Formulation of keratin based cosmetic products from chicken feathers

Project report submitted in partial fulfillment of requirement for

the degree of

Masters of Technology

in

Biotechnology

By

Megha Sharma (212555)

Under the supervision of

Dr. Ashok Kumar Nadda

to

Department of Biotechnology and Bioinformatics,

Jaypee University of Information Technology, Waknaghat

Solan-173234, Himachal Pradesh

Candidate's Declaration

I hereby declare that this **project "Formulation of Keratin based Cosmetic Products from Chicken Feathers"** has been done by me under the supervision of **Dr. Ashok Kumar Nadda, Assistant Professor, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology.** I also declare that neither this project nor any part of this project has been submitted elsewhere for award of any degree or diploma.

Megha Sharma

212555

Date:

Supervisor's Certificate

This is to certify that the work which is being presented in the project report entitled **"Formulation of Keratin based Cosmetic Products from Chicken Feathers"** in partial fulfilment of the requirements for the award of the degree of **Masters in Technology in Biotechnology;** submitted to the Department of Biotechnology And Bioinformatics , Jaypee University of Information Technology, Waknaghat is an authentic record of work carried out by during the period from July 2022 to May 2023 under the supervision of **Dr. Ashok Kumar Nadda, Department of Biotechnology And Bioinformatics, Jaypee University of Information Technology, Waknaghat Solan, India.**

I also authenticate that I have carried out the above-mentioned project work under the proficiency stream.

This is to certify that the above statement made is correct to the best of my knowledge.

Dr. Ashok Kumar Nadda
Assistant Professor
Department of Biotechnology & Bioinformatics,
Jaypee University of Information Technology,

Date:

Acknowledgement

Firstly, I express my heartiest thanks and gratefulness to almighty God for his divine blessing that made it possible to complete the project work successfully.

I express my deep sense of gratitude to **Dr. Sudhir Kumar, Professor and Head, Department of Biotechnology and Bioinformatics**, Jaypee University of Information Technology, Waknaghat for his kind support that helped me in carrying out the dissertation work in a good manner.

I am really very grateful and wish my profound indebtedness to my Supervisor **Dr. Ashok Kumar Nadda, Assistant Professor, Department of Biotechnology and Bioinformatics,** Jaypee University of Information Technology, Waknaghat. His deep Knowledge, keen interest, endless patience, scholarly guidance, continual encouragement, constant supervision, and valuable advice have made it possible to complete this project. I express my deepest sense of gratitude towards him for kind cooperation in every condition.

I would like to express my gratitude and thank Miss Megha Maurya for her help, constant support and guidance throughout my project.
I would also like to thank all my lab mates and my friend Alka Sharma for constant encouragement and support till the completion of my project work.

Finally, I must acknowledge with due respect the constant support and patience of my parents.

Table of Content

List of Figures

List of Tables

List of Abbreviation

S. No.	Abbr.	Abbreviation(s)
1.	^{0}C	Degree Celsius
2.	Cm	Centimeter
3.	F1	Formulation 1
4.	F2	Formulation 2
5.	gm	Gram
6.	HCl	Hydrogen Chloride
7.	ml	Milliliter
8.	M	Molarity
9.	NaOH	Sodium Hydroxide
10.	N	Normality
11.	qs	Quantity Sufficient
12.	rpm	Revolution per minute
13.	S1	Sample 1
14.	S2	Sample 2
15.	S3	Sample 3

Abstract

Keratin; a fibrous protein that is mainly present in humans and animals in the different forms of such as nails, hair, hooves and feathers. Although it is a very important protein for the human body, but its source causes a lot of environmental damage. Dumping of human hair and chicken feathers is a global environmental concern also threatening the living beings. Feathers, bones, beaks, and other hard tissues are only a few examples of the massive amounts of keratin waste produced by slaughterhouses. Highly contaminated effluent from slaughterhouses has been described as having a detrimental effect on the biodiversity of the ecosystem. Chicken feathers is a great, abundant and an inexpensive source of keratin that can be utilized to produce different valuable products. In the duration of this project, alkaline hydrolysis is a method that has been used to extract the keratin from waste chicken feather biomass procured form local slaughter house. In this study the extracted keratin has been used in the formulation of shampoo and cream as a method of utilizing waste biomass and creation of valuable products. All the formulations are prepared using the dialyzed Keratin solution. The formulated products were then evaluated on the basis of their physico-chemical characterization such as color, appearance, pH, viscosity and stability. The results indicated that the formulations were stable when stored at different temperature and had a correct range of pH and viscosity.

Keywords: *Keratin; feathers; alkaline hydrolysis; biomass; formulation*

Chapter 1 Introduction

Growing environmental concerns have driven the need for safe and sustainable bio-based materials, forcing the use of available natural by-products as alternatives. Keratin is one of the most abundant proteins which is present in the body of all birds, and mammals. It is a major component of nails, feathers, wool, and hooves and gives strength to the muscles and body [1]. Keratin was first used as a medicine in 16th century.

The techniques of waste management, deals with the suitable treatment of waste to eliminate commercial and industrial, and household wastes to limit environmental contamination and maximize the product recovery. Several industries, however, continue to have difficulties in disposing of garbage in an environmentally responsible manner [2]. Long term and innovative solutions are required to effectively minimize the rate of pollution. Harmful environmental effects can be prevented to a large extent by replacing, fossil fuels with bio-based materials or with the renewable resources. Highly contaminated effluent from slaughterhouses has been described as having a detrimental effect on the biodiversity of the ecosystem. The biological oxygen demand and chemical oxygen demand of the waste water are high because of the breakdown of organic materials such blood, flesh, excrement, and fat [3]. Waste water that is discharged into water bodies contains significant levels of nitrogen and phosphorus [4].

Keratin protein belongs to a group of fibrous proteins which is also known as sclerosing proteins. Keratin is an insoluble form of protein and has very stable structure, a small protein and is uniform in size. There are two forms of keratin which are α- and β-keratins; rich in glycine and hydroxyproline and proline. Keratin from chicken feathers is a major source of cheap, environmentally friendly and commercial biomaterials [4]. There are various methods to extract keratin from the waste biomass of chicken feathers, including acid hydrolysis, mechanical and enzymatic methods [3]. The extracted Keratin is a highly resourceful biopolymer which could be modified in numerous forms for example; microparticles, beads, gel, films, cosmetic products and nanoparticles [3]. The modified forms of keratin have various applications in food sciences, green chemistry, pharmaceuticals and also in cosmetic industries.

The cosmetic industry is growing day by day and numerous cosmetics products are formulated using Keratin hydrolysates such as shampoos, hair cream, conditioners, body lotions,

hair serum, mascaras, nail polish and nail serums. Human hair contains around 89% of Keratin, 8% moisture and 3% minerals. The hydrolysate of Keratin is used to nourish the hair for the smoothening effect. Now a days Keratin treatment of hair is being done by many individuals to get smooth, silky and shiny straight hair [5]. This Keratin treatment is very costly and also not good for the health of hair. It just makes hair to look smooth, silky and straight for some period of time. Harmful chemicals are used in the Keratin hair treatment such as formaldehyde which is a carcinogen and causes many allergic reactions and side effects such as vomiting, cough and dizziness.

In this study we have tried to formulate some of the hair care products such as shampoo and hair cream using dialyzed Keratin solution and aloe vera extract. *Aloe barbadensis miller* is commonly known as Aloe vera. It grows in all over the world under both hot and dry conditions. Aloe vera has applications in pharma and cosmetic industries. Different antioxidants are present in aloe vera like Vitamin A, C, and E which has antibacterial and anti-inflammatory properties. Aloe vera has benefits in hair products. It provides moisture to hair and scalp and prevents dandruff, promotes hair growth, and protects hair from UV radiations. Aloe vera works great to strengthen the hair and to prevent itchy scalp or greasy strands.

This study was done to formulate Keratin shampoo and cream to evaluate its physico-chemical characterizations. The physico-chemical characterizations are done on the basis of visual inspection, determination of pH, stability test and viscosity.

OBJECTIVES

1. Collection and pretreatment of chicken feather biomass.
2. Extraction of Keratin from waste Chicken feather biomass.
3. Formulation of keratin-based shampoo and cream for topical use.
4. Physico-chemical characterization of prepared product.

