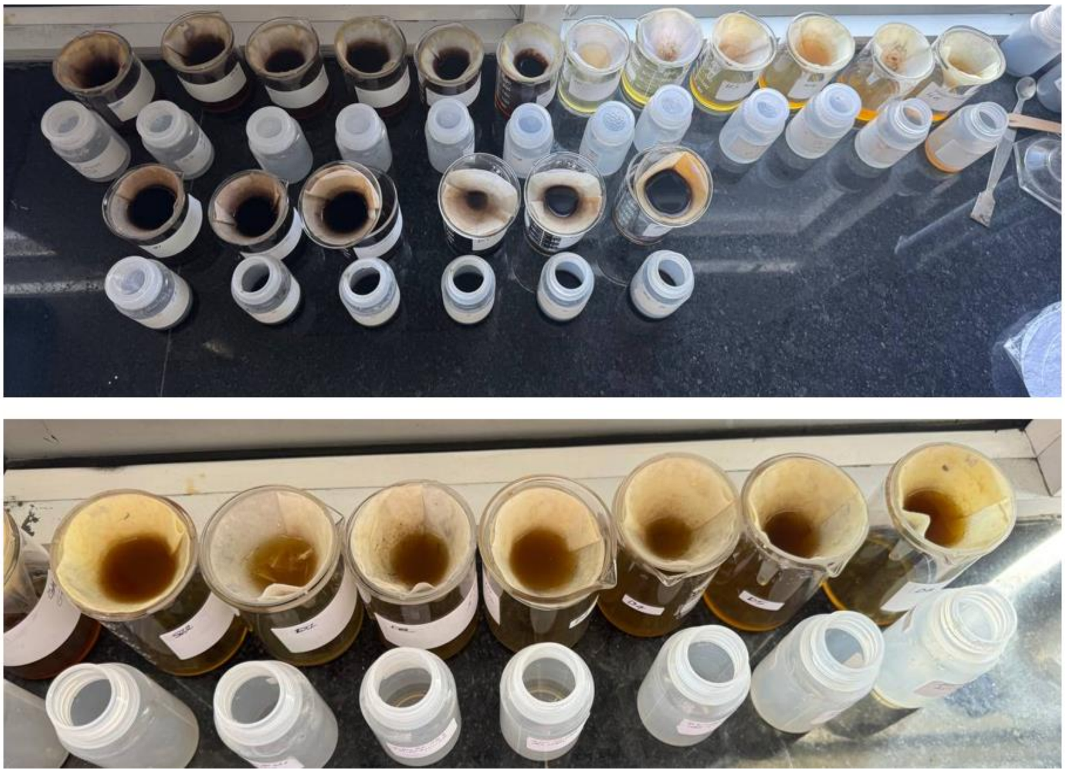

Figure 17: Filtration of samples

5.7 Filtration process

1. Bottles were removed from the hot-air oven after 24 hours.
2. The filtration apparatus was prepared with filter paper and a funnel.
3. 250ml beakers were cleaned and dried for filtrate collection.
4. Extracted material was slowly poured on filter paper.
5. Occasionally stir and add the distilled water to avoid blocking due to solid residue.

5.8 Precipitation method

1. pH was adjusted to the iso-electric point with the help of a pH meter, and 1M Hydrochloric acid (HCL).
2. HCL was added drop by drop into the solution.
3. Stir the solution for proper mixing of chemicals into the solution.
4. pH was adjusted with 1M HCL to 4.4 for precipitation.
5. The solution was covered with aluminum foil and a retention period of 5 hours was provided to settle down the keratin solution to the bottom.

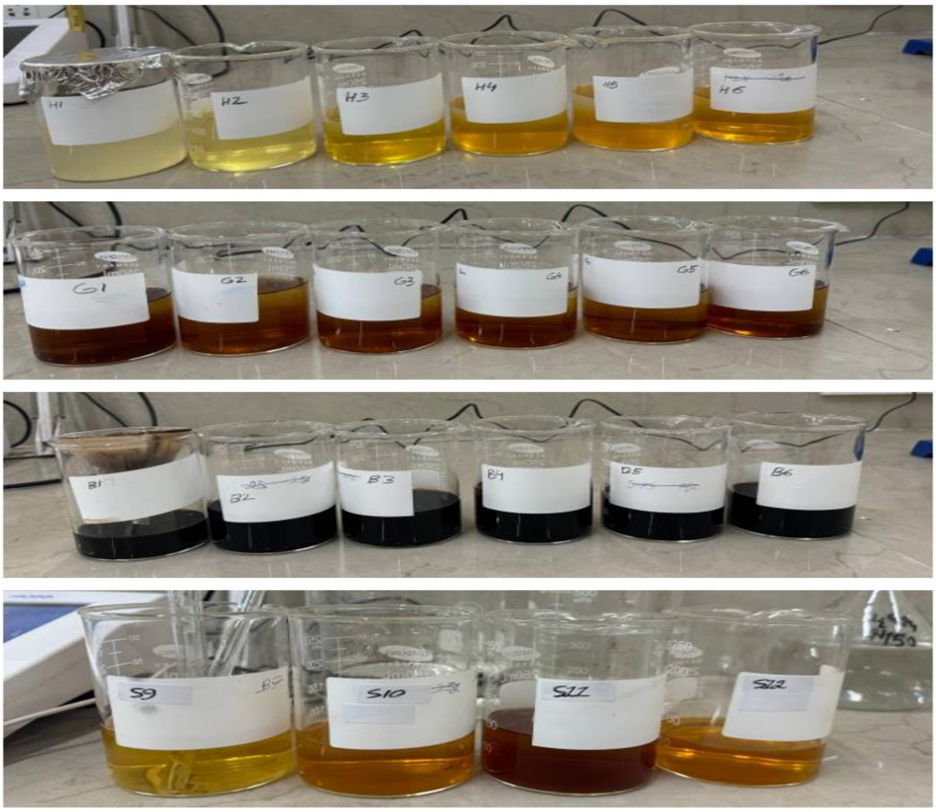

Figure 18: Precipitation process

5.9 Centrifuge process

1. After the precipitation process there were suspended particles in the solution.
2. The supernatant was shifted to a centrifuge tube of 50 ml each.
3. The solution was centrifuged for 10 minutes at 5000 RPM.
4. The supernatant was discarded safely.
5. With the help of a spatula, distilled water precipitate was collected into the pre-weighted crucible.

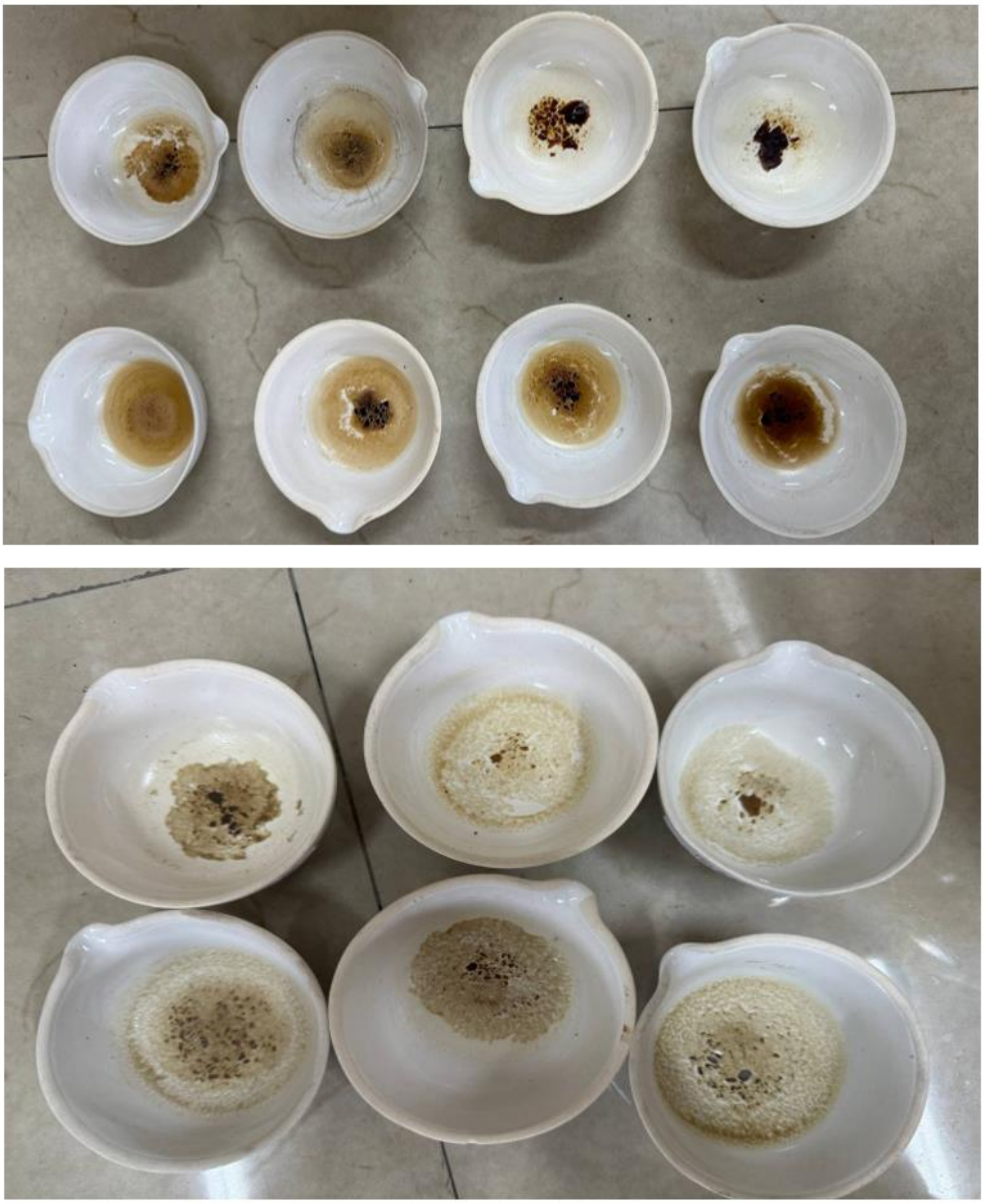

Figure 19: Extracted samples

CHAPTER 6: DETERMINATION OF KERATIN YIELD

6.1 Yield Calculation

1. Wash the crucibles with distilled water to remove any contamination.
2. Oven-dried at 100oc for 20 minutes to remove the remaining moisture.
3. Kept in a desiccator for 10 minutes.
4. Use a weighing machine to measure the exact weight of the crucible and label it afterward.
5. The Keratin solution was poured into the crucible and kept in the oven for 24 hours at 45
6. Weight is calculated

Extraction = initial weight of crucible (pre-weight of crucible) – final weight of crucible

Say,

The initial weight of the crucible is = 57.444g

After 24hours at 45^{o}c

The final weight of the crucible is = 57.542g

= 57.542 – 57.444

=0.098g

Yield = $\frac{Extracted\ keratin}{Total\ raw\ material} * 100$

= $\frac{0.098}{2} * 100$

= 4.9%

6.2 Yield at NaOH amount (1:40) (0.5M)

Sample	***Initial*** *pH*	***HCL Added (ml)***	***Final*** *pH*	***Crucible (IW)(g)***	***Crucible (FW)(g)***	*Extraction*	***Yield***
Hen feather (1)	*12.6*	*20*	*5.25*	*57.444*	*57.542*	*0.098*	***4.9***
Hen feather (2)	*12.3*	*19.1*	*5*	*65.245*	*65.331*	*0.086*	***4.3***
Sheep wool (1)	*12.27*	*17.5*	*4.42*	*70.396*	*70.453*	*0.057*	***2.85***
Sheep wool (2)	*12.6*	*21*	*4.43*	*49.656*	*49.714*	*0.058*	***2.9***
Sheep wool (3)	*11.7*	*16.4*	*4.32*	*59.473*	*59.56*	*0.087*	***4.35***
Goat hair (1)	*12.54*	*21.7*	*4.26*	*63.892*	*63.965*	*0.073*	***3.65***
Goat hair (2)	*12.48*	*22.3*	*4.4*	*61.464*	*61.522*	*0.058*	***2.9***
Goat hair (3)	*12.35*	*21.3*	*4.46*	*56.478*	*56.535*	*0.057*	***2.85***
Buffalo hair (1)	*12.37*	*16.8*	*4.48*	*61.642*	*61.73*	*0.088*	***4.4***
Buffalo hair (2)	*12.46*	*16.7*	*4.49*	*65.476*	*65.543*	*0.067*	***3.35***
Buffalo hair (3)	*12.22*	*17.5*	*4.49*	*64.586*	*64.76*	*0.174*	***8.7***

Table 12: Extraction at NaOH (1:40) at temp 65^{o}c of various samples

6.3 Effect of varying concentrations of sodium hydroxide (NaOH) (1:30)

6.3.1 Hen feather

Hen feather samples were divided into 12 categories H1-H6 at 60ºc and H7- H12 at 40ºc and the highest yield was in the H7 sample with a NaOH concentration of 0.5M with a ratio (1:30) kept at 40ºc for 24 hours. while the lowest yield was in the H5 sample with a NaOH concentration of 1.5M with a ratio (1:30) kept at 60ºc for 24 detention period.