Chapter 2 Review of Literature

2.1 Sources of Keratin

Keratin protein is a unique protein found in the outer layer of hair, which is made up of hair and other parts of the body [1]. This protein keeps hair healthy and strong. It also makes your hair shinier and smoother. There are many different sources of keratin, including cowhide, wool, silk, and feathers. For those wanting to know the best source of keratin, the best ones are feathers or wool [4]. Keratin is the protein that makes up the outer layer of hair, nails and teeth. It is also found in the cells of our skin, organs, and other connective tissues. Keratin is made up of a protein called keratin sulfate, which is made up of sulfur and oxygen [2]. The body can make keratin from sulfur and oxygen in three different ways: 1) through digestion, 2) through respiration, and 3) through exposure to sunlight. Since keratin is produced in the intestines, the main source is diet.

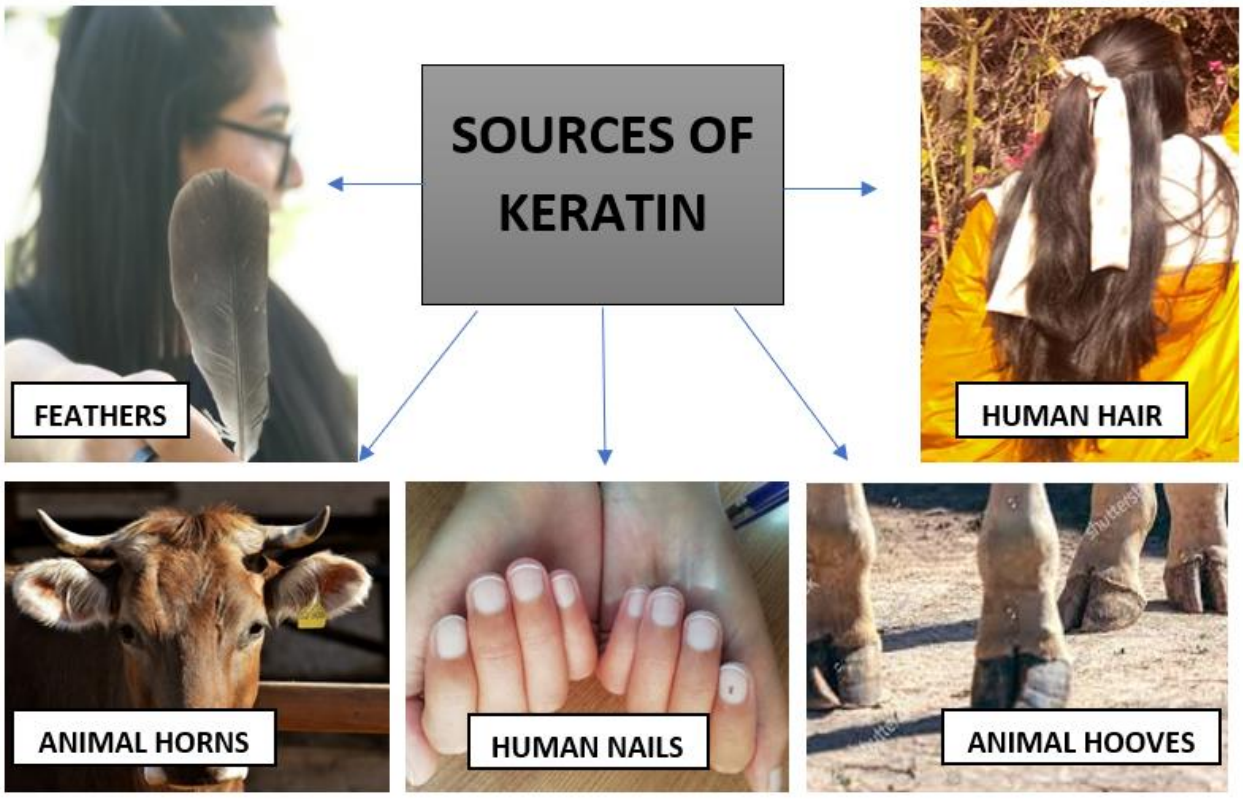

Fig 2.1: Different Sources of Keratin

2.2 Forms of Keratin

Keratin is a broad protein group that may be defined as a fibrous protein which produces the major structural elements of feathers, hair, horns, and claws [2].

There are two major forms of keratin [6]:

- α-keratin;
- β-keratin;

Keratin; subdivided in two major forms alpha- keratin; and beta- keratin. The main difference between alpha and beta keratin is their structure and their different sources. Alpha keratin is mainly found in mammals for example; human hair, nails and animal wool and is present in helical structure [7]. Whereas, beta keratin is found in reptiles and birds for example; bird and chicken feathers and in skin of reptiles and is present in the form of beta sheets [6].

TABLE 1: Difference between alpha (α) and beta (β) keratin

	ALPHA-KERATIN	**BETA-KERATIN**
DEFINITION	A structural protein which is mainly found in mammals.	Protein mainly occurs in epidermis of reptiles.
SOURCES	Wool; Hair (human and animals); Hooves; Horns; Fingernails (human and animals); Quills.	Feathers (Chicken and birds); Reptilian claws; Scales; Claws; Avian beaks.
STURUCTURE	Helical structure.	Beta sheets.
FUNCTION	Provides structural stability	Adds more rigidity to reptile's skin; Provides protection and waterproofing.

2.3 Methods of Keratin Extraction

Keratin is hydrophobic in nature and is insoluble protein. It has tightly packed chain of polypeptides, disulfide bonds, strong hydrogen bonding and strong hydrophobic interactions. The extraction and dissolution of keratin is difficult and time taking [8]. By using various reducing agent's natural keratin protein can be obtained from chicken feathers. These chemicals will dissolve the keratin fibers by breaking down their disulfide and hydrogen bonds [9].

Over the past few years, different techniques to extract keratin from waste biomass are used such as chemical hydrolysis, microbial and enzymatic treatment, microwave technique, technique of steam explosion and process of thermal hydrolysis [10].

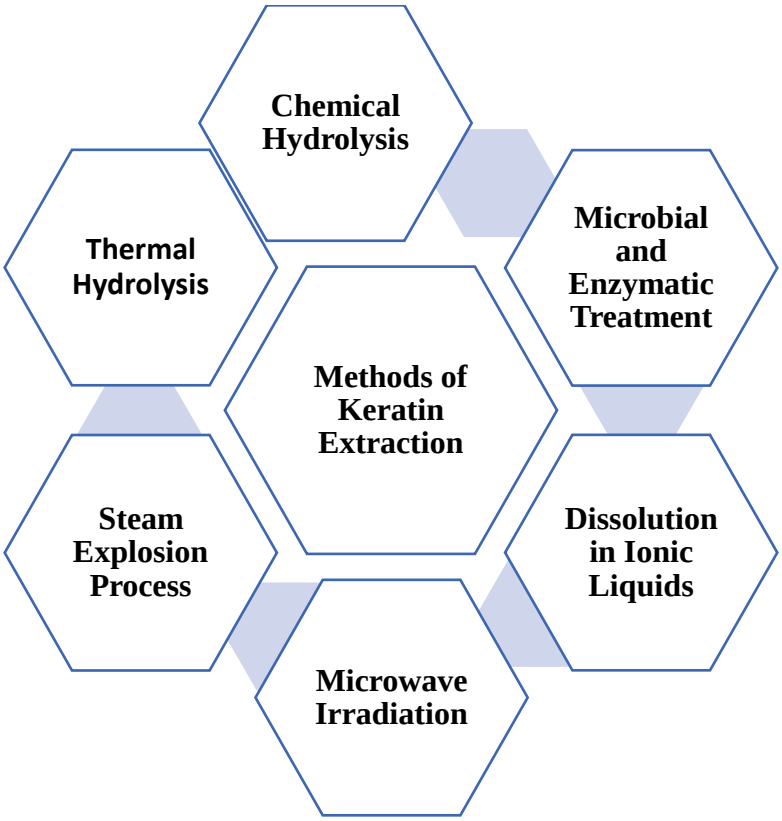

Fig 2.3: Different Methods of Extraction of Keratin

2.3.1 Chemical Hydrolysis

For the extraction of keratin using chemical hydrolysis the total keratin yield is governed by temperature, pH, the concentration of acid or base and the total time of reaction are used for the dissolution of feathers. In chemical hydrolysis the maximum yield is accompanied by constant heating and shaking for feathers at constant temperature; however, high temperature also tends to destroy the amino acids [11]. The oxidation is done using 2N HCl solution or using 2% mild ammonia or peracetic acid solution [12].

2.3.2 Enzymatic Treatment

Low amount of energy is required for enzymatic hydrolysis, it occurs while affected by the reducing agents that break the disulfide bonds present in keratin. Sulfitolysis, deamination and proteolysis are the major mechanisms used to break down the keratin. Protein disulfide links and S-sulfocysteine and cysteine are broken during sulfitolysis and proteolysis using sulfite material which is produced by bacteria [8,13].