Sample	Weight	NaOH conc.	NaOH(ml)	Initial pH	final pH	Yield (%)
H1	1	0.5	30	12.885	4.424	5
H2	1	0.75	30	13.666	4.434	3.7
H3	1	1	30	13.628	4.411	3.7
H4	2	1.25	60	13.789	4.421	4.2
H5	2	1.5	60	13.9	4.4	2.55
H6	2.002	2	60	14.333	4.403	4.795204795
H7	1.003	0.5	30	13.637	4.423	10.36889332
H8	1	0.75	30	13.887	4.402	7.3
H9	1.004	1	30	14.075	4.438	6.573705179
H10	2.001	1.25	60	14.549	4.401	7.346326837
H11	2.001	1.5	60	14.33	4.472	5.497251374
H12	2	2	60	14.181	4.474	5

Table 13: Hen feather keratin yield

6.3.2 Goat hair

Goat hair samples were divided into 12 categories G1-G6 at 60ºc and G7- G12 at 40ºc and the highest yield was in the G7 sample with a NaOH concentration of 0.5M with a ratio (1:30) kept at 40ºc for 24 hours. while the lowest yield was in the G4 sample with a NaOH concentration of 1.25M with a ratio (1:30) kept at 60ºc for 24 detention period.

Sample	**Weight**	**NaOH conc.**	**NaOH(ml)**	**Initial pH**	**final pH**	**Yield (%)**
G1	1	0.5	30	12.15	4.472	5.3
G2	1	0.75	30	13.396	4.6	3.2
G3	1.002	1	30	13.772	4.4	2.395209581
G4	1.001	1.25	30	15.18	4.44	1.398601399
G5	1.004	1.5	30	13.7	4.455	2.19123506
G6	1.004	2	30	14.8	4.465	2.689243028
G7	1.002	0.5	30	13.948	4.481	18.36327345
G8	1.004	0.75	30	14.18	4.468	8.366533865
G9	1	1	30	14.052	4.465	6.1
G10	1.007	1.25	30	14.213	4.427	4.667328699
G11	1.004	1.5	30	13.933	4.438	3.784860558
G12	1.001	2	30	14.556	4.446	3.596403596

Table 14: Goat hair keratin yield

6.3.3 Buffalo hair

Buffalo hair samples were divided into 12 categories B1-B6 at 60ºc and B7- B12 at 40ºc and the highest yield was in the B1 and B7 samples with NaOH concentration of 0.5M with a ratio (1:30) kept at 40ºc and 60ºc for 24 hours. while the lowest yield was in the B6 sample with a NaOH concentration of 2M with a ratio (1:30) kept at 60ºc for 24 detention period.

Sample	**Weight**	**NaOH conc.**	**NaOH(ml)**	**Initial pH**	**final pH**	**Yield (%)**
B1	1	0.5	30	13.33	4.453	23.4
B2	1	0.75	30	13.632	4.45	8.5
B3	1	1	30	14.164	4.46	7.7
B4	1	1.25	30	13.786	4.464	4.6
B5	1	1.5	30	14.19	4.459	4.1
B6	1	2	30	14.62	4.464	2.5
B7	1	0.5	30	12.936	4.401	19.5
B8	1	0.75	30	13.422	4.401	15.6
B9	1	1	30	14.38	4.471	13.2
B10	1	1.25	30	14.705	4.431	6.8
B11	1	1.5	30	12.576	4.484	10.4
B12	1	2	30	14.4	4.473	7.9

Table 15: Buffalo hair keratin yield

6.3.4 Sheep wool

Sheep wool samples were divided into 12 categories S1-S6 at 60ºc and S7- S12 at 40ºc and the highest yield was in the S7 sample with a NaOH concentration of 0.5M with a ratio (1:30) kept at 40ºc for 24 hours. while the lowest yield was in the S6 sample with a NaOH concentration of 2M with a ratio (1:30) kept at 60ºc for 24 detention period.

Sample	**Weight**	**NaOH conc.**	**NaOH(ml)**	**Initial pH**	**final pH**	**Yield (%)**
S1	1	0.5	30	13.372	4.44	5
S2	1	0.75	30	13.938	4.426	5.1
S3	1	1	30	14.245	4.466	5
S4	1	1.25	30	14.772	4.403	3.1
S5	1	1.5	30	14.736	4.421	3.2
S6	1	2	30	15.047	4.442	2.8
S7	1	0.5	30	12.75	4.403	20.9
S8	1	0.75	30	13.801	4.453	13.6
S9	1	1	30	14.111	4.405	10.2
S10	1	1.25	30	14.154	4.413	8
S11	1	1.5	30	14.601	4.46	12.3
S12	1	2	30	14.692	4.474	8.2

Table 16: Sheep wool keratin yield

6.3.5 Duck feather

Duck feather was divided into 6 categories D1-D6, and the highest yield was expected in the sample with a concentration of 1.25M NaOH with a ratio (1:30) when kept at 60^{o}c and the least was in NaOH concentration of 1M at the same temperature.

Sample	**Weight**	**NaOH conc.**	**NaOH(ml)**	**Initial pH**	**final pH**	**Yield (%)**
D1	1	0.5	30	12.963	4.436	2.4
D2	1	0.75	30	13.592	4.439	2.4
D3	1	1	30	13.893	4.425	2
D4	1	1.25	30	13.793	4.445	3
D5	1	1.5	30	14.169	4.462	2.6
D6	1	2	30	14.015	4.411	2.8

Table 17: Duck feather keratin yield

CHAPTER 7: RESULTS

7.1 Yield analysis

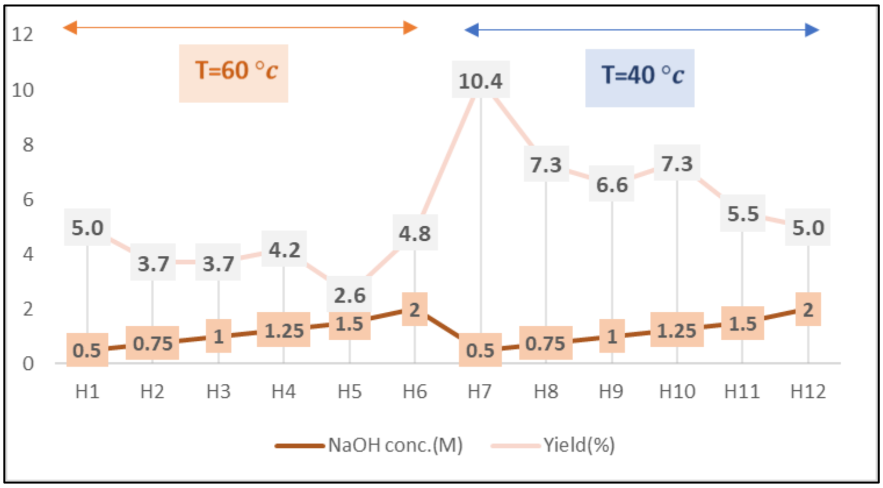

Figure 20: keratin yield from hen feather

The diagram depicts that around 12 samples from H1 to H12 were taken with variations in concentration of NaOH and temperature range of 40 and 60 degrees Celsius. Hen feather describes the yield of keratin that changes with the change in the concentration of NaOH. At 60 degrees Celsius, the H1 sample with a concentration of 0.5M provides a yield of 5%, followed by H6 with a 2M concentration that gives a yield of approximately 4.79%. With temperature deduced to 40 degrees Celsius, the yield doubled in the H7 sample with 0.5M NaOH, providing nearly 10%. However, output initially increases, providing another peak at sample H9 with 0.75M, but beyond that, it slightly decreases but remains high as if compared to the extraction at the 60-degree samples. It states that lower temperature and NaOH concentration can provide higher output of keratin. The optimal concentration for better output is 0.5M at 40 degrees Celsius.

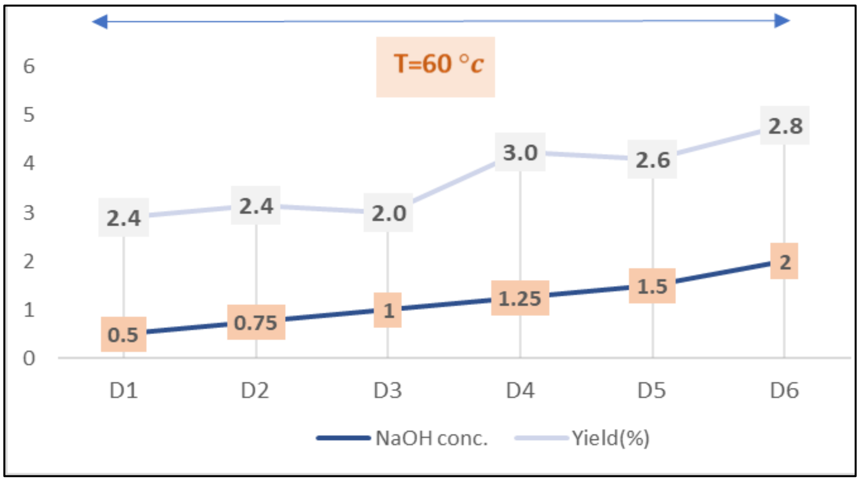

Figure 21: keratin yield from duck feather

Duck feather illustrates that with the increased concentration of NaOH, the yield of keratin extracted exceeds. D1 provides a yield of 2.4% at a concentration of 0.5M with small fluctuation over concentration slight changes, and the D4 sample has the highest yield of 3%, which in comparison to other livestock keratin waste like hen feather is the lowest. The optimal concentration for extraction from duck feathers for the highest yield was at 1.25M at 60.

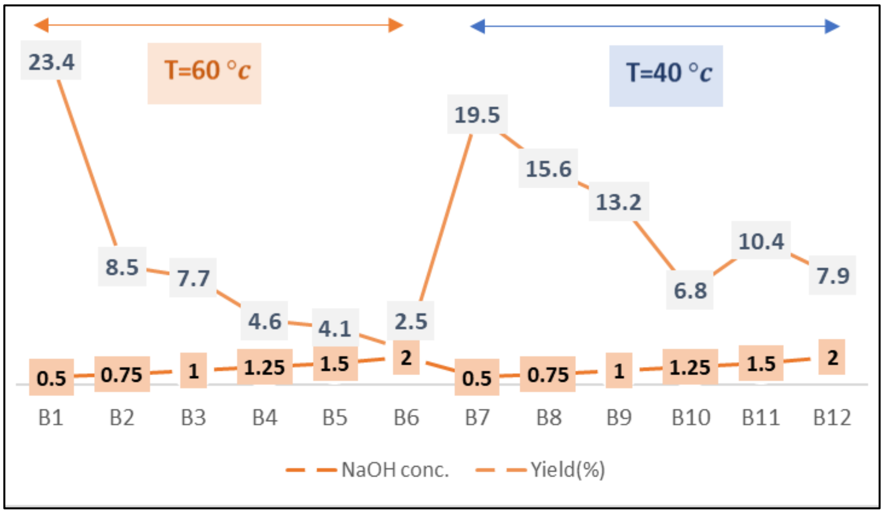

Figure 22: keratin yield from buffalo hair

Buffalo hair extract with the same protocol showed opposite results. The highest yield can be accomplished at 60 degrees Celsius with a NaOH dose of 0.5M. As the concentration increased to above 0.75M drop in the output but decreased in oven temperature to 40 degrees the yield increased and output at similar concentrations were high as for 0.75M, 1M, and 1.25M where 8.5%, 7.7%, and 4.6% yield at the same concentration at 40 almost doubled to 15.6%, 13.2%, and 6.8%. The optimal dose for extraction of keratin from buffalo hair is 0.5M NaOH at both 60 and 40.

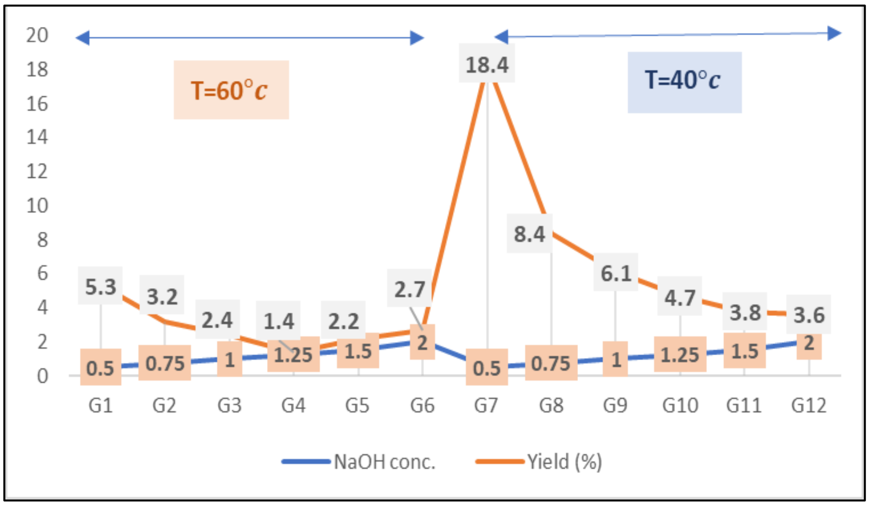

Figure 23: keratin yield from goat hair

Goat hair was divided into 12 categories from G1 to G12. With the increase in the concentration of NaOH, the output shows an almost similar result where the yield is nearly or below 5% when kept at 60oc for 24 hours of retention time. When the temperature for samples G7 - G12 was reduced by 20, the yield doubled for the sample of NaOH: 0.75M from 3.2% to 8.3%, 1M from 2.3% to 6.1%, 1.25M from 1.25% to 4.5%, 1.5M from 2.1% to 3.7% and 2M from 2.6% to 3.6%. However, for the 0.5M, the yield increased from 5.3% to 18.3%, and that is the optimal concentration for extracting keratin from the goat hair.

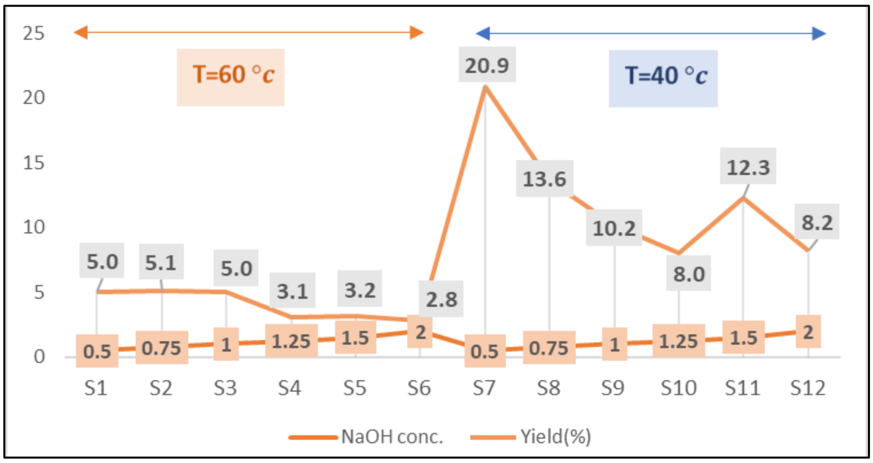

Figure 24: keratin yield from sheep wool

For the extraction of keratin from rejected wool 12 samples were taken from S1 to S12 with variation in concentration and temperature. In a temperature range of 60, the yield at S1 starts at 5% and remains nearly the same till the S3 sample and decreases to 3.1%,3.2%, and finally 2.8% until the S6 sample. The yield spikes dramatically with the lowering temperature to 40 degrees for S7 to 20.9% but decreases to 8.2% for S12 progressively with concentration of NaOH changes. Data comparison to the extraction of hen and duck feathers, the extraction of sheep wool is comparably higher at similar concentrations and temperatures making it an efficient source for extraction. Table 15 depicts that 1g of sample dissolved in 40ml of NaOH provides a 3-5% yield. Hence, the amount of NaOH used also affects the output. Thus, lowering the NaOH amount and concentration improved yield can be achieved. The optimal dose for extraction of keratin from sheep wool is NaOH 0.5M at 40 degrees Celsius.

From the various samples taken into consideration: hen feather, duck feather, sheep wool, buffalo hair, and goat hair, it was examined that with an increase in concentration of the NaOH, output also decreases, and the temperature increases output decreases. The productivity of keratin from livestock keratin waste is inversely proportional to the temperature and NaOH concentration. Table 15: proves that yield decreases as the amount of NaOH increases.

The main finding was that the yield of keratin for the alpha-helices containing samples of sheep wool, buffalo, and goat hairs was higher comparatively to extraction from samples containing beta-sheet: hen and duck feather. The highest yield achieved in the hen and duck feathers was 10% and 3% respectively. Whereas, the alpha-keratin provides a yield of 20% which is nearly twice that.

7.2 FT-IR Spectroscopy

FT-IR TABLE FOR KERATIN VERIFICATION

FREQUENCY, cm^{-1}	**BOND**	**FUNCTIONAL GROUP**
3640-3610 (s) (sh)	O-H stretch, free hydroxyl	Alcohols, phenols
3500-3200 (s) (b)	O-H stretch, H-bonded	Alcohols, phenols
3400-3250 (m)	N-H stretch	1^o, 2^o amines, amides
3300-2500 (m)	O-H stretch	Carboxylic acids
3100-3000 (m)	=C-H stretch	Alkenes
3000-2850 (m)	C-H stretch	Alkanes
1760-1665 (s)	C=O stretch	Carbonyls (general)
1760-1690 (s)	C=H stretch	Carboxylic acids
1730-1715 (s)	C=O stretch	α, β saturated esters
1715 (s)	C=O stretch	Ketones
1710-1665 (s)	C=O stretch	α, β – saturated aldehydes, ketones
1650-1580 (m)	N-H bend	1^0 amines
1470-1450	C-H bend	Alkanes
1335-1250 (s)	C-N stretch	Aromatic amines
1320-1000 (s)	C-O stretch	Alcohols, carboxylic acids, esters, ethers
1250-1020 (m)	C-N stretch	Aliphatic amines
1000-650 (s)	=C-H bend	Alkenes
950-910 (m)	O-H bend	Carboxylic acids
910-665 (s) (b)	N-H wag	$1^{0,}$ 2^0 amines
Where, M= medium, w=weak, s=strong, b =broad, sh=sharp		

Table 18: FT-IR vibration for keratin identification

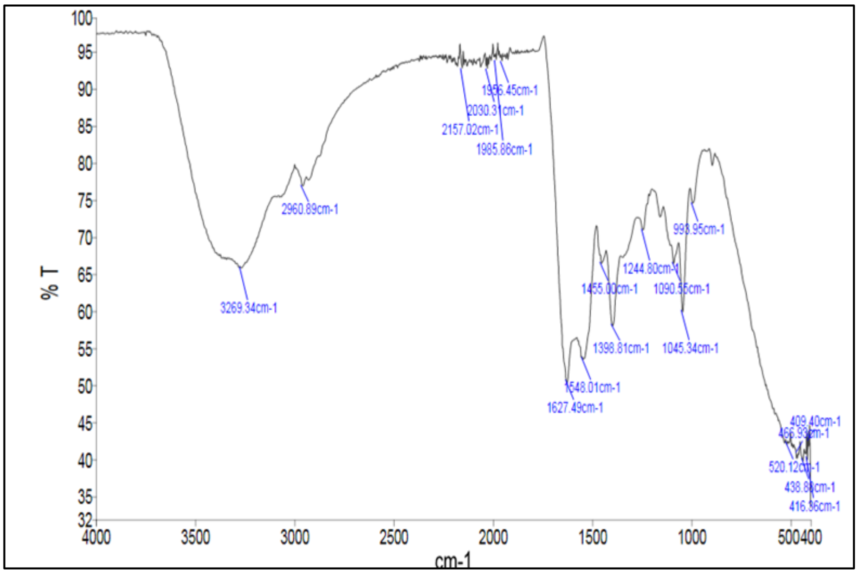

Figure 25: FT-IR analysis of keratin extracted from Goat hair

FT-IR image confirms the chemical composition of the extracted keratin from goat hair in figure-31. The characteristic peaks at 3269.34 cm^{-1} signify the presence of 0-H stretching. However, the peak at 2960.89 cm^{-1} represents the C-H Stretching. The existence of the C=0 Stretch amide-I at 1627 cm^{-1} whereas the peaks at 1548.01 cm^{-1} and 1398.8 cm^{-1} clarify the existence of the C-H stretch and N-H stretch of amide II. Strong peaks at the 1045.34 cm^{-1} and 1090,55 cm^{-1} give the presence of the carboxylic acids while 409.40, 438.88, and 416.36 cm^{-1} are associated with the mono-sulfide and disulfide bonds.

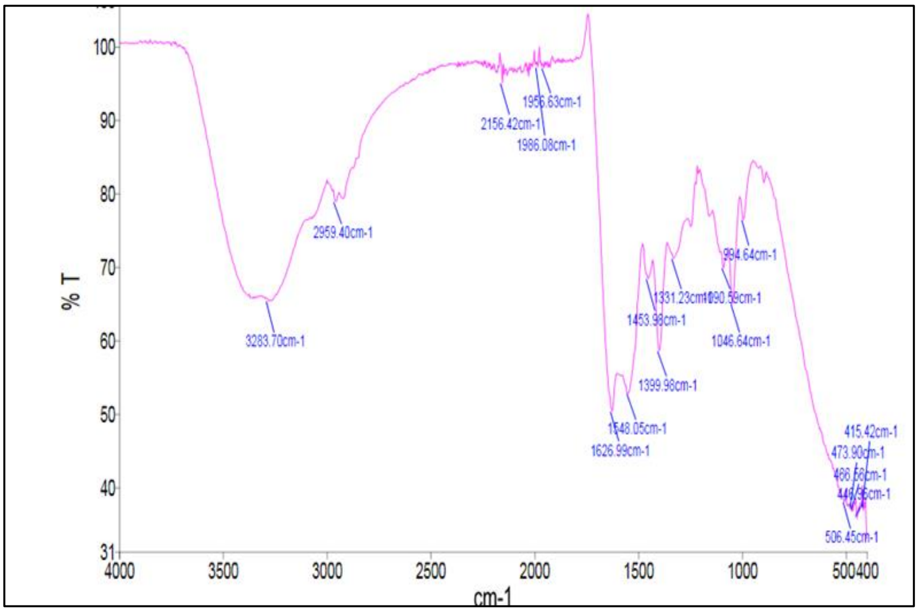

Figure 26: FT-IR analysis of keratin from sheep wool

Sheep wool under FT-IR shows almost similar results the typical protein vibrations at 3283.70 cm^{-1} show the N-H vibration of amide 10 and O-H Stretch at 2959.40 cm^{-1}. The peak characteristics of the C=O at 1626.99 cm^{-1} of amide-I and 1548.05 cm^{-1} of amide II. The various peaks at the range between 1400-1200 cm^{-1} are due to amide-III.

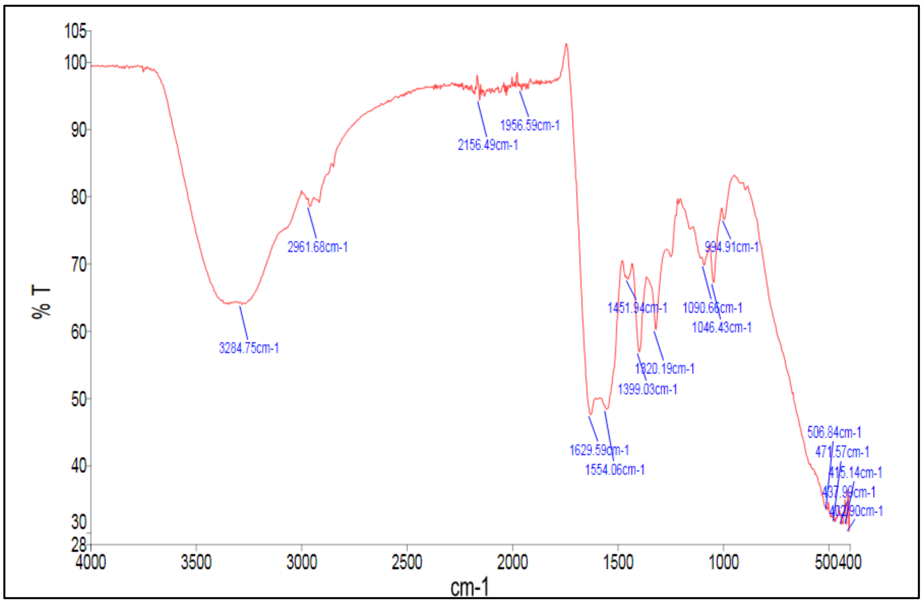

Figure 27: FT-IR analysis of keratin from buffalo hair

The chemical composition of buffalo hair under FT-IR spectroscopy shows bands at 2960.68 and 1629.59 cm^{-1} due to the presence of amide I and amide II in the protein. Whereas the absorption band of C=O can be determined between 1629.59 and 2554.06 cm^{-1}. The peak at 2961.68 cm^{-1} corresponds to the bending vibration of N-H. The peaks and the amino groups observed during the spectroscopy of the extracted keratin from buffalo hair are similar to Wang et al. 2020 in the rabbit hair keratin extraction.

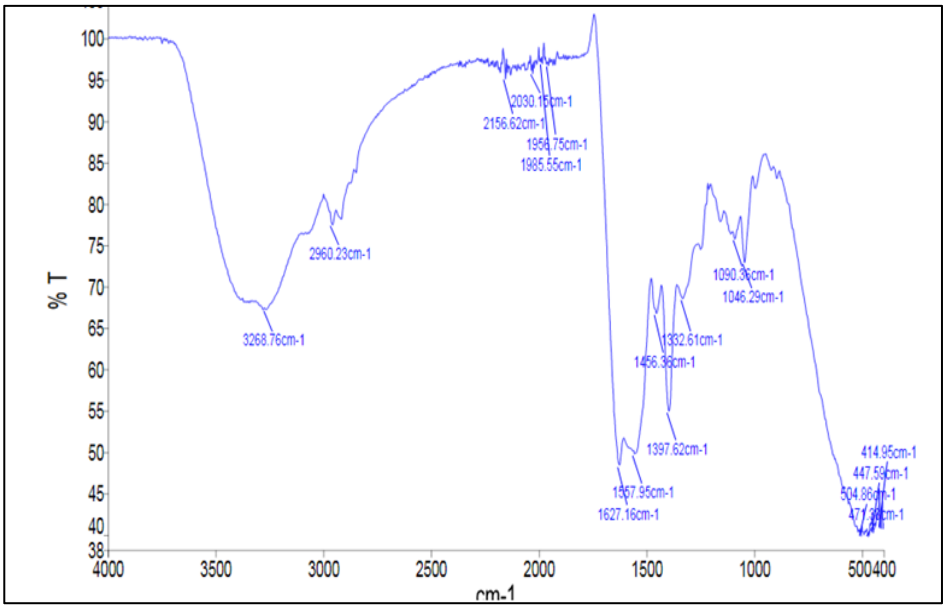

Figure 28: FT-IR analysis of keratin from hen feather

The observation of the FT-IR spectroscopy of the hen feather depicts the presence of an amide A band connected to the stretching vibration of N-H bonds at 3268.76 cm^{-1}. C=O stretch vibration at the 1627.16 cm^{-1} verifies the presence of the amide I band. Whereas, the amide II can be visible at 1567.95 cm^{-1} and related to the N-H bending and C-H stretching. A strong peak at the range between 1332.61-1046.29 cm^{-1} observes the amide III to aromatic amides (C-N) and carboxylic acid (C-O) stretching. Overall, these FT-IR spectra indicate there is the presence of protein in the extracted material.