2.3.3 Microwave irradiation

In this method, the keratinous mass obtains uniform heating whereas the activation energy needed for keratin extraction is decreased. Due to the rapid homogeneous heating that takes place, the keratin degradation and extraction process is efficient. As the temperature of reactor rises, the molecules involved in the microwave-assisted heating have a tendency to uniformly absorb energy. It has been observed in different studies that the temperature and time of incubation plays a very important role in keratin extraction from microwave irradiation. At temperature 150 to 180 °C for 1hr the total extraction yield observes was 60% whereas; at 180 °C for about 30 min yield of 31% was observed and at 160–200 °C for around 20 min the total yield was 71% [14-16].

2.3.4 Steam explosion

This technique was first used in the year 1982 using wool biomass for the extraction of keratin [17]. This process is a hydrothermal process known as steam explosion. The process uses very high-pressure and saturated steam over short period of time that is 1 to 10 minutes. In the reactor, the biomass of keratin is kept at a high temperature of 180–230 °C. In the final step the pressure is released which results in the decompression, dissolution and breaking of the keratinous

biomass fibers [18]. The results are significantly influenced and are affected by the number of parameters including resistance time, moisture, particle size, pressure and temperature. In a study (2015) it was reported that the total keratin yield extracted was maximum at high pressure [19].

2.3.5 Thermal hydrolysis

This is a superheated process to extract keratin using the chicken feathers biomass. This process is carried out in a pressure cell which is completely sealed with 20mg/ml water in it, then the preparation is kept in a preheated oven. In this hydrolysis, the biomass is exposed to different temperature and pressure settings while the biomass is being treated with water until the protein is broken down into the oligopeptides. According to a study conducted in year 2007, the incubation time and heat both affect the reaction and dissolution rate of biomass [20].

Thermal hydrolysis is a process of two steps: (i) breaking down the fibers-containing keratin proteins and; (ii) cleaving the bonds of sulfide that hold the keratinous fibers together.

This thermal hydrolysis process of two steps has shown approximately 70% keratin yield in a recent study [21].

2.4 Applications of Keratin

The degradation of keratin and its disposal can have detrimental effects on the environment. For this reason, extensive research has focused on the utilization of keratin waste. Thousands of tons of human hair, animal horns, hooves and feathers containing keratin are discarded each year [4].

The degradation and disposal of keratin can have harmful effects on the nature and environment. Therefore, many researches had been conducted on the recycling of keratin waste [8]. In today’s world many researches are done to utilize the keratin to produce environment friendly and biodegradable materials. Now a days many pharmaceuticals and cosmetic industries are using Keratin hydrolysate to produce valuable product's [22].

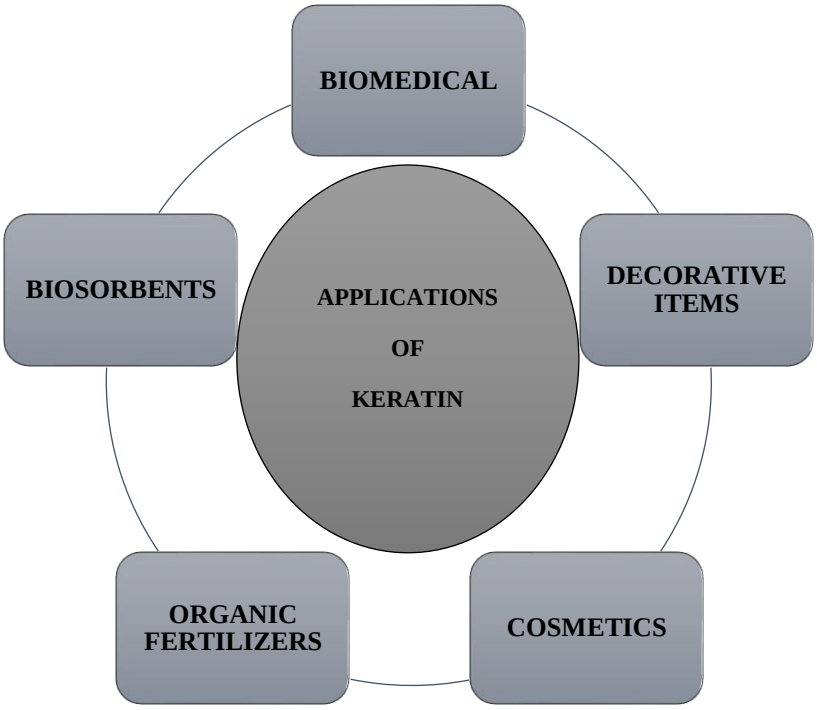

Fig 2.4: Applications of Keratin

2.4.1 Biomedical

Materials based on keratin have excellent mechanical resistance, are very biocompatible and are easily biodegradable. These materials can be easily processed into complex 3D scaffolds, films, sponges, and hydrogels for number of biomedical applications. Hydrogel of keratin (9% purity) has demonstrated the reduction ability of the progression of burns and promotes regeneration of skin. A mixture of 5% of keratin hydrogen gel and 5% polyvinyl alcohol demonstrates an in vitro wound healing process. Keratin film is an excellent alternative for ocular surface reconstruction due to its ability to effectively transmit light and high mechanical strength [23].

Hydrogel is a well-known polymer gel due to its superior biocompatibility and wide range of biomedical uses, including wound healing, implants, and in drug delivery systems [24]. The hydrogels were created by using the keratin powder extracted from waste chicken feather. Furthermore, because of their non-toxicity and water solubility, starch, polyvinyl alcohol (PVA)

and polyvinylpyrrolidone (PVP) had been widely used for biomedical applications as hydrogels [25].

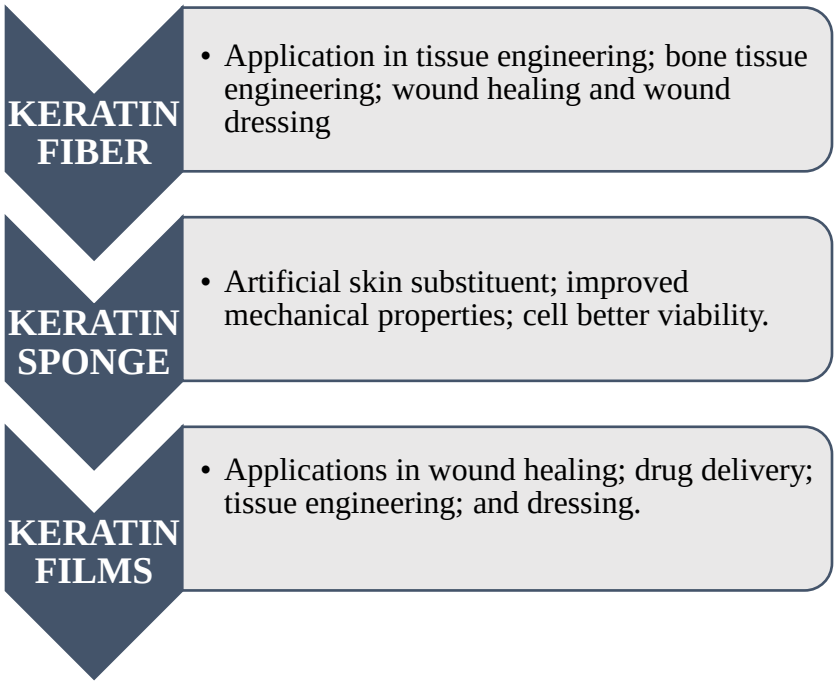

Fig 2.4.1: Biomedical Applications

2.4.2 Biosorbents

Keratin biomaterials help remove heavy metals from water by having active polar sites; which are present on their surface that attract metal ions (charged) through surface chemical and physical mechanisms. As a biosorbent, keratin comes from three different sources: animal hooves and horns, human hair, and feathers. Chicken feather keratin could also be used for the absorption of Cd, Zn, Ni, and Cr metal ions [26].

2.4.3 Organic Fertilizers

Waste containing keratin is used as an organic fertilizer due to the presence of (N) and (C). Chicken Feathers are an excellent source of organic fertilizer as they are rich in amino acid, peptides, and minerals. Feather hydrolysates remediate contaminated soil and promote plants to grow [27]. As a source of nitrogen, the amino acids present in hydrolysate of keratin will influence the rhizospheric microbes, which will ultimately have an impact of microbes on the roots of the plant. As a result, the hydrolysate made from waste biomass of keratin is a potential biofertilizer. According to studies, bacterial hydrolysate of keratin can act as a biofertilizer that helps in the development of plants [27]. The microorganisms contribute in the prevention of nutrient loss by absorbing extra inorganic nutrients present in the soil by synthetic fertilizers [28]. Finally, combining more microorganisms employed in biofertilizers with keratin wastes that have undergone microbial treatment would provide a novel fertilizer that brings together the benefits of both biofertilizers and organic fertilizers [29]. The microbe employed in biofertilizers that has the capacity to break down keratin is optimal.