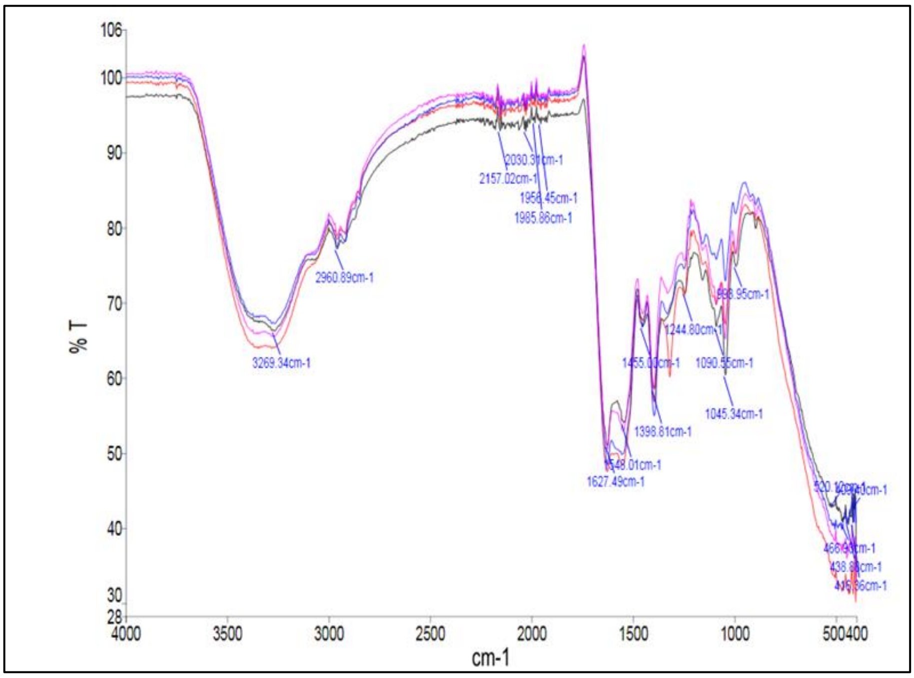

Figure 29: FT-IR analysis comparison of all samples

All -FT-IR

The figure shows the FT-IR spectra of the keratin extracted from wastes like buffalo hair, goat hair, sheep wool, and hen feathers. Range with 4000-400 cm^{-1} applied at the resolution range of 4 cm^{-1} assigned to the peptide bonds (-CONH-). The peak at 3269.34 cm^{-1} is related to the O-H Vibration and N-H stretching. The peak at 1627.49 cm^{-1} is due to the C-O stretch depicting the amide-I. Amide II at 1548.01 cm^{-1} is due to the N-H and C-H bending vibrations. Broad peaks at the 1244.80 cm^{-1} explain the presence of the amide III caused by the C-N and N-H stretching. The FT-IR Image of all four samples shows a variation. Extracted from the buffalo hair has high peaks compared to the others that depict a high amount of alpha-helices in the hair. High peaks of the hen feather show a high amount of the beta-sheets present.

7.3 SEM Analysis

The scanning electron microscopy analysis of the extracted keratin was done to know the morphology and size evaluation of the keratin structure at the x100, x500, x1000, and x2000. For the observation of keratin, it was gold coated with a thickness of 150-200 A.

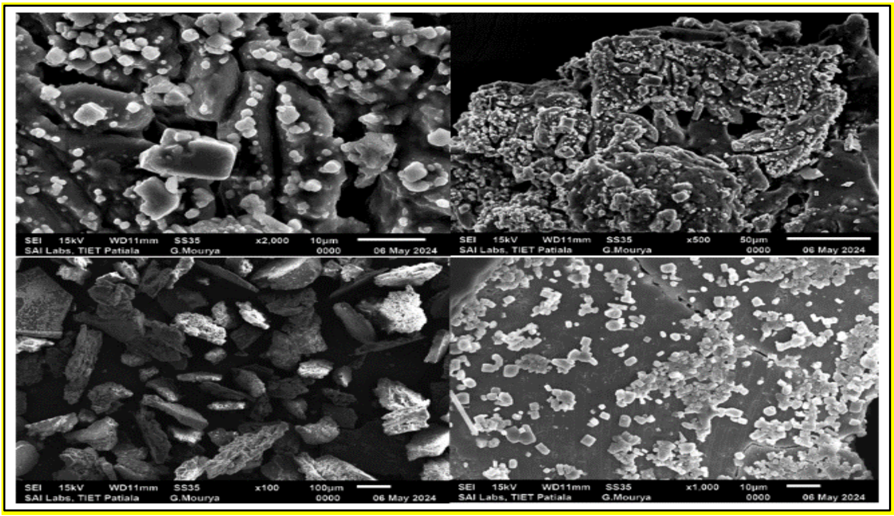

Figure 30: SEM image of keratin extracted from goat hair

Goat hair was observed under SEM with x100 depicting that the average size of the keratin molecules is 100µm and has separated particles with uneven and irregular surfaces. At x500 it shows the unsmooth structure. but, connectivity between the particles makes it a potential material for implementation in the biomedical and textile industry. Full coverage of keratin can be seen at x1000 and x2000 with particle diameter 10µm due to strong interaction between the matrix.

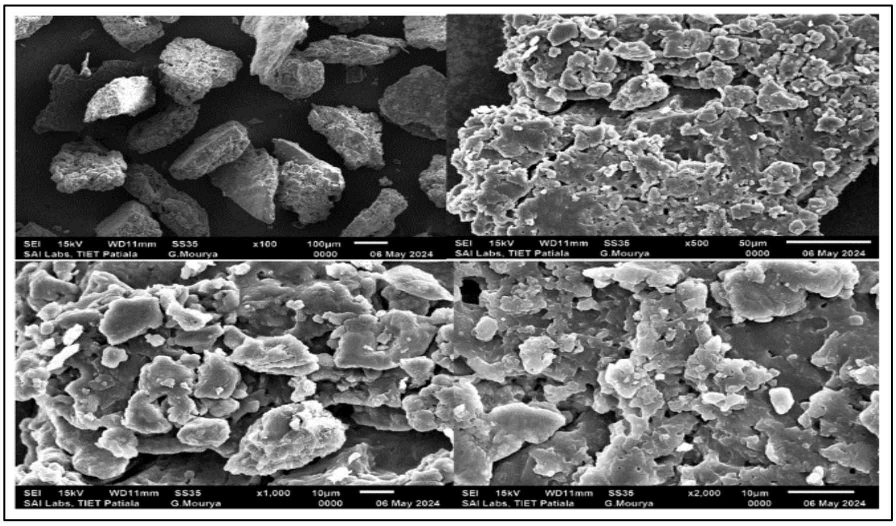

Figure 31: SEM image of keratin extracted from duck feather

Duck feathers under SEM analysis under x100 magnification ranges depict the grainy structure of the keratin. However, various ranges at x500, x1000, and x2000 show irregularity but interconnectivity between the structure.

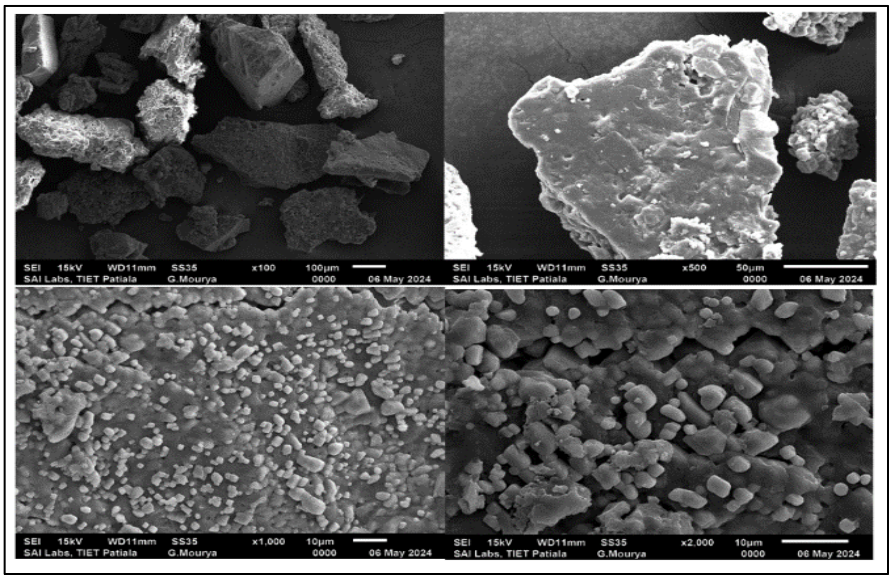

Figure 32: SEM image of keratin extracted from hen feather

Hen feather due to strong interaction between the molecules depicts the sizeable and rougher morphology with interconnected architecture arrangements, visible cracks can be seen at x1000 and x2000 magnification.

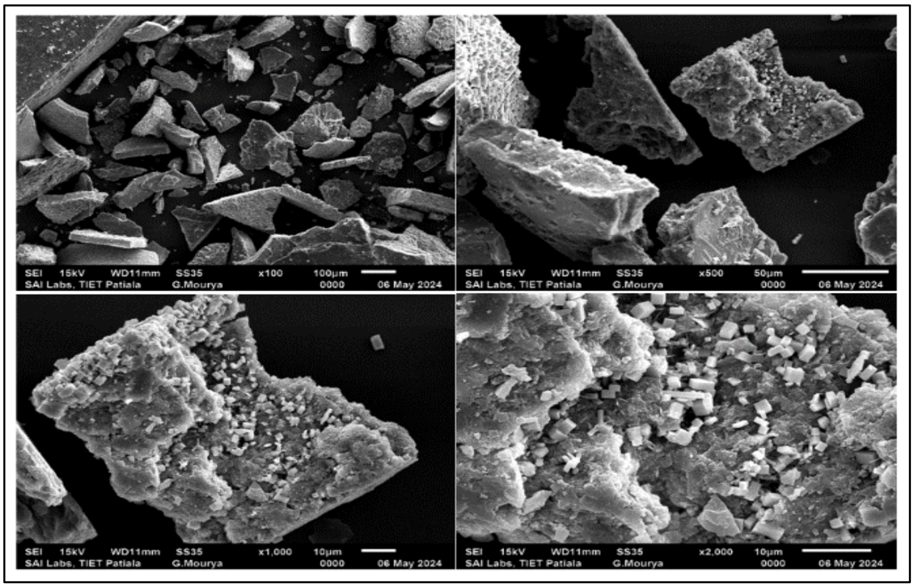

Figure 33: SEM image of keratin extracted from buffalo hair

Buffalo hair keratin observed at x100 shows an elongated, thread-like structure, and x500, and x1000x amplification show a large porous morphology of irregularly shaped particles with less propagation of cracks making it suitable for feed and nutrient supplements. At x2000 magnification alpha keratin appears to have a slightly rough surface with a tightly packed structure.

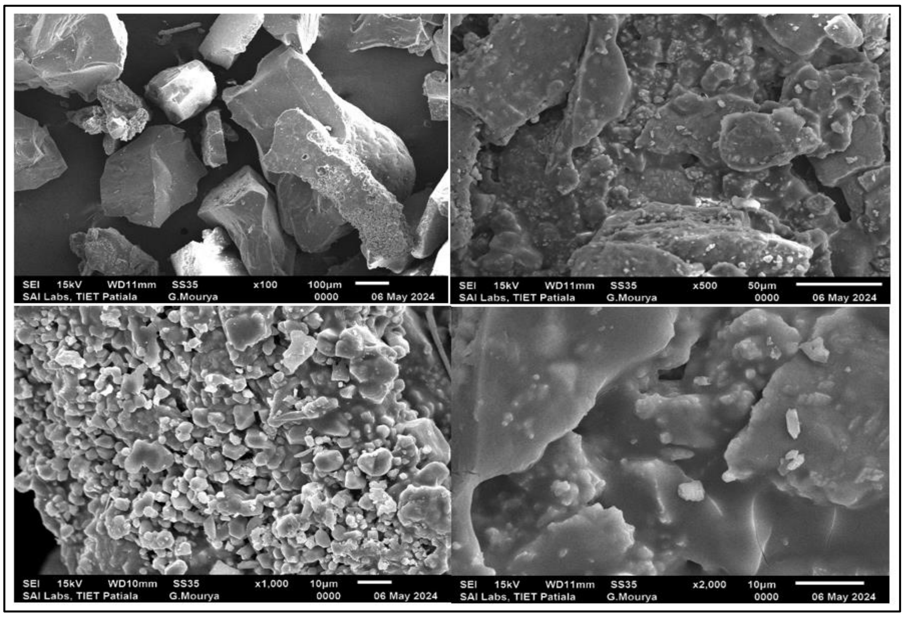

Figure 34: SEM image of keratin extracted from sheep wool

SEM image of the wool fiber keratin hydrolyzed through the alkaline hydrolysis method depicts the clear and smooth structure with uneven shape and crack-less morphology. The structure at x2000 appears to have an interconnected matrix with less porosity, even toughness, and appropriate weight distribution enabling the utilization in the development of sustainable material manufacturing.

7.4 DSC analysis

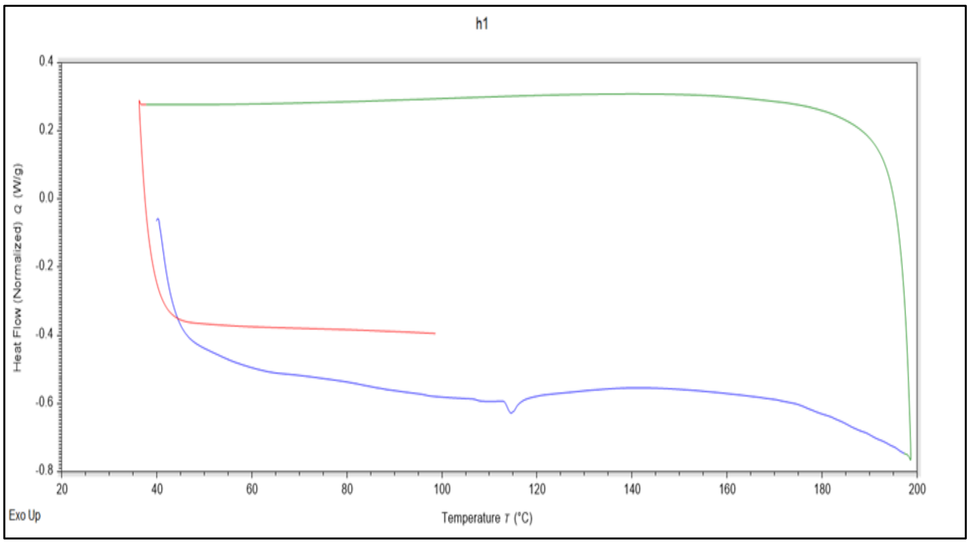

Figure 35: DSC Analysis of hen feather-derived keratin

DSC analysis was done on TA DSC 25 by initially measuring the 5 mg of extracted keratin into the sample pan and sealed inside the compartment. The heat-cool-heat procedure was followed at 20ºC/min with a temperature range between 40 to 200ºC under a nitrogen gas cell purge rate of 180.05ml/min.

Hen feather under the above conditions shows the exothermic trough from 40 to 60 degrees Celsius due to loss of moisture or water in the sample. However, the transition remains almost steady till 110c when the melting of the keratin converts solid into liquid at this stage. Some heat absorption at this stage causes an endothermic phase, the transition remains almost constant until 200c when another phase of exothermic takes place.

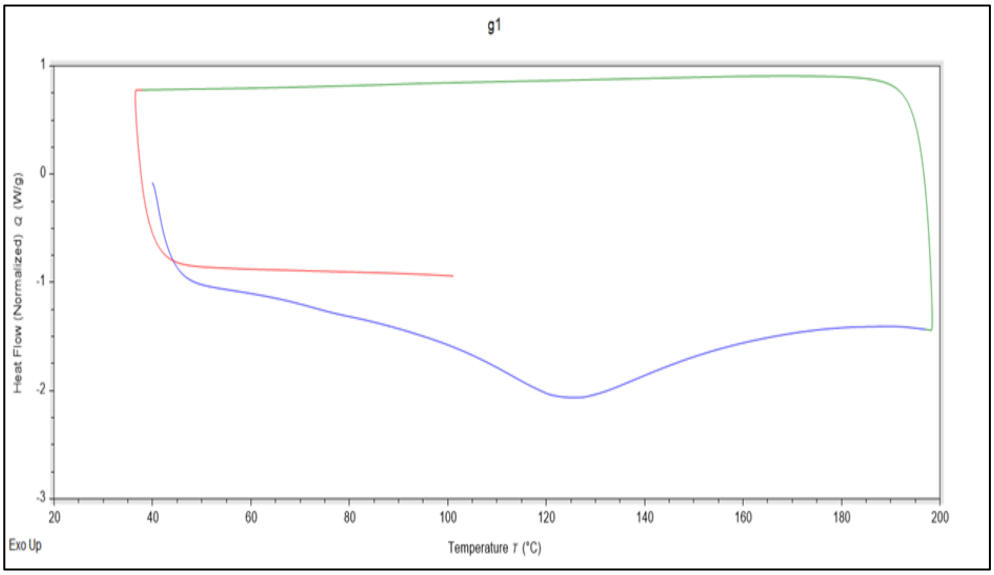

Figure 36: DSC Analysis of goat hair-derived keratin

Goat hair under a similar condition shows the opposite condition with temperature rises above 40 there is an exothermic trough with steady fall due to evaporation of moisture in alpha-helices until the temperature reaches 120 degrees Celsius when due to melting of the keratin some absorption of heat causes the endothermic trough until the temperature reaches the 200. The next phase of heating after cooling shows a steady result due to the complete melting of the keratin has already happened.

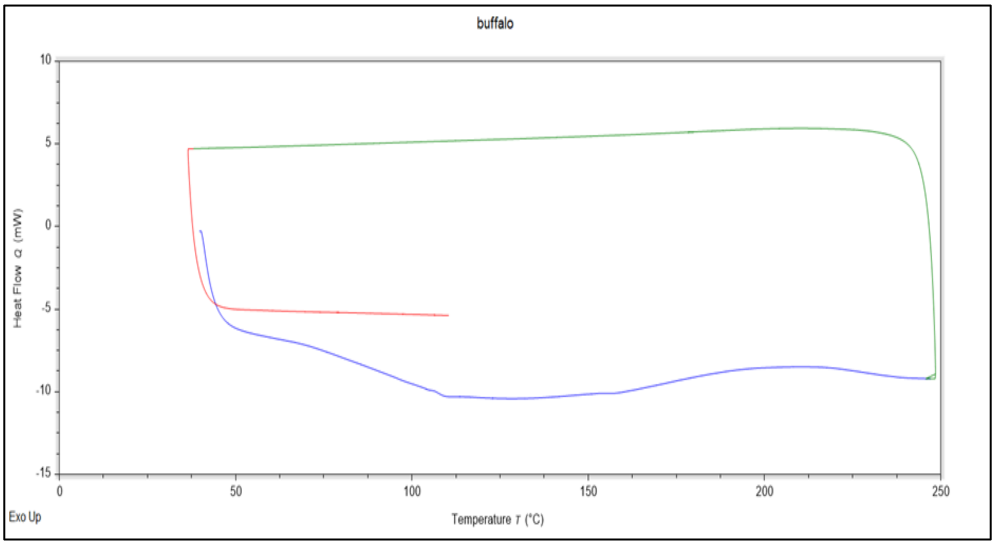

Figure 37: DSC Analysis of buffalo hair-derived keratin

Buffalo hair undergoes mass degradation until 100 degrees at the heat flow from 0 to -10 due to ignition of the oxygen-containing thiol groups present in the alpha helices. The breakdown of the keratin structure converts the crystalline structure into a liquid. Thus, the sample remains almost constant until the temperature reaches the 200°C.

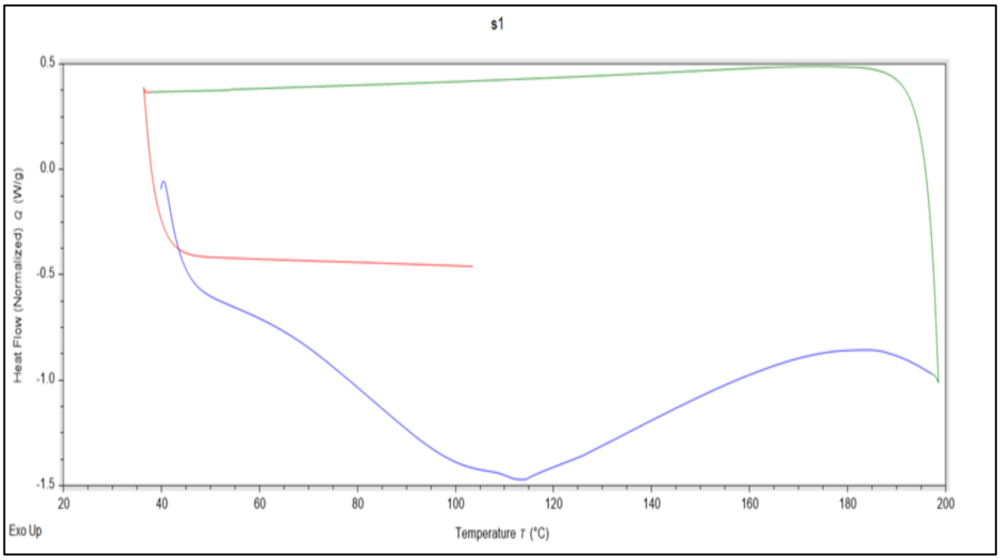

Figure 38: DSC Analysis of sheep wool-derived keratin

DSC analysis of the sheep wool shows the small absorption causing an endothermic peak at 30c followed by a large exothermic peak at 110c due to evaporation of the water molecules from the wool keratin. The endothermic peak after 120c causes the thermal decomposition of the keratin.

CHAPTER 8: SUSTAINABLE APPROACH

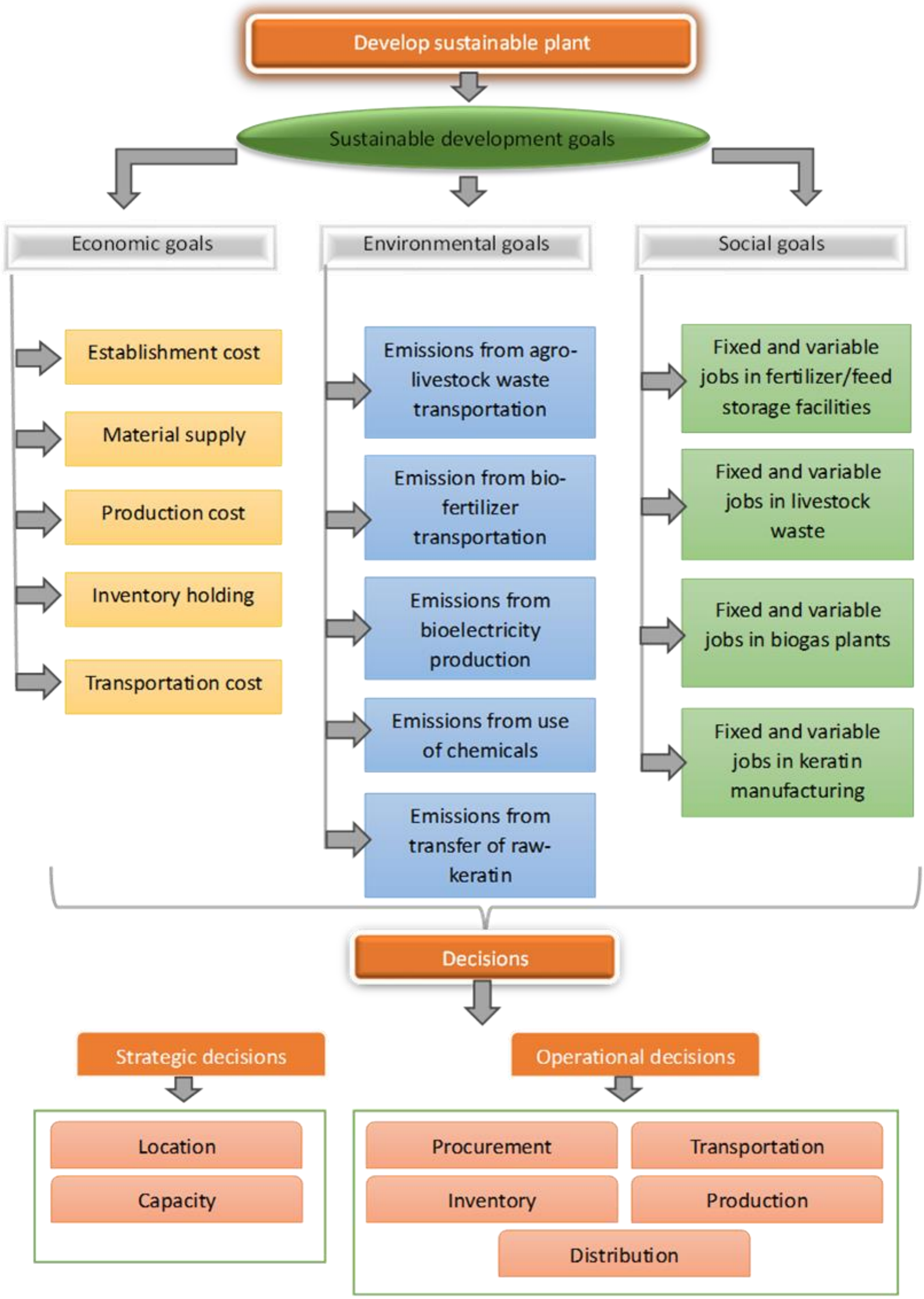

Figure 39: Sustainable Approach

8.1 Technical Analysis

The section examines technical analysis of the extraction of keratin from livestock waste. The following diagram depicts a schematic representation of the different components required for the extraction.