2.4.4 Cosmetics

Keratin is used in a variety of cosmetic applications, including skin and hair applications. Peptides of keratin help in improving the moisture content of hair, by giving it shine and suppleness. Keratin acts as a humectant, drawing moisture from the lower layers of the skin to the stratum corneum.

Keratin was able to improve both the thermal and mechanical properties of normal hair [7]. Peptide bonds (mainly containing the amino acid; cysteine) were found to be non-cytotoxic. A wide variety of creams, shampoos, and conditioners are currently available in hair straightening compositions, but correct compositions and procedures are required to produce better and safer formulations [30].

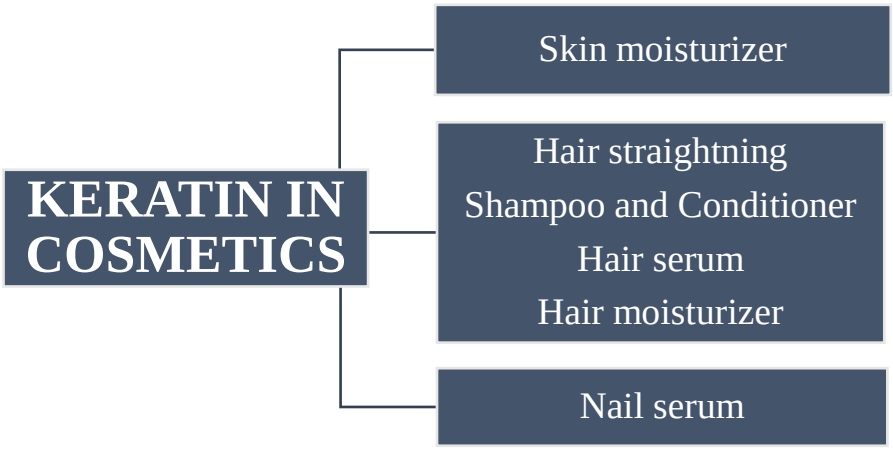

Fig 2.4.4: Applications of Keratin in Cosmetics

Skin Care Product

keratin protein can be applied alone or in combination with lipids to improve skin elasticity, hydration and moisture absorption profile. Different skin care products can be produced using keratin such as face cream, soap, facewash and scrub. Keratin based products are good for skin as it has antiaging and healing properties [31].

Nail Cosmetic Products

Keratin can be extracted using reducing conditions and its film is prepared by a solvent evaporation process [32]. The produced films were suitable for penetration tests with respect to water repellency and mechanical stability. Formulation of keratin-based nail serum can also be a good option as it will help in the nourishment of nails and helps to make them stronger and shinier [30].

Hair Care Products

Keratin protein reduces frizz and calms overlapping hair cells. The use of keratin rich products helps to prevent dryness while also making the hair healthier and glossier. Keratin based products help to strengthen the roots of hair [33]. This helps to prevent breaking as your hair develops, allowing to boost growth without fear of hair loss.

Hair Keratin Treatment

Keratin is naturally present in hair and nails of humans. In Keratin hair treatment additional Keratin is added to the hair, which reduces frizz, boost shine and also strengthen the hair. Keratin treatment is done to straighten the wavy or curly hair [34].

However, keratin treatments may cause certain side effects and some safety concerns. Formaldehyde is a chemical used in keratin hair treatment which is a carcinogenic compound and can cause cancer and hair loss [33]. The use of formaldehyde can cause eye irritation, headaches, nausea, dizziness, vomiting and skin rash.

Chapter 3 Material and Methods

Material Required

NaOH (purchased from Hiedia), Xanthan gum, Sodium Lauryl Sulphate, HCl, Polysorbate 80 (were purchased from Lobachemic), Petroleum ether, Cetyl Alcohol (were purchased from SRL), Glycerol (purchased from Sigma), Propylene Glycol (purchased from Qualigens), Stearic Acid (purchased from Laboratory Rasayan), Citric Acid (purchased from Fisher scientific), Benzyl Alcohol, Sodium Alginate, Gaur gum Chicken feathers, Detergent, Coconut oil.

Method

3.1 Collection of Chicken Feathers

Chicken Feathers were collected from nearby Slaughter house located in Shimla.

Location: Modern Slaughter house, M.C. Abattoir Shimla

(Krishna Nagar, Lalpani Shimla; Himachal Pradesh**)**

Fig 3.1 A: Waste Chicken Feathers in Slaughter house

3.2 Pretreatment of Feathers:

Soaked feathers in detergent for overnight to remove all the impurities and then in petroleum ether for 2-3 hr [11]. Sundried the feathers and stored in plastic bags.

Fig 3.2 (A): Feathers soaked in detergent; **(B):** Feathers soaked in petroleum ether; **(C):** Drying of feathers in sunlight; **(D):** Stored clean feathers in plastic bags

3.3 Extraction of keratin

Dissolved the pretreated feathers in 0.2M of NaOH solution and kept for overnight incubation at 50^0C and continuous shaking at 150 rpm [35]. Filtered the solution to remove insoluble residues. Using 2N HCl the neutralization of filtered solution was carried out in fume hood [36]. The precipitates of keratin were filtered, lyophilized and stored for future use.

3.4 Dialysis of Keratin solution

Filtered Keratin solution was transferred into dialysis membrane. The solution was dialyzed against the distilled water (5L). Replaced distilled water after every 12 hours [37]. The dialyzed solution was collected after 48 hours and stored in a container.

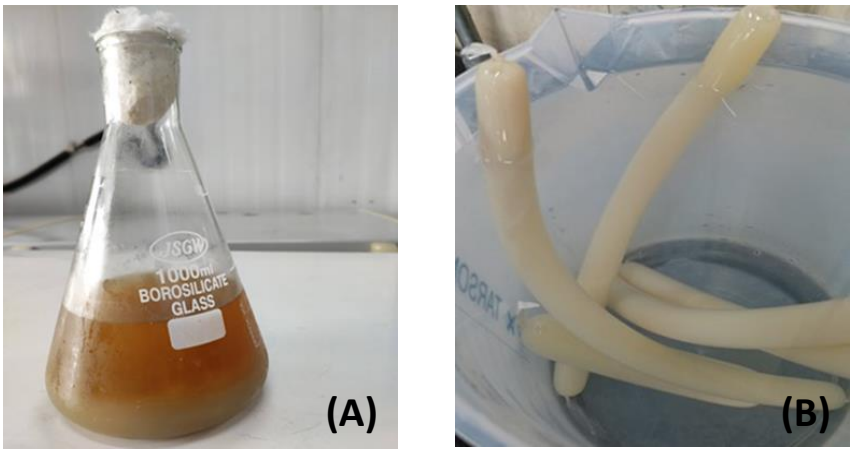

Fig 3.4 (A): Filtered solution of dissolved feathers in NaOH Solution, **(B):** Filtered solution transferred to dialysis membrane

3.5 Aloe vera Extract

Washed the aloe vera plant, separated the pulp and grinded it to get a uniform mixture. Kept the grinded mixture on hot plat at 40^0C for 10 min and let it cool. A mixture of glycerol and xanthan gum was added to the aloe vera extract, transferred the extract to glass jar and stored for future use.

3.6 Formulation of Keratin Shampoo

Formulated the shampoo using the ingredients mentioned in Table.1. The components were mixed together one by one. Mixture of gaur gum and glycerol was slowly added to all the components by constant stirring to get the viscous formulation.

Table 2: Composition of Keratin Shampoo for sample 1, 2 and 3

Material Used	S-1	S-2	S-3
Dialyzed Keratin (ml)	10	30	40
Sodium Lauryl Sulphate (gm)	0.5	0.5	0.5
Gaur gum (gm)	1	0.5	0.7
Glycerol (ml)	10	10	10
Aloe vera Extract (ml)	10	10	10
Propylene Glycol (ml)	1	1	1
Citric Acid (3%)	qs	qs	qs
Distilled Water	qs	qs	qs

3.7 Physico-chemical Characterization of formulated Shampoo

Physical Appearance: The test of physical appearance is done by visual inspection. Color, and appearance of the formulated shampoo was observed.

pH Determination: pH is determined on a scale of 10. To determine the pH 1% solution of formulated shampoo was prepared and evaluated by using a digital pH meter. Most of the shampoos has a pH of slightly acidic or neutral.

Dirt Dispersion: A drop of ink was added to 1% solution of formulated shampoo in a test tube. Covered the test tube and shake for 2 to 3 minutes. The amount of ink present in the water showed that the formulated shampoo can remove dirt.

Foam Stability and Foam Formation: 1% solution of shampoo was prepared and shaken for 1 min. The volume of foam was measured and recorded and then the stability of foam was observed for 1 minute.

Phase Separation: To check the phase separation the formulated shampoo was centrifuged for 10 min. at 8000 rpm.

Spreadability: The spreadability of formulated product is calculated by measuring the diameter. 1ml of the product is poured on a glass plate using a pipette and leave it for 5 minutes. The diameter is measured after 5 minutes using a scale.

Stability: The formulated product is stored at different temperature that is at -4^0C, room temperature and 40^0C and parameters such as color, appearance, pH and foam formation was observed for 30 days from the day of formulation.

Viscosity: The viscosity of formulated shampoo was measured by viscometer using spindle 63 at different rpm. The change in the value of viscosity was recorded as the speed increases.

3.8 Formulation of Keratin Cream

The formulation of Keratin cream was done using all the ingredients mentioned in Table.2. Three different phases water phase, oil phase and cool down phase was prepared separately and mixed together by constant stirring. After mixing all the components the homogenization is done by constant stirring at constant speed for 20 and 40 minutes.

Table 3: Composition of Keratin Cream for formulations 1 and 2

INGREDIENT	F-1	F-2
WATER PHASE		
Glycerol (ml)	4	4
Xanthan Gum (gm)	0.2	0.2
Sodium Alginate (gm)	0.2	0.2
Propylene Glycol (ml)	4	4
Distilled Water	qs	qs
OIL PHASE		
Stearic Acid (gm)	5	5
Cetyl Alcohol (gm)	4	4
Coconut Oil (ml)	5	5
Polysorbate 80 (ml)	3	3
COOL DOWN PHASE		
Dialyzed Keratin (ml)	30	60
Benzyl Alcohol (ml)	1	1
Aloe vera Extract (ml)	5	5
Citric Acid (3%)	qs	qs

3.9 Physico-chemical Characterization of formulated Cream

Physical Appearance: The test of physical appearance is done by visual inspection. Color, appearance and texture of the formulated products are observed.

pH Determination: pH is determined on a scale of 10. To determine the pH 1% solution of formulated cream was prepared and evaluated by using a digital pH meter.

Phase Separation: The phase separation is checked by centrifugation. The formulated cream was for 5 minutes at 8000 rpm.

Stability: The formulated product is stored at different temperature that is at -4^0C, room temperature and 40^0C and parameters such as color, texture, pH and homogeneity was observed for 30 days from the day of formulation.

Viscosity: The viscosity is measured by viscometer using spindle 63 at different rpm. The change in the value of viscosity was recorded as the speed increases.

Chapter 4 Results and Discussions

4.1 Extraction of Keratin Powder

The extracted Keratin pellet was kept in a Lyophilizer for almost 24 hours. The Keratin pellet was crushed using mortar and pestle.

205 grams of Keratin powder were obtained and stored for future use.

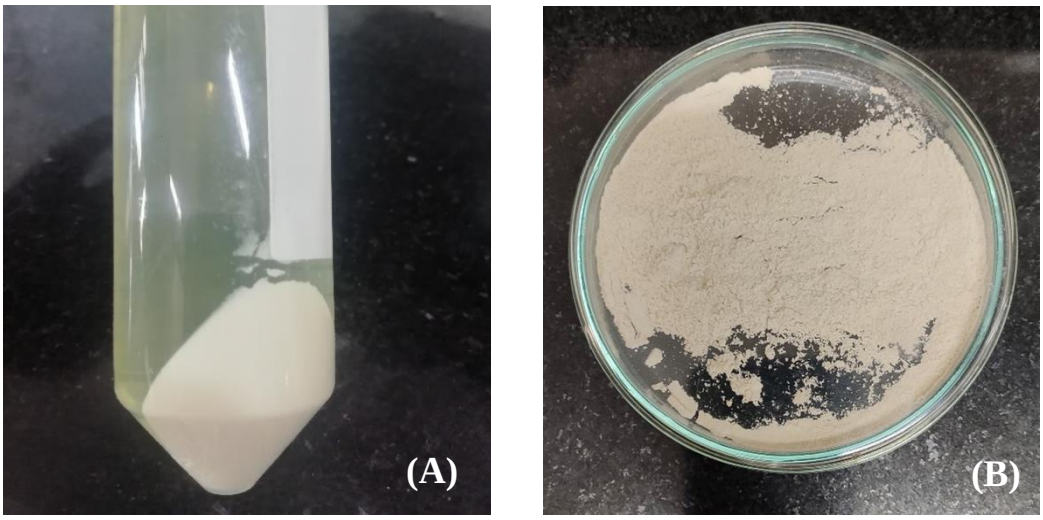

Fig 4.1 (A): Keratin pellet after 24 hours of lyophilization, **(B):** White Keratin Powder

4.2 Total Keratin Yield

The total percentage of Keratin yield and Keratin loss was calculated from 300 grams of Chicken Feathers.

205 grams of Keratin Powder was obtained from 300 grams of chicken feathers.

$$\textbf{Total Yield (\%)} = \frac{\text{weight of keratin X 100}}{\text{Initial weight of the biomass}}$$

$$\textbf{Total Yield (\%)} = \frac{\textbf{205 X 100}}{\textbf{300}} = \textbf{68.3 \%}$$

$$\textbf{Total Keratin loss (\%)} = \frac{\text{(Initial weight of the biomass –weight of keratin) X 100}}{\text{Initial weight of the biomass}}$$

$$\textbf{Total Keratin loss (\%)} = \frac{\textbf{(300 - 205) X 100}}{\textbf{300}} = \textbf{31.6\%}$$

Schrooyen et al. [38] in a study reported that the total yield of keratin extracted using waste chicken feathers was 75% Poole et al. [39] reported the total keratin yield of 62 % using sodium sulphite.

After all the calculation the total Keratin Yield was 68.3% and 31.6% was the total Yield of Keratin loss from 300 gram of feathers by using 0.2M NaOH solution for the dissolution of chicken feathers.

4.3 Shampoo Formulation

The 3 different samples were prepared using different concentrations of Keratin and Gaur gum. The composition is given in Table.2.

Fig 4.3 (A): 3 samples of Shampoo formulation

Physical Observations: Different parameters were observed of 3 different formulations (S-1; S-2; S-3) from the day of formulation for 30 days. Table.4 represents the observations on the same day of formulation such as appearance, color, pH, foam production and stability. Whereas; Table.5 represents 30 days of observations for appearance, phase separation and Spreadability at room temperature. The results are recorded based on visual inspection. It was recorded in Table.4 and 5 that the formulated shampoo is opaque and has an appearance of gel [40].

pH Determination: The pH is determined on a scale of 10 using a digital pH meter. According to a study majority of formulated shampoos have a pH range of 5 to 7 [41]. The pH value of formulated samples was observed at room temperature on the day of formulation and was noted in Table4. The values of pH of 3 samples were 6.4, 6.9, 6.7 on the day of formulation [42].

Dirt Dispersion: This test is a very important parameter to evaluate the cleaning action of shampoo. Shampoos that concentrate the ink in foam are considered to have a poor action of cleaning. [44] The results showed that it can remove dirt as the proportion of ink was observed in water and not in foam after shaking the mixture of water, ink and shampoo.

Table 4: Different parameters observed on the day of formulation.

Parameters	Observations		
	S-1	**S-2**	**S-3**
Color	Opaque	Opaque	Opaque
Appearance	Gel	Gel	Gel
pH	6.4	6.9	6.7
Foam Production ability	Good	Good	Good
Foam stability	Good	Good	Good
Dirt Dispersion test	Positive	Positive	Positive
Phase separation	No	No	No

Foam Stability and Foam Formation: The formulated product showed foam formation as SLS was added as a surfactant. The foam was also stable as observed for 1min after vigorously shaking the 1% prepared solution of distilled water and shampoo [43]. All the observations were recorded in Table.4. and Table.5.

Phase Separation: After centrifugation there was no phase separation in all the different formulations. The observations of phase separation on the day of formulation were recorded in Table.4 and for 1 month it was recorded in Table.5.