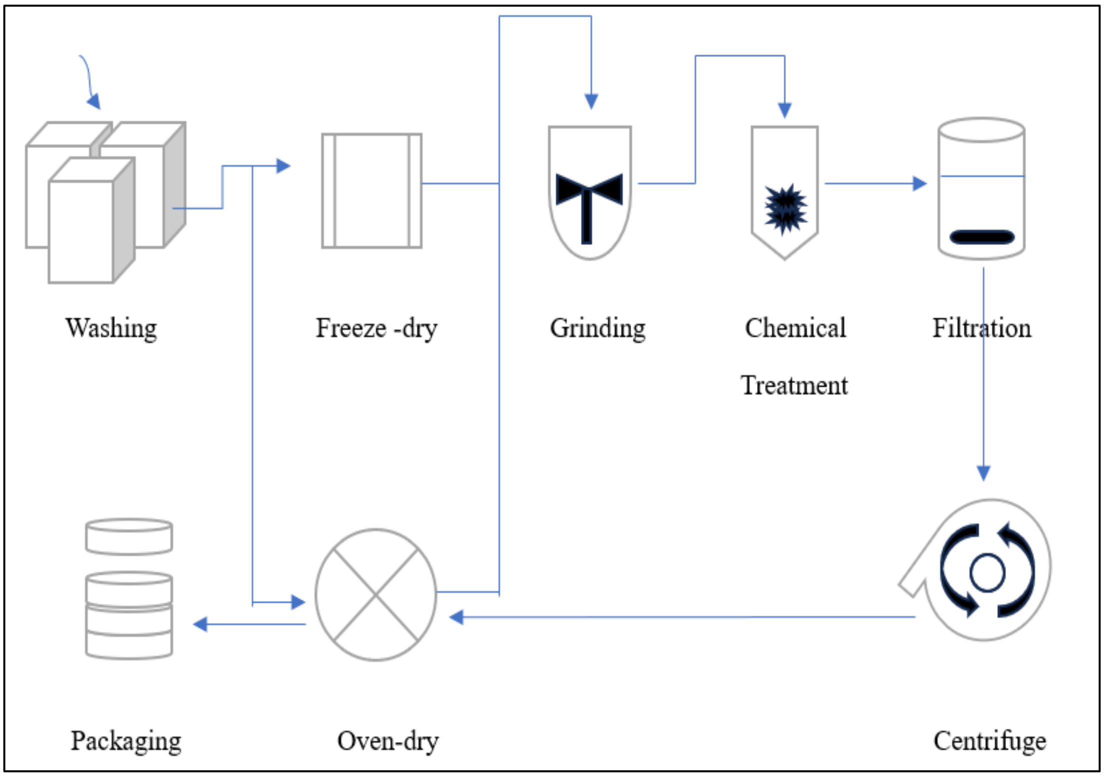

Figure 40 Plant operations

Plant operation procedure

Waste can be gathered from slaughterhouses, dairy and poultry farms, and the meat processing and textile industry, where waste is typically rejected or dumped into landfills or open spaces.

To ensure smooth operation and better handling, we subdivide waste into batches. Each batch will contain 10-15kg of keratin waste, allowing 35-40 kg utilization per day. Examine if the waste contains a variety of waste, and segregation and sorting of waste is required.

We suppose we have three forms of keratin waste with the highest yield potential of around 20%: batch 1-buffalo hair, batch 2-goat hair, and batch 3-sheep wool, further improvement by research and development in the field.

The plant will work for 24 days each month, for a total of 288 days each year. The operation in the plant will take as follows: -

I. After 30-40 minutes of washing remove dirt, dust, and blood from the raw material. Material can be dissolved into acetone or ethanol for about 1-2 hours for additional removal of contaminants.
II. Dried to eliminate moisture from the samples. During the summer, direct sunlight will be sufficient. However, in the winter, oven-drying can be employed.
III. Size reduction increases the contact surface area of the material and can take up to 30-40 minutes for the whole batch.
IV. Extraction of the keratin can take up to 8- 20 hours to completely dissolve at a regulated temperature.
V. Filter the extracted keratin and centrifuge the solution to precipitation solution at a retention period of 1-2 hours. The precipitate can be collected and Supernate disposed of securely.
VI. Dry the sample at room temperature for 2-3 hours.
VII. Packed according to industrial standards.

Extraction of keratin from 35kg of total waste with NaOH 0.5M concentration amount: -

CONDITIONS	**1g**	**35kg**
NaOH concentration	0.5M	0.5M
NaOH amount	30ml	30ml
HCL amount	2.5ml	2.5 ml
Sample amount	1g	35kg
Temperature	45^{o}c	45^{o}c
Detention period	24 hours	8-12 hours
For the preparation of 1L NaOH solution		
NaOH amount	20g	**2.1Kg**
HCL amount	82ml	**2.8 litre**
Water amount	1L	**105L**

Table 19: chemical requirement for 35kg

8.2 Financial Analysis

8.2.1 Capital cost

ITEMS	DAILY SUPPLY	ANNUAL SUPPLY	COST
Buffalo Hair	10-15kg	3360kg	0
Goat Hair	10-15kg	3360kg	0
Sheep Wool	10-15kg	3360kg	0
TOTAL WASTE RECYCLED		**10,080kg**	**0**
# Assumed the price of raw material=0, due to no- economic value			

Table 20: Raw material cost

ITEMS	QUANTITY	UNIT	UNIT COST	TOTAL COST
Dumping Area	800	Gaj	3000	24,00,000
Laboratory Space	400	Gaj	3000	12,00,000
Renovation and Setup	1	Rs	400000	4,00,000
HVAC System	1	Rs	300000	3,00,000
Storage Area	1	Rs	150000	1,50,000
Furniture, Tables, Chairs	8	Rs	15000	1,20,000
TOTAL COST (Rs)				**45,70,000/-**

Table 21: Infrastructure cost

ITEMS	UNIT COST	NO. OF UNITS	TOTAL COST
Vehicle	650000	1	6,50,000
Safety Equip.	1500	100	1,50,000
Portable Shredder	35000	1	35,000
Polythene Bags	50	500	25,000
RFID	15500	1	15,500
Storage Bins	2500	5	12,500
Handling Training	10000	1	10,000
Vehicle Tracking Sys.	7500	1	7,500
Weighting Machine	7000	1	7,000
Sanitation Equip.	2000	3	6,000
Phone Service	2500	2	5,000
TOTAL COST (Rs)			**9,23,500/-**

Table 22: Collection cost

ITEM	UNIT COST	NO. OF UNITS	TOTAL COST
Ventilation system	120000	1	1,20,000
Grinder	43000	2	86,000
shredders	55000	1	55,000
Computer	35000	1	35,000
Segregation bins	3500	10	35,000
Indus. water storage tank	25000	1	25,000
Temp. controller	17500	1	17,500
Indus. Tank mixer	10500	1	10,500
Pumping system	6500	1	6,500
Storage racks	2500	2	5,000
Communication	1500	1	1,500
TOTAL COST (Rs)			**3,97,000/-**

Table 23: Segregation and Cleaning cost

ITEM	UNIT COST	NO. OF UNITS	TOTAL COST
Analytical instruments (UV-spectroscopy)	150000	1	1,50,000
Centrifuge machine	45,000	2	90,000
Computer system	35,000	1	35,000
Hot-air oven	35,000	1	30,000
Filtration setup	15,000	2	30,000
Water Purification	25,000	1	25,000
Ph meter	11,000	2	22,000
Safety equipment (PPEs, fire extinguisher)	16500	1	16,500
Weighing machine	5,000	2	10,000
Vortex shaker	3,700	2	7,400
Magnetic stirrer	3600	2	7,200
TOTAL COST (Rs)			**4,23,100/-**

Table 24: Equipment cost

ITEM	UNIT COST	NO. OF UNITS	TOTAL COST
Ethanol	290	10 * 12 *12	4,17,600
Sodium Hydroxide	650	2.1 * 24 *12	3,93,120
Hydrochloric Acid	135	2.8 * 24 * 12	1,08,864
Detergent	200	1.5* 24 * 12	86,400
TOTAL COST (Rs)			**10,05,984/-**

Table 25: Chemical cost

8.2.3 Operation Cost

ITEMS	UNIT COST	NO. OF UNITS	TOTAL COST
Driver	700	1 * 24 *12	2,01,600
Labor	500	1 * 24 * 12	1,44,000
Diesel	90	1 * 80 * 12	98,800
Tires	8000	4	32,000
Unexpected Expenses	2500	1 * 12	30,000
Repair and Maintenance	15000	1	15,000
Insurance	14400	1	14,400
Staff Benefits	1000	1 * 12	12,000
Cleaning and washing	150	1 * 52	7,800
Zip-Lock Bags	5	5 * 24 * 12	7,200
Road Permit	5000	1	5,000
Oil	250	15	3,750
Phone Expenses	300	1 * 12	3,600
Fire Extinguisher, PPEs	2500 + 14000	1	16.500
TOTAL COST (Rs)			**5,91,650/-**

Table 26: Collection operation cost

ITEM	UNIT COST	NO. OF UNITS	TOTAL COST
Un-Skilled Labour	500	2 * 24 * 12	2,88,000
Lab-Technician	800	1 * 24 *12	2,30,400
Electricity	19095	1 * 12	2,29,140
Skilled Labour	700	1 * 24 * 12	2,01,600
Lab Supplies	500	1 * 24 * 12	1,44,000
Aluminum Foil, Tissue paper	440	1 * 24 * 12	1,26,720
Cleaning and Sanitation	10000	1 * 12	1,20,000
Crucible	250	20 * 2 * 12	1,20,000
Glassware	250	35 *12	1,05,000
Sanitation	350	1 * 24 * 12	1,00,800
Filter Paper	1700	3.5 * 12	81,600
Centrifuge Tubes	150	16 * 2 * 12	64,800
Packaging	20	10 * 24 * 12	57,600
Miscellaneous	1000	1 * 52	52,000
Quality control	45000	1	45,000
Safety Equipment	30	5 * 24 * 12	43,200
Benefits	12000	3 * 12	36,000
Equipment Maintenance	30000	1	30,000
Consumables	100	1 * 24 * 12	28,800

Facility Maintenance	25000	1	25,000
Sewage	2000	1 * 12	24,000
Office Supplies	1500	1 * 12	18,000
Gas	1500	1 * 12	18,000
Regulatory Fees	15000	1	15,000
Approvals	15000	1	15,000
Water	1000	1 * 12	12,000
Training	10000	1	10,000
Software	800	1 * 12	9,600
Spare Parts	8500	1	8,500
TOTAL COST (Rs)			**22,59,760/-**

Table 27: Extraction operation cost

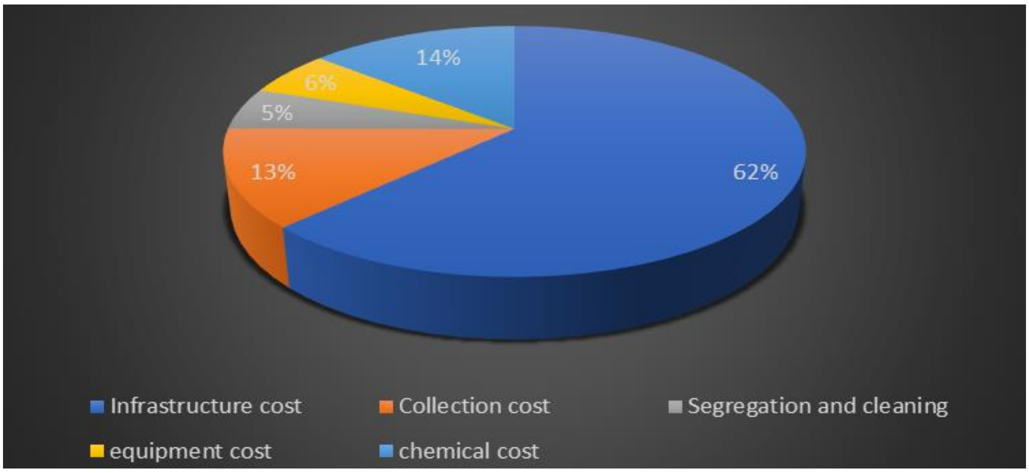

Figure 41: Capital cost

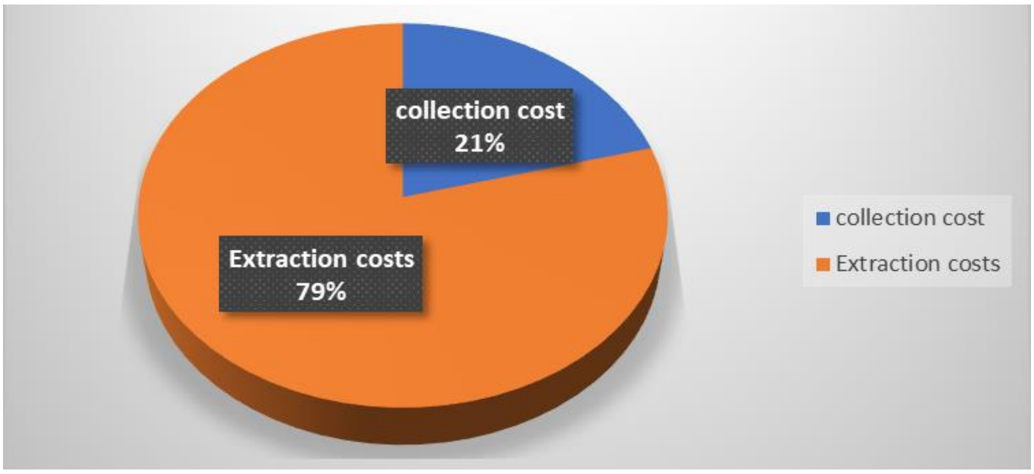

Figure 42: Operational cost

8.3 Cost-Benefit Analysis

QUANTITATIVE ANALYSIS		YEAR 1	YEAR 2	YEAR 3
Opening Balance	**0**	**-47,94,378**	**-17,72,206**	**46,34,935.24**
COSTS				
Capital Costs				
Infrastructure cost	45,70,000	0	0	0
Collection cost	9,23,500	0	0	0
Segregation and cleaning	3,97,000	0	0	0
equipment cost	4,23,100	0	0	0
chemical cost	10,05,984	0	0	0
TOTAL	***73,19,584***	**0**	**0**	**0**
Operational Costs				
collection cost	5,91,650	6,03,483.00	6,09,517.83	6,21,708.19
Extraction costs	22,59,760	22,82,358	23,05,181	23,51,285
TOTAL	***28,51,410***	**28,85,840**	**29,14,699**	**29,72,992.99**
Benefits				
Keratin-product Sales	34,56,000	56,44,800	83,23,200	97,92,000
By-product utilization	0	0	2,08,080	2,44,800
Sustainability marketing	0	0	14,400	36,000
Financial assistance	18,29,896	0	504000	1008000
Cost-reduction	0	52,203.55	0	0
Recurring benefits	90,720	1,58,760	2,72,160	0
Labour-cost reduction	0	0	0	0
Decreased Overhead	0	27,249.30	0	0
Intangible benefits		25,000	0	0
Other Benefits		0	0	10,800
TOTAL	**53,76,616**	**59,08,012.85**	**93,21,840**	**1,10,91,600**
Benefits - Cost	**-47,94,378**	**-17,72,205.75**	**46,34,935**	**1,27,53,542.26**

Figure 43: Cost-Benefit Analysis

CHAPTER 9: PROJECTED DEPLOYMENT AND OBSTACLES

9.1 Utilization of extracted keratin

9.1.1 *Bio-degradable materials*

Keratin implemented in drug delivery, cosmetics, and bioplastics provides an affordable, eco-friendly, and sustainable replacement for petroleum-based plastics [52,53].