Spreadability: The diameter of 1ml of shampoo was noted for 30 days and recorded in Table.5. The results showed that all three formulated samples had different Spreadability due to different concentration of Gaur gum. The Spreadability of sample 1, 2 and 3 was in range 2.2 to 2.5 cm; 3.3 to 3.7cm and 3.0 to 3.5 cm.

Table 5: Parameters observed for 30 days from the day of formulation.

	S-1 (10 ml Keratin)			S-2 (20 ml Keratin)			S-3 (30 ml Keratin)		
No. of Days	**Appearance**	**Phase Separation (Centrifugation)**	**Spreadability (Diameter in cm)**	**Appearance**	**Phase Separation (Centrifugation)**	**Spreadability (Diameter in cm)**	**Appearance**	**Phase Separation (Centrifugation)**	**Spreadability (Diameter in cm)**
0	Gel	No	2.3	Gel	No	3.5	Gel	No	3.2
2	-	-	-	Gel	No	3.4	Gel	No	3.5
4	Gel	No	2.4	Gel	No	3.6	Gel	No	3.4
6	Gel	No	2.5	-	-	-	Gel	No	3.0
8	Gel	No	2.3	Gel	No	3.5	Gel	No	3.0
10	Gel	No	2.4	Gel	No	3.4	-	-	-
12	Gel	No	2.3	Gel	No	3.7	Gel	No	3.4
14	Gel	No	2.5	Gel	No	3.5	Gel	No	3.2
16	-	-	-	Gel	No	3.3	Gel	No	3.3
18	Gel	No	2.2	Gel	No	3.6	Gel	No	3.0
20	Gel	No	2.4	-	-	-	Gel	No	3.1
22	Gel	No	2.3	Gel	No	3.7	Gel	No	3.3
24	Gel	No	2.5	Gel	No	3.5	-	-	-
26	Gel	No	2.3	Gel	No	3.6	Gel	No	3.5
28	Gel	No	2.2	-	-	-	Gel	No	3.0
30	Gel	No	3.1	Gel	No	3.0	Gel	No	3.2

Stability Test: The prepared formulations (S-1; S-2; S-3) were stored at different temperature and different parameters as color, appearance of shampoo, pH and foam formulation were observed for 30 days from the day 1 of formulation. The observations were recorded in Table.6. After 30 days of observation all the formulated samples showed no change and were stable at -4^0C, room temperature and 40^0C.

Table 6: Observations of stability test of formulated shampoo.

NO. OF DAYS	PARAMETERS	-4^0C (S-1; S-2; S-3)	ROOM TEMP. (S-1; S-2; S-3)	40^0C (S-1; S-2; S-3)
1	**Color**	Unchanged	Unchanged	Unchanged
	Appearance	Gel	Gel	Gel
	Foam Formation	Observed	Observed	Observed
2	**Color**	Unchanged	Unchanged	Unchanged
	Appearance	Gel	Gel	Gel
	Foam Formation	Observed	Observed	Observed
6	**Color**	Unchanged	Unchanged	Unchanged
	Appearance	Gel	Gel	Gel
	Foam Formation	Observed	Observed	Observed
10	**Color**	Unchanged	Unchanged	Unchanged
	Appearance	Gel	Gel	Gel
	Foam Formation	Observed	Observed	Observed
16	**Color**	Unchanged	Unchanged	Unchanged
	Appearance	Gel	Gel	Gel
	Foam Formation	Observed	Observed	Observed
20	**Color**	Unchanged	Unchanged	Unchanged
	Appearance	Gel	Gel	Gel
	Foam Formation	Observed	Observed	Observed
25	**Color**	Unchanged	Unchanged	Unchanged
	Appearance	Gel	Gel	Gel
	Foam Formation	Observed	Observed	Observed

Viscosity: The viscosity of formulated shampoo was measured by viscometer using spindle 63 at different rpm. Table.7 represents the viscosity value of formulation 1, 2, and 3. The viscosity of product is an important key factor in controlling and defining different attributes as shelf life, flow rate, clarity and the spreading ability.

The observations of viscosity are mentioned in Table.7. The value of viscosity should decrease with the increase in spindles speed (rpm) therefore, it will confirm that the formulations of shampoo are pseudo-plastic in nature and this pseudo-plastic behavior is a necessary characteristic in all the shampoos [41].

Figure 4.3 (B) represents a graphical representation of viscosity which showed that the nature of all the formulations were non-Newtonian.

Table 7: Viscosity of different formulations of shampoo at different rpm.

SPINDLE NO.	SPEED (rpm)	VISCOSITY (cP)		
		S-1	S-2	S-3
63	1.0	1800	-	3820
63	2.0	1500	420	3000
63	4.0	1440	720	2480
63	6.0	1360	720	2100
63	10	1188	636	1600

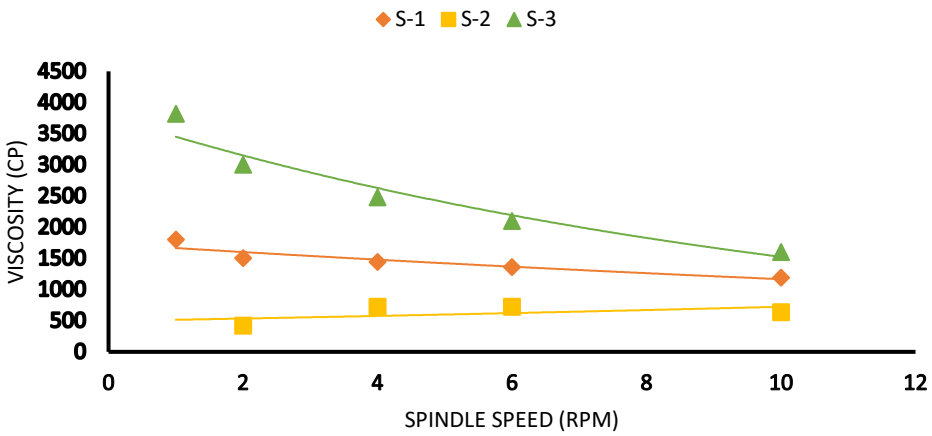

Fig 4.3 (B): Graphical representation of viscosity

4.4 Cream Formulation

The 2 different samples of Cream were formulated using different concentrations of Keratin and all the other parameters were kept constant. The composition for the same is given in Table.3.

Fig 4.4 (A): 2 samples of Cream formulation

Physical observations: Different parameters were observed of 2 formulations (F-1, F-2) from the day of formulation for 30 days. Table.7 represents the observations on the same day of formulation such as color, appearance, pH, texture and phase separation. According to the observations the formulated cream was white in color and has a creamy texture [45].

pH Determination: The pH value of formulated samples was observed at room temperature on the day of formulation and was noted in Table8. The values of pH of 2 formulated samples were 5.6 and 5.9 on the day of formulation. The reading of pH denoted that the formulated cream is slightly acidic in nature [46].

Phase Separation: The phase separation is checked by centrifugation. The formulated cream was centrifuged at 8000 rpm for 5 minutes.

Table 8: Parameters observed on the day of formulation.

Parameters	Observations	
	F-1	**F-2**
Color	White	White
Appearance	Semi solid	Semi solid
Texture	Creamy	Creamy
Smell	Distinct Smell	Distinct Smell
Homogeneity	Homogenous	Homogenous
pH	5.6	5.9
Phase Separation	No	No

Stability Test: The prepared formulations (F-1; F-2) were stored at different temperature and different parameters as color, texture and homogeneity of cream were observed for 30 days from the day 1 of formulation. After 30 days of observation the formulated cream samples was stable at -4^0C, room temperature and 40^0C as there was no change in observations [47].

Table 9: Observations of Stability test of formulated cream

NO. OF DAYS	PARAMETERS	-4^0C (F-1; F-2)	ROOM TEMP. (F-1; F-2)	40^0C (F-1; F-2)
Day 1	**Color**	Unchanged	Unchanged	Unchanged
	Texture	Creamy	Creamy	Creamy
	Homogeneity	Homogenous	Homogenous	Homogenous
Day 4	**Color**	Unchanged	Unchanged	Unchanged
	Texture	Creamy	Creamy	Creamy
	Homogeneity	Homogenous	Homogenous	Homogenous
Day 8	**Color**	Unchanged	Unchanged	Unchanged
	Texture	Creamy	Creamy	Creamy
	Homogeneity	Homogenous	Homogenous	Homogenous
Day 12	**Color**	Unchanged	Unchanged	Unchanged
	Texture	Creamy	Creamy	Creamy
	Homogeneity	Homogenous	Homogenous	Homogenous
Day 16	**Color**	Unchanged	Unchanged	Unchanged
	Texture	Creamy	Creamy	Creamy
	Homogeneity	Homogenous	Homogenous	Homogenous
Day 20	**Color**	Unchanged	Unchanged	Unchanged
	Texture	Creamy	Creamy	Creamy
	Homogeneity	Homogenous	Homogenous	Homogenous

Viscosity: The viscosity of cream samples were measured by viscometer using spindle 63 at different rpm. Table.10 represents the viscosity value of formulation 1 and 2.