- For the manufacturing of bio-degradable plastic, keratin is blended with other bio-polymers like starch or PVA and plasticizers like glycerol or urea to make it more flexible and processible. Naturally-available fibers like chitosan or re-enforcing agents improve their mechanical properties [4,9,54].
- Bio-degradable films and preservatives can extend the product shelf life of food products. Many disposable items bags, wraps, etc can disposed of in nature. Furthermore, in many studies, they work as cushioning materials and protection of materials [4,55].

9.1.2 Bio-medical materials

The extraction of keratin from the chicken feather waste through alkaline hydrolysis is the easiest, economically and environment-friendly method, and the extracted product applied in animal feed, bio-compote, and biomedical in the formation of gel or film can be employed [17,55].

- Keratin is a bio-degradable, bio-compatible product that helps accelerate cell growth and healing tissues thus, reduces the need for dressing changes [9,48,56-58].
- Keratin-based nanoparticles, hydrogels, and biofilms with therapeutic agents provide benefits like drug delivery improving the efficiency, biocompatible, biodegradable solution, and providing controlled and sustainable release of drugs [3,9,14,54].

9.1.3 Agricultural uses

- Hydrolyzing keratin into smaller peptides and amino acids can be used as a fertilizer. It has proven results in improving the soil structure and plant growth by enhancing microbial activity and nutrient quantity [3,39,48,56,59].
- It can work as an animal feed supplement when hydrolyzed into digestible proteins, and provide nutrients and growth to livestock while reducing the need for synthetic protein.

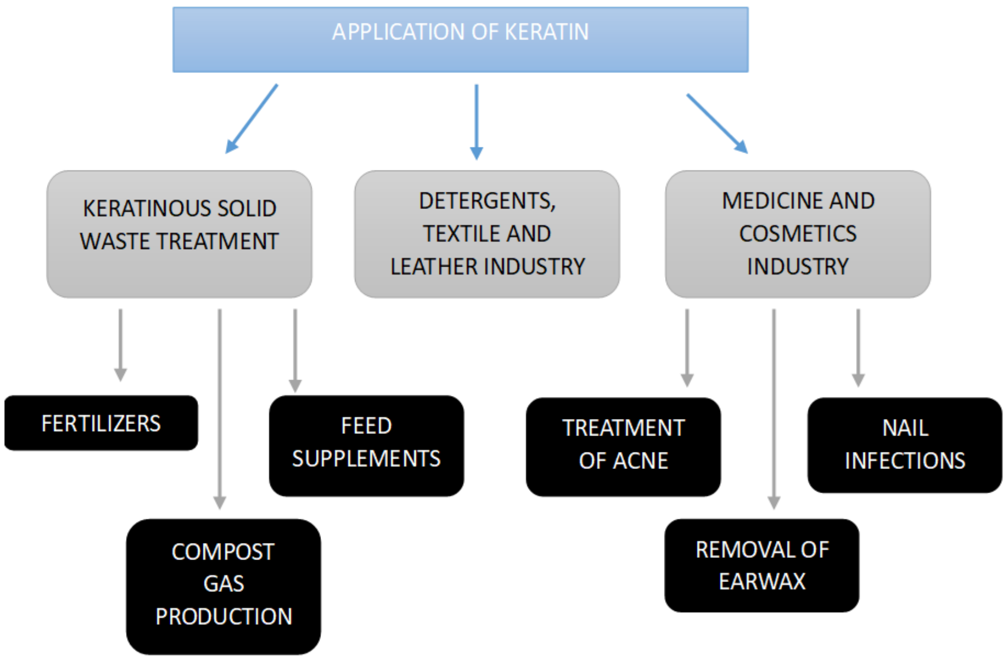

Figure 44: Application of keratin

9.1.4 Textile Industry Applications

- Keratin modification with natural and synthetic textile fibers enhances their properties like elasticity, moisture retention, and strength providing resistance to bacterial growth, softening, and UV protection agents in textile makes comfortable to wear. It is a bio-degradable material and eco-friendly material [48,59].
- Improve fibers and fabric properties like moisture management and thermal regulation to regulate the body temperature throughout the year. Overall, Keratin can help develop innovative and sustainable textiles [47,60].

9.1.5 Cosmetic applications

Keratin operates in the cosmetic industry for manufacturing haircare, skincare, and nailcare products. It can be blended to get modification for immediate and long-term solutions [9,48,57,59].

- Keratin treatment for hair strengthening and repair reinforces the hair structure, reducing breakage and splitting ends. Many shampoos and conditioners use keratin formula for straightening and protective layers to provide shine, silkiness, and softness to hair [14].

- Keratin helps maintain skin hydration and natural skin barrier function protects skin from pollution and harsh weather conditions [36]. Sensitive and irritated skin has also proven results in skin healing and cell regeneration [3,56].

9.2 Successful businesses utilizing keratin utilization

Keraplast technologies

- New Zealand is the world's 3rd largest wool producer after Australia and China. However, waste generated during the trimming process and a declining wool demand possess no value, and large amounts of wool get rejected or wasted.
- Company based in New Zealand that utilizes keratin from wool for sustainable purposes. They source products from the wool industry, more precisely by-products of the wool industry with proprietary technologies and processes that extract the keratin from the wool fiber.
- The company produces in-house production of keratin-based personal care products for hair and skin care. They have also developed some medical products for wound care and cell regeneration and sell products in the global market through various sales channels.
- Also, the company focuses on continuous R&D for the development of new products with innovation and a competitive edge.

Keratin bio-sciences

- The company is based in the United States and transforms keratin waste from poultry farms for value-added products. They source their raw material from the poultry industry and slaughterhouses and extract keratin from rejected by-products through environmentally friendly methods.
- Using the feather waste reduces environmental impact, and promotes sustainability.
- This company uses poultry feather fiber and uses this product for many industrial applications.
- They collect and process feathers through advanced technology and convert them into high-quality keratin.
- Extracted keratin implied in the textiles industry, construction material, automotive, and packaging industries promotes resource efficiency as this waste is landfilled and increases carbon footprint.

9.3 Potential environmental and economic benefits in aligning with SDGs.

SDG 1: No Poverty

Developing a new industry based on keratin extraction will assist in creating jobs and generate revenue in both rural and urban communities, and selling the extracted material to other industries and provide business opportunities in rural and agricultural regions because keratin waste is typically abandoned or burned and considered as rejected waste, assuming no commercial value [44]

SDG 2: Zero Hunger

Keratin extraction can be utilized in food processing industries Hence, promoting SDG 2 by utilizing livestock waste effectively without compromising other food resources [61]. In China, keratin waste is used as eatable food and turned into food products due to being rich in protein, which does not interfere with food production but rather enhances the overall value derived from livestock sources. [**Error! Reference source not found.**]

SDG 3: Good Health and Well-Being

Keratin has already been recycled for the medical industry for the production of wound healing and drug delivery as well as for hair and skin treatment and contributing to the health benefits of developing keratin-based products [9].

SDG 6: Clean Water and Sanitation

Converting the keratin waste produced into value-added goods, environmental pollution caused by the waste can be minimized, and improved water quality in the agriculture sector. As keratin waste takes a longer duration to degrade in nature, causing soil and water contamination [3,55,61].

SDG 8: Decent Work and Economic Growth

The Keratin industry has the potential to provide opportunities for the generation to come as livestock product demand has drastically increased over the years, and the by-product generated during the process. Thus, it can promote economic growth and development, preferably in rural areas [12,63].

SDG 9: Industry, Innovation and Infrastructure

Extraction of keratin from waste promotes innovation in waste management as the growing demand for livestock products led to various forms of pollution, encouraging the researcher to develop alternatives or innovations that will provide a business opportunity and promote waste-to-wealth [55,58].

SDG 12: Responsible Consumption and Production

Reduce, reuse, and recycle are the 3Rs to achieve sustainable goals. Therefore, the effective utilization of waste for the production of goods helps to ensure sustainable production practices and also, encourages the recycling of waste, aligning the production and consumption patterns [48,63].

SDG 13: Climate Action

Various gases and foul smells are emitted from waste when dumped in open space. It also promotes the growth of many microbes and diseases. Eliminating keratin waste and better management practices, greenhouse gas emissions, and climate impact can be reduced [3,49].

9.4 Technical challenges

Separation and collection

- Different types of keratin waste are generated daily, and sorting the waste is a very significant
- There are two forms of keratin waste found: -
    1. Alpha-keratin – found in hairs, wool, horns, and nails (found in mammals)
    2. Beta-keratin – found in feathers, claws, beaks, and scales of birds (found in birds and reptiles), and keratins vary in color and composition.

  As a result, segregating the types of waste is crucial to achieve high yield and purity. Mixing impacts purity fluctuation and will not receive good profit margins and impact our reputation in the market.
- Pre-wash these collected raw materials are required to remove dirt, blood, grease, and manurer, which might reduce output and efficiency.

Chemicals used

- The presence of the chemicals used in the alkaline hydrolysis methods might be costly and may affect the environment. Nowadays, businesses need to focus on the greener solution for the long run and adopt alternatives.

Quality control

- Quality control gets disregarded sometimes, but crucial because it creates a good reputation and goodwill for the company. Quality control is required to review the optimal dose for pH, temperature, and retention time required for the extraction if the targeted market is the pharmaceutical and cosmetic industry.

Environmental and safety concerns

- Disposal of chemicals used is one of the challenges. Either reducing the molarity of the chemical or additional treatment plants employed is a time-consuming process. So, use safer chemicals to prevent environmental pollution.
- Handling the chemicals required special care and experience.

9.5 Economic challenges

Cost barriers

- The setting of the facility requires a significant amount of capital investment
- Need for expensive equipment like filtration and extraction systems.
- Equipment like hot air ovens and mechanical separation are energy-intensive
- High-purity chemicals can be pricey
- Skilled labor supervision for the advanced extraction process
- Collection and transportation can be expensive if not located near sources
- A large amount of by-products generated requires a waste-treatment plant.
- Demand for keratin is volatile and fluctuates

Funding barrier

- Banks and other financial institutes often hesitate to find start-ups
- Funding for new and niche industries due to a lack of historical data is a challenging
- Continuous R&D is required to get aid and central assistance
- Getting government is a challenging and competitive process

9.6 Regulatory challenges

Environmental regulation

- Chemicals used required proper disposal or treatment plant to prevent environmental contamination
- Effluent from industry must meet the water quality standard or require contamination removal
- Proper air pollution control systems like scrubbers and filters required
- A process related to extraction is water-intensive and requires a recycling system

Safety regulations

- Proper safety equipment like PPEs, gloves, masks, and fire extinguishers for the safety of workers and facility
- Regular safety inspections and maintenance to maintain safety standards
- Local building codes and emergency response systems require additional safety

9.7 Proposed solutions

9.7.1 Innovations and Improvements

- Adoption of an automated segregation system to reduce the potential risk and labor cost
- Advance cleaning and pre-treatment methods to increase efficiency by reducing contaminants
- Do an evaluation survey for strategic advancement
- Use eco-friendly chemical extraction process to minimize waste
- Upgrade extraction methods and expand to get more flexibility
- Software and monitoring systems can help maintain product quality
- Use of renewable energy sources can cut costs and decrease dependence
- Water treatment and recycling systems can reduce water consumption and discharge
- By-products can be used for bio-energy and compost to minimize waste and increase profit margins
- Proper ventilation systems and facility design can reduce risk
- Partnership with government and research institutes to improve output
- Continuous improvement by customer reviews and feedback and also by adoption of new technology

CONCLUSION

Due to shifting consumption trends, many undesirable by-products emerge at different stages of production, resulting in consequences; waste management strategies must be implemented for the well-being of society and the environment. Keratin is a by-product of many processing and manufacturing industries and millions of tons every year are abandoned or scorched. Waste consists of keratin compounds with a high content of amino acids such as cysteine, alanine, and many more. The study establishes utilizing extracted keratin as raw material for other processing industries. Analyses such as FT-IR, SEM, and DSC were conducted to identify the keratin composition, results show that buffalo hair and sheep wool produce a yield above 20% which may vary with varying in the concentration and amount of NaOH, detention period, and temperature. Whereas the alpha-helices buffalo hair exhibits sharper vibration peaks, sheep wool has the smoothest structure and hen feather has a more rigid architecture. DSC analysis shows that hen feather has the highest thermal stability while buffalo hair has the least. Further, technical analysis followed by financial, cost-benefit, and market analysis were undertaken for a plant with 2 tons annual keratin production capacity and an average yield of 21% from livestock waste. Potential challenges and solutions for plant optimization were provided, for better waste management and sustainability.