As the speed of spindle increases the value of viscosity decreases. The observation of viscosity was mentioned in Table.9. Therefore; the formulation of shampoo is pseudo-plastic in nature and this pseudo-plastic behavior is a necessary characteristic in all creams [41]. Figure B shows a graphical representation of viscosity.

Figure 4.4 (B) represents a graphical representation of viscosity which showed that the nature of all the formulations were non-Newtonian.

Table 10: Viscosity of different formulations of cream at different rpm.

SPINDLE NO.	SPEED (rpm)	VISCOSITY (cP)	
		F-1	F-2
63	1.0	40200	62880
63	2.0	26580	49060
63	3.0	20400	38440
63	4.0	15630	23340
63	5.0	13180	19900
63	6.0	11880	15880
63	10	8808	10200

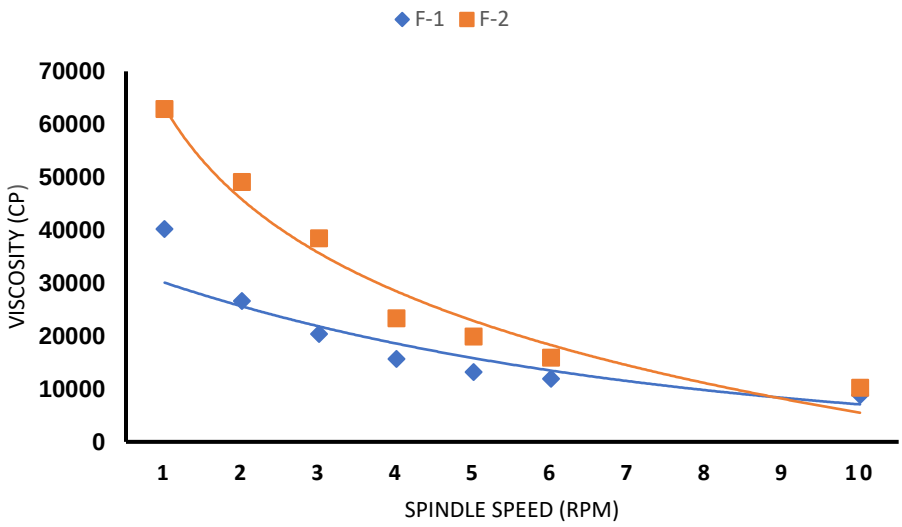

Fig: 4.4 (B): Graphical representation of viscosity

Chapter 5 Conclusion

Keratin is a very important fibrous and structural protein which is found in humans and as well as in animals. There are different sources of keratin such as in animals it is found in hooves, wool and in feathers of chickens and birds whereas in humans it is found in nails, hair and also in intestine. The structure of keratin is very stable and has cysteine, strong disulfide bonds, hydrogen bonds and hydrophilic interactions due to which it is insoluble in many organic solvents including water. According to its structure keratin is divided into two forms which are alpha and beta keratin. The main difference between the two forms of keratin is that the alpha keratin is present in form of helical structure whereas the beta keratin is present in the form of sheets (beta sheets). There are different methods which could be used for the extraction of keratin using animal waste. From past many years, several techniques had been used for the method of keratin extraction such as reduction, oxidation, chemical hydrolysis, microbial and enzymatic method, process of steam explosion and thermal hydrolysis. This protein has several applications in biomedical, pharmaceuticals, cosmetic industries and also in the field of agriculture. Different valuable products such as organic fertilizers, biosorbents, bioplastics and cosmetic products can be formed using keratin. Animal waste such as chicken feathers and sheep wool are the major source of keratin and around 70% off keratin yield can be extracted by using sheep wool and chicken feather waste. The residual chicken feather waste is a current concern that is affecting the environment globally. This waste when dumped in the ETP plant at slaughter houses used harmful chemicals like caustic, alum, Urea and DAP. This is affecting the ground water. In this study, waste chicken feathers were used for the extraction of keratin using alkaline hydrolysis method to formulate different valuable cosmetic products such as cream and shampoo. The waste chicken feathers had been collected from a nearby slaughterhouse in Shimla. The collected chicken feathers were pretreated using distilled water and petroleum ether so that all the unwanted residues can be removed and clean feathers can be used for the extraction purpose. The alkaline hydrolysis is a cheap and time-consuming method for the extraction of keratin. The clean pretreated waste chicken feathers were dissolved in 0.2 molar NaOH solution for overnight incubation at 50^0C and 150 rpm. 2N HCl solution was prepared for the precipitation of keratin then the precipitates of keratin were filtered, lyophilized and stored for further use. White keratin powder was obtained using the method of alkaline hydrolysis. The total keratin yield was calculated after the extraction

and it was observed that 68.3% was the total yield and 31.6% was keratin loss. For the formulation of hair products such as hair cream and shampoo the dialysate of keratin and prepared aloe vera extract was used. The formulation of keratin-based cream and shampoo was done using different concentrations of dialysate. 3 different samples of shampoo and 2 different samples of cream was formulated in this study. After formulation the physical and chemical characterization was done on the basis of visual inspection. Different parameters of formulated cream and shampoo were observed such as color, appearance and pH. The samples of formulated shampoo were opaque and showed gel like appearance. The pH value of 3 samples were 6.4, 6.9 and 6.7 which is a correct range of pH as shampoo should have a pH value between 6 to 7. To check the stability formulated samples were stored at -4^0 C room temp, and 40^0 C and for 25 days its color, appearance and foaming ability was observed. The results of stability test showed that the shampoo was stable at different temperature and showed no change in color and appearance. Same parameters were observed for the 2 samples of formulated cream using different concentration of dialysate. On the day of formulation, it was observed that the cream was white in color and has a semi solid appearance and creamy texture. The pH of cream was 5.6 and 5.9 which was acidic in nature. The formulated cream was also stable when stored at different temperature of -4^0 C room temp, and 40^0 C for 20 days. No change in color, texture and homogeneity was observed in the formulated samples of cream. The viscosity of cream and shampoo was measured by a viscometer at different rpm using spindle no. 63 at room temp. The graph was plotted using all the values of viscosity for the different samples of cream and shampoo. The graph represented that the formulated samples were pseudo-plastic in nature as the value of viscosity decreases with increase in speed (rpm) of spindle and this pseudo-plastic behavior is a necessary characteristic in shampoos and cream. All the results were positive for different formulations of cream and shampoo.

REFERENCES

[1] S. Sharma and A. Kumar, Eds., *Keratin as a protein biopolymer: Extraction from waste biomass and applications*, 1st ed. Cham, Switzerland: Springer Nature, 2018.

[2] R. Fraser, T. Macrae, D. Parry, and E. Suzuki, "The structure of feather keratin," *Polymer (Guildf.)*, vol. 12, no. 1, pp. 35–56, 1971.

[3] S. Sharma and A. Gupta, "Sustainable management of keratin waste biomass: Applications and future perspectives," *Braz. Arch. Biol. Technol.*, vol. 59, no. 0, 2016.

[4] J. C. Joardar and M. M. Rahman, "Poultry feather waste management and effects on plant growth," *Int. J. Recycl. Org. Waste Agric.*, vol. 7, no. 3, pp. 183–188, 2018.

[5] A. L. V. Villa *et al.*, "Feather keratin hydrolysates obtained from microbial keratinases: effect on hair fiber," *BMC Biotechnol.*, vol. 13, no. 1, p. 15, 2013.

[6] E. G. Bendit, "Infrared absorption spectrum of keratin. I. Spectra of ?-, ?-, and supercontracted keratin," *Biopolymers*, vol. 4, no. 5, pp. 539–559, 1966.

[7] J. McKittrick, P.-Y. Chen, S. G. Bodde, W. Yang, E. E. Novitskaya, and M. A. Meyers, "The structure, functions, and mechanical properties of keratin," *JOM (1989)*, vol. 64, no. 4, pp. 449–468, 2012.

[8] C. R. Chilakamarry *et al.*, "Extraction and application of keratin from natural resources: a review," *3 Biotech*, vol. 11, no. 5, 2021.

[9] H. Admassu, "Chicken feathers based keratin extraction process data analysis using response surface - box-Behnken design method and characterization of keratin product," 2020.

[10] Extraction and Characterization of Natural Protein (Keratin) From Waste Chicken Feather.