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ANNEXURE I: PROCESS FOLLOWED FOR EXTRACTION

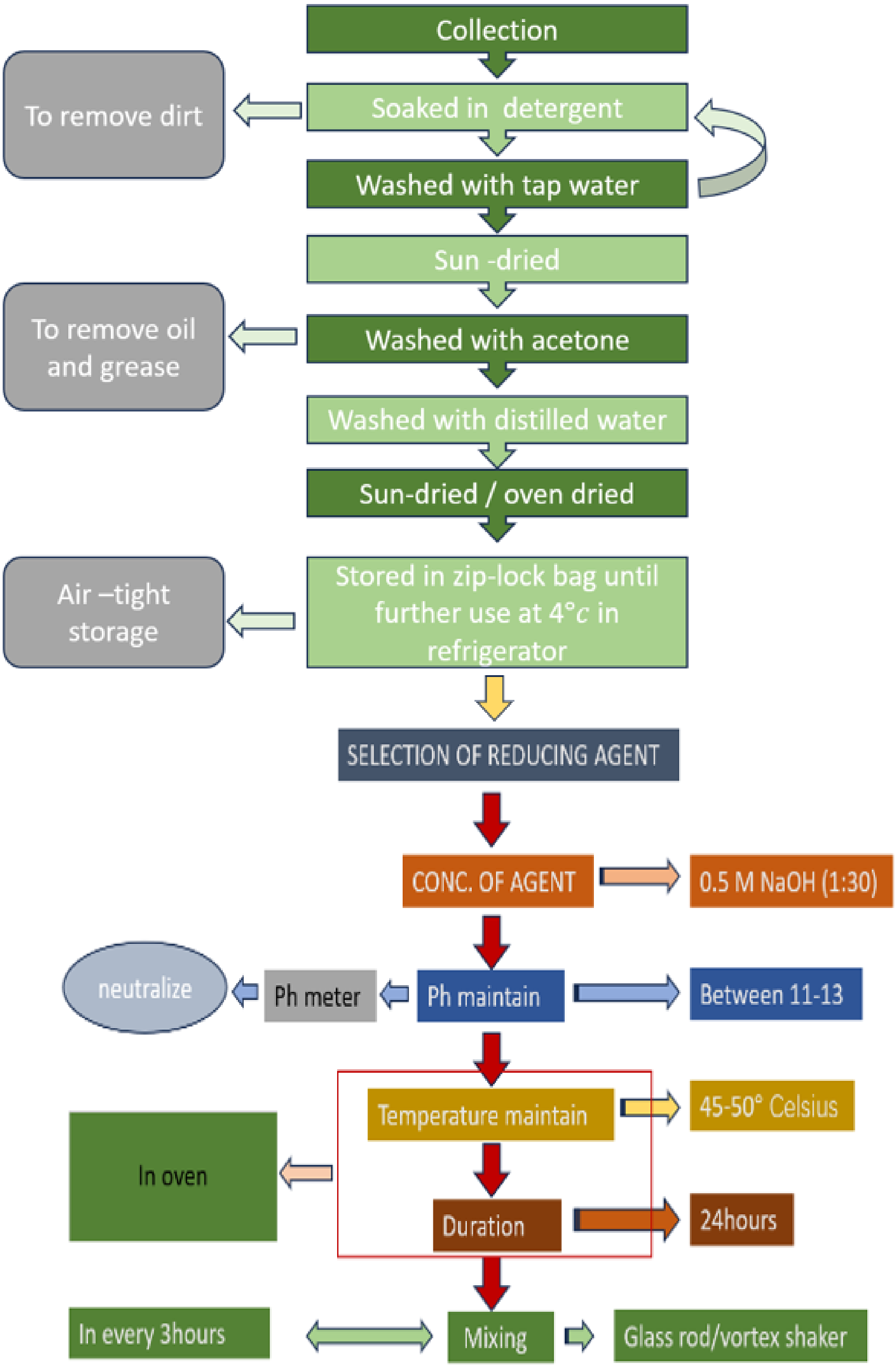

Figure 45: Extraction process

ANNEXURE II: CALCULATION

Preparation of NaOH solution

Preparation of the solution of sodium hydroxide pellets 98% was used in distilled water.

1) Molarity = $\frac{weight\ of\ NaOH*1000}{250*Molecular\ weight\ of\ NaOH}$
2) n=cV

where,

c= concentration in M (moles per litre)

n= number of moles

V= volume in liters

The volume of distilled water required-

1000ml *$\frac{1L}{1000mL}$ =1L

Say we are preparing the 0.5M NaOH solution

n= cV

= (0.5M) (1L) = 0.5 mol

Conversion of the mole to mass

m=nM

where,

m= mass in grams

n= number of moles

M= molar mass in gram per mol.

Where,

M = atomic molar masses of each atom of NaOH

For NaOH,

M= (22.99 g/mol) + (16.00 g/mol) + (1.01 g/mol)

= 40.00g/mol

Thus, for the preparation of 0.5M NaOH in 1L distilled water

m= nM

= (0.5) (40.00 g/mol)

= 20 g

Preparation Of HCL Solution

For the preparation of 1M HCL in 1L

We know,

$$C_f * V_f = Cs * V_s \qquad -(1)$$

where,

C_f = concentration of the final diluted solution

V_f = volume of final diluted solution

C_s = concentration of the stock solution

V_s = volume of the stock solution

C_s can be calculated by

We know,

Density of HCL =1.19 g/l

The molecular weight of HCL = 36.5

Thus,

370 * 1.19 = 440.3 g/l

Molarity of HCL = $\frac{440.3}{36.5}$= 12.0630 or 12M

So, putting values in (1)

We get,

$1 * 1 = 12.0630 * V_S$

V_S =82ml in 1L

1000- 82 = 918ml

For the preparation of 1M HCL in 1L distilled water

We need 82ml of HCL and 918ml of distilled water

QUESTIONNAIRE

DATE OF VISIT ☐☐☐ TIME ☐☐☐

NAME ______________________ Gender ☐ M ☐ F

ADDRESS ______________________

CONTACT NO. ______________________ Locality ☐ Urban ☐ Rural

Operation

Operation period ☐ <6mon. ☐ 6m-1yr. ☐ 1-5yr. ☐ >5yr. ☐ >10yr ______________

Employees ☐ nil ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ________

Environment ☐ Very safe ☐ Safe ☐ Neutral ☐ Unsafe ☐ Very unsafe

Training ☐ Yes ☐ No

Injury ☐ Yes ☐ No

Cleanliness ☐ Very clean ☐ Clean ☐ Neutral ☐ Dirty ☐ Very dirty

Income source

Investment (daily/weekly/monthly/yearly) (product+expenses) ______________ Profit/Margin______________

Main consumer ☐ Hotels ☐ Local customers ☐ Other______________

Which product gives high margin __________ and low margin __________ (acc. to them)

Waste generated

Types of animals ☐ Chicken ☐ Goat ☐ Sheep ☐ Pig ☐ Other ______________

Waste generated animal blood/ feces/ bone/ fat/ animal trimmings / stomach content / urine / feather or hairs / head

Which animal leave large amount of waste and low waste ______________________

Does religious/cultural belief effect the amount of waste generated ☐ Yes ☐ No

Other waste ☐ Animal byproducts ☐ Packaging materials ☐ Organic waste ☐ Hazardous waste ☐ Other

Quantity of waste generated daily/weekly/monthly ______________

Quantity of water used in washing/ other purposes ______________ Source of water ______________

Waste management

How they handle different kind of waste generated______________________

Equipment used in handling/ management waste ______________________

Any special technique they used to minimize waste ______________

Are there designated waste collection bins or area ☐ Yes ☐ No

Are those collection area marked and labelled ☐ Yes ☐ No

Segregation of waste types (recyclable, hazardous) ☐ Yes ☐ No

Training for separation and disposal ☐ Yes ☐ No

Aware about potential of different kind of waste ☐ Yes ☐ No

What type of material recycled ☐ Plastic ☐ Paper ☐ Metal ☐ Other ______________

Figure 46: Survey questionnaire

Questionnaire for yourself

Technical review

Raw material

- What types of livestock waste are available in the local/ national market in sufficient quantities?

  Feather/wool/hairs/horns/hooves

- Are there reliable suppliers/ partnerships for the supply of raw materials throughout the year?
- How will the collection/ procurement process take place?
- Which location is best suitable for a steady supply of raw materials?

Extraction process

- Which process is most suitable for our raw material available?
- What can be the potential yield or purity of the method applied?

Quality control

- Which quality control measure to take to ensure product consistency?
- Which certification or regulations are required for targeted market application?

Environmental and safety compliances

- What are the state and central-level environmental regulations required?
- What safety protocols are required for proper chemical handling and operations?

Energy and resources required

- What are the expected water and energy requirements for the treatment facility?
- Which renewable energy resources and treatment systems can be incorporated?

Economic feasibility review

Initial investment

- What is the initial capital investment required for setting up the facility?
- What funding options are available?

Operational costs

- Amount of operation costs for labor, energy, chemicals and other required?
- How can processes be optimized to reduce the cost?

Market analysis

- What is the current market demand and trends for keratin-based products?
- Who are potential competitors and their strengths and weaknesses in the industry?

Potential Revenue

- What will be the pricing strategy for better profitability?
- What can be estimated sales monthly/yearly?

Risk assessment analysis

Technical risk

- What are the potential technical risks and how to minimize them?
- What if the technologies applied failed and what measure can be taken?
- Are there any alternative technologies available that can be adopted?

Market risk

- Who and where to sell the finished goods?
- What is the demand stability of keratin-based products?
- Which strategies to apply to mitigate the market?
- What are your strengths and weaknesses?
- What were the threats and opportunities during a market search?

Regulatory risk

- What risk is associated with non-compliance with safety standards and regulations?
- How can regulations be monitored and managed?

Implementation strategies

Project Timeline

- What is the timeline and key milestone the project can achieve?
- What if project delays then what are contingencies available?

Partnerships and collaborations

- With whom partnership or collaboration can achieve desired results?
- How will we manage and maintain partnership?

ANNEXURE V: PURPOSED LAYOUT OF PLANT

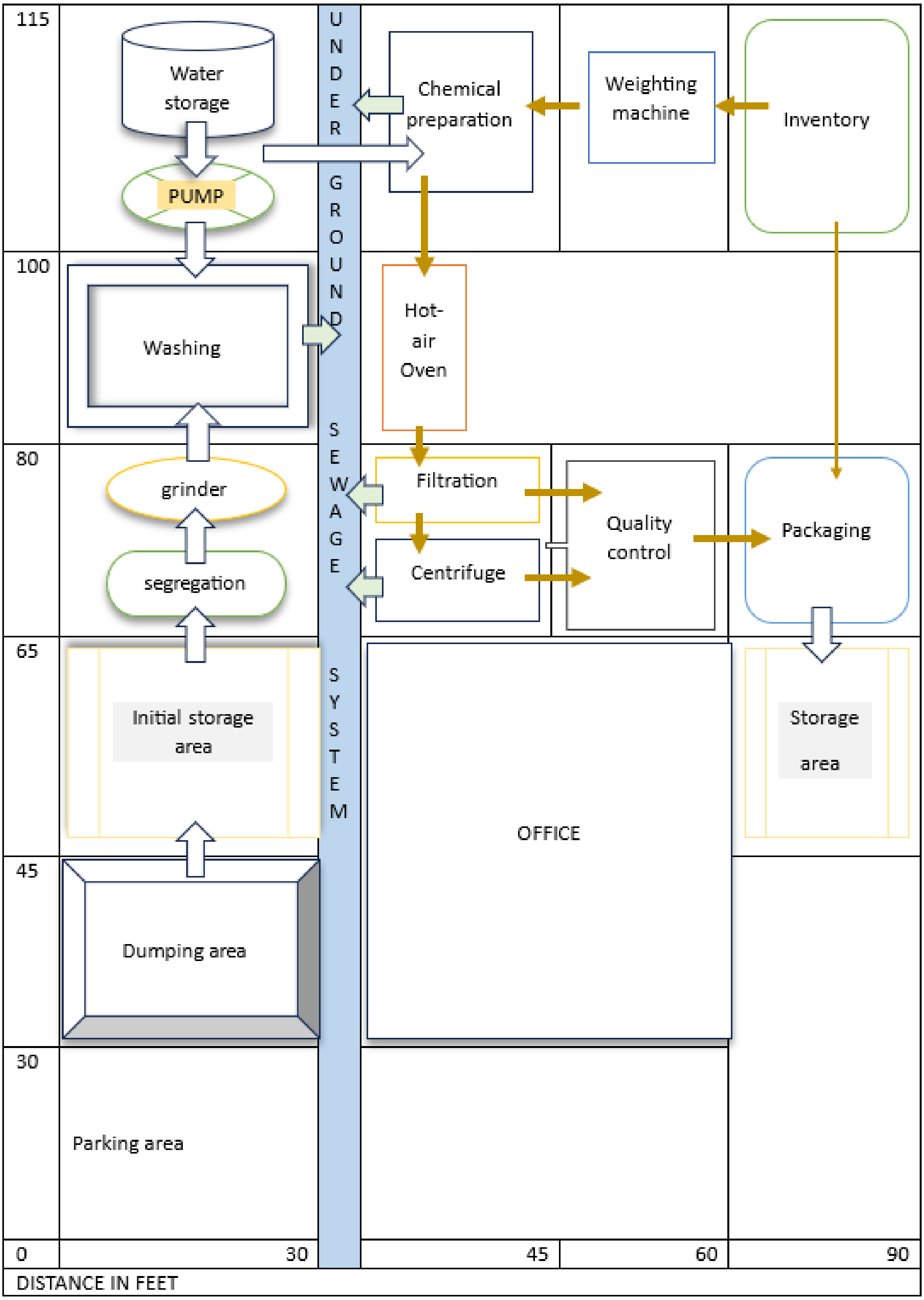

Figure 47: Plant layout

ANNEXURE VI: PHOTOGRAPHS

Figure 48: Photographs