[11] I. Sinkiewicz, A. Śliwińska, H. Staroszczyk, and I. Kołodziejska, "Alternative methods of preparation of soluble keratin from chicken feathers," Waste Biomass Valorization, vol. 8, no. 4, pp. 1043–1048, 2017.

[12] E. M. Brown, K. Pandya, M. M. Taylor, and C.-K. Liu, "Comparison of methods for extraction of keratin from waste wool," Agric. Sci., vol. 07, no. 10, pp. 670–679, 2016.

[13] S. Feroz, N. Muhammad, J. Ratnayake, and G. Dias, "Keratin - Based materials for biomedical applications," Bioact. Mater., vol. 5, no. 3, pp. 496–509, 2020.

[14] M. Zoccola et al., "Microwave-assisted chemical-free hydrolysis of wool keratin," Text. Res. J., vol. 82, no. 19, pp. 2006–2018, 2012.

[15] F. Bertini, M. Canetti, A. Patrucco, and M. Zoccola, “Wool keratin-polypropylene composites: Properties and thermal degradation,” Polym. Degrad. Stab., vol. 98, no. 5, pp. 980–987, 2013.

[16] S. Feroz, N. Muhammad, J. Ranayake, and G. Dias, “Keratin - Based materials for biomedical applications,” Bioact. Mater., vol. 5, no. 3, pp. 496–509, 2020.

[17] A. Miyamoto, K. Kobayashi, M. Inomata, and Y. Murakami, “Nitrogen-15 tracer investigation of the mechanism of the reaction of nitric oxide with ammonia on vanadium oxide catalysts,” J. Phys. Chem., vol. 86, no. 15, pp. 2945–2950, 1982.

[18] W. Xu, W. Guo, and W. Li, “Thermal analysis of ultrafine wool powder,” J. Appl. Polym. Sci., vol. 87, no. 14, pp. 2372–2376, 2003.

[19] Y. Zhang, R. J. Alsop, A. Soomro, F.-C. Yang, and M. C. Rheinstädter, “Effect of shampoo, conditioner and permanent waving on the molecular structure of human hair,” PeerJ, vol. 3, no. e1296, p. e1296, 2015.

[20] S. Yin, F. Ding, and N. V. Dokholyan, “Eris: an automated estimator of protein stability,” Nat. Methods, vol. 4, no. 6, pp. 466–467, 2007.

[21] K. Tasaki, “A novel thermal hydrolysis process for extraction of keratin from hog hair for commercial applications,” Waste Manag., vol. 104, pp. 33–41, 2020.

[22] R. K. Donato and A. Mija, “Keratin associations with synthetic, biosynthetic and natural polymers: An extensive review,” Polymers (Basel), vol. 12, no. 1, p. 32, 2019.

[23] J. G. Rouse and M. E. Van Dyke, “A review of keratin-based biomaterials for biomedical applications,” Materials (Basel), vol. 3, no. 2, pp. 999–1014, 2010.

[24] F.-M. Chen and X. Liu, “Advancing biomaterials of human origin for tissue engineering,” Prog. Polym. Sci., vol. 53, pp. 86–168, 2016.

[25] M. S. Zafar et al., “Biomimetic aspects of restorative dentistry biomaterials,” Biomimetics (Basel), vol. 5, no. 3, p. 34, 2020.

[26] S. Saha, M. Zubair, M. A. Khosa, S. Song, and A. Ullah, “Keratin and chitosan biosorbents for wastewater treatment: A review,” J. Polym. Environ., vol. 27, no. 7, pp. 1389–1403, 2019.

[27] Q. Li, “Perspectives on converting keratin-containing wastes into biofertilizers for sustainable agriculture,” Front. Microbiol., vol. 13, 2022.

[28] S. Maitra *et al.*, "Bioinoculants-natural biological resources for sustainable plant production," *Microorganisms*, vol. 10, no. 1, p. 51, 2021.

[29] B. Mitter, G. Brader, N. Pfaffenbichler, and A. Sessitsch, "Next generation microbiome applications for crop production - limitations and the need of knowledge-based solutions," *Curr. Opin. Microbiol.*, vol. 49, pp. 59–65, 2019.

[30] P. Mokrejš, M. Huťťa, J. Pavlačková, and P. Egner, "Preparation of keratin hydrolysate from chicken feathers and its application in cosmetics," J. Vis. Exp., no. 129, 2017.

[31] C. Barba, S. Méndez, A. Roddick-Lanzilotta, R. Kelly, J. L. Parra, and L. Coderch, "Cosmetic effectiveness of topically applied hydrolysed keratin peptides and lipids derived from wool," Skin Res. Technol., vol. 14, no. 2, pp. 243–248, 2008.

[32] S. Murdan, "Drug delivery to the nail following topical application," Int. J. Pharm., vol. 236, no. 1–2, pp. 1–26, 2002.

[33] A. Tinoco, M. Martins, A. Cavaco-Paulo, and A. Ribeiro, "Biotechnology of functional proteins and peptides for hair cosmetic formulations," Trends Biotechnol., vol. 40, no. 5, pp. 591–605, 2022.

[34] A. L. V. Villa et al., "Feather keratin hydrolysates obtained from microbial keratinases: effect on hair fiber," BMC Biotechnol., vol. 13, no. 1, 2013.

[35] P. Mokrejs, P. Svoboda, J. Hrncirik, D. Janacova, and V. Vasek, "Processing poultry feathers into keratin hydrolysate through alkaline-enzymatic hydrolysis," *Waste Manag. Res.*, vol. 29, no. 3, pp. 260–267, 2011.

[36] M. Dąbrowska, A. Sommer, I. Sinkiewicz, A. Taraszkiewicz, and H. Staroszczyk, "An optimal designed experiment for the alkaline hydrolysis of feather keratin," *Environ. Sci. Pollut. Res. Int.*, vol. 29, no. 16, pp. 24145–24154, 2022.

[37] P. M. M. Schrooyen, P. J. Dijkstra, R. C. Oberthür, A. Bantjes, and J. Feijen, "Stabilization of solutions of feather keratins by sodium dodecyl sulfate," *J. Colloid Interface Sci.*, vol. 240, no. 1, pp. 30–39, 2001.

[38] P. M. Schrooyen, P. J. Dijkstra, R. C. Oberthür, A. Bantjes, and J. Feijen, "Partially carboxymethylated feather keratins. 1. Properties in aqueous systems," *J. Agric. Food Chem.*, vol. 48, no. 9, pp. 4326–4334, 2000.

[39] A. J. Poole, R. E. Lyons, and J. S. Church, "Dissolving feather keratin using sodium sulfide for bio-polymer applications," *J Polym Environ*, vol. 19, no. 4, pp. 995–1004, 2011.

[40] M. Azadbakht, T. Monadi, Z. Esmaeili, A. Chabra, and N. Tavakoli, “Formulation and evaluation of licorice shampoo in comparison with commercial shampoo,” *J. Pharm. Bioallied Sci.*, vol. 10, no. 4, pp. 208–215, 2018.

[41] A. R. Mainkar and C. I. Jolly, “Evaluation of commercial herbal shampoos,” *Int. J. Cosmet. Sci.*, vol. 22, no. 5, pp. 385–391, 2000.

[42] J. Tarun, J. Susan, J. Suria, V. J. Susan, and S. Criton, “Evaluation of pH of bathing soaps and shampoos for skin and hair care,” *Indian J. Dermatol.*, vol. 59, no. 5, pp. 442–444, 2014.

[43] K. Al Badi and S. A. Khan, “Formulation, evaluation and comparison of the herbal shampoo with the commercial shampoos,” *Beni-Suef Univ. J. Basic Appl. Sci.*, vol. 3, no. 4, pp. 301–305, 2014.

[44] Ali Heyam Saad and Rasool Bazigha Kadhim and Bazigha Kadhim Abdul Rasool, “Formulation and evaluation of herbal shampoo from Ziziphus spina leaves extract,” 2011.

[45] S. R. L. Avish D. Maru1, formulation and evaluation of moisturizing cream containing sunflower wax. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2018.

[46] P. L. Tan *et al.*, “Formulation and physicochemical evaluation of green cosmeceutical herbal face cream containing standardized mangosteen peel extract,” *Cosmetics*, vol. 9, no. 3, p. 46, 2022.

[47] Abhay Prakash Mishra, Sarla Saklani, Luigi Milella, Priyanka Tiwari, Formulation and evaluation of herbal antioxidant face cream of Nardostachys jatamansi collected from Indian Himalayan region. *Asian Pacific Journal of Tropical Biomedicine*, 2014.