“Extraction of keratin from waste biomass for novel biomaterial synthesis”

A PROJECT

Submitted in partial fulfillment of the requirement for the award of the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

Under the supervision of

Dr. Ashok Kumar

By

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To

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CERTIFICATE

This is to certify that the work entitled **"Extraction of keratin from waste biomass for novel biomaterial synthesis"** pursued by Tania Sharma (151830) and Pratibha Pandey (151801) in a partial fulfillment for the award of degree Bachelor of Technology in Biotechnology from Jaypee University of Information Technology, Waknaghat has been carried out underneath my supervision. This a part of work has now not been submitted in part or wholly to some other University or Institute for the award of any degree or appreciation.

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The scrutinize opportunity we had become a remarkable chance for learning and professional improvement. We are grateful for having a favorable opportunity to meet and introduce our research to professionals who led us through this venture. We have had such a lot of rich experiences and participation that we believe it will forever shape and influence our professional lifestyles whilst fostering personal increase and development.

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Sincerely,

Tania Sharma (151830)

Pratibha Pandey (151801)

Date:

DECLARATION

We hereby confirm that the work proclaimed in the B-Tech thesis entitled **"Extraction of keratin from waste biomass for novel biomaterial synthesis"** submitted at Jaypee University of Information Technology, Waknaghat, Solan is credible record of our work carried out under the supervision of **Dr. Ashok Kumar**. The results embodied in this thesis have not been submitted to any other university or institute for the award of any degree or diploma.

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LIST OF ABBREVIATIONS

Symbol	Abbreviation
°C	Degree Celsius
%	Percentage
pH	Power of Hydrogen
g	Gram
v/v	Volume/Volume
w/v	Weight/Volume
ml	Milliliter
h	Hour
min	Minute
dH_2O	Distilled Water
rpm	Rotation per minute
NaOH	Sodium Hydroxide
HCl	Hydrochloric acid
SEM	Scanning Electron Microscopy
XRD	X-Ray Diffraction

ABSTRACT

Keratin is a structural protein which is highly stable and a biodegradable biopolymer. Hair composes of roughly 65–95% protein. Keratin protein is organized into filaments of hair cells forming a polypeptide chain. Due to the potential of keratin derived biomaterials being intrinsically biocompatible, ecological and naturally abundant has various applications in the field of biomedical technology. In this study, human hairs were collected from a hairdressing salon in Waknaghat, Himachal Pradesh India. Hairs were thoroughly washed with detergent and dried using a ventilated oven at 40°C for 24 h. Further washing with ethanol (90 % v/v) to disinfect and sterilize hair followed by chopping (20-25 cm) using a scissor and stored in a sealable bag. Hair was subjected to dissolution in an alkaline solution of 8% NaOH. Dissolution of hairs was done at 37°C in an incubator for 6-8 h. The dissolved mixture was filtered and human hair hydrolysate was precipitated with HCl (concentrated) under shaking conditions. Then, dialysis was performed for 2 h to remove unwanted salts and chemicals. The dialyzed sediments were crushed using liquid N2 in mortar and pestle to procure keratin powder. The total yield of extracted keratin powder was 83.41%. Furthermore, the extracted keratin powder could be used for producing a novel bio-product as we know waste biomass is posing a great threat to the environment. Thus, this study provided an easy and efficient method to extract keratin from waste biomass that could raise up the pharmaceutical industry in conjunction with the cosmetic industry.

Keywords: Keratin, biomedical, disulfide bonds, waste biomass, industrial application

CHAPTER 1

INTRODUCTION

1. Introduction

Keratin, the call circle for relatives of structural proteins that are abundant within the outer layer of human skin, hair, and nails. It is a complicated moreover structurally stable protein commenced in tough human and animal tissues. Eminently rich in cysteine content which is an amino acid, and it owns the ability to self-bring together into cluster of fibers. Individual strands within the bundles of fibers further pass-linked through S-S (sulfur-sulfur) bonds concerning the facet chains of cysteine. This is how, keratin especially known to be tough, insoluble structures that are strongest non-mineralized tissues found in nature. Other simplest non-mineralized tissue that truly duplicates the sturdiness of keratin is "Chitin". These are the considerable proteins that exhibit a molecular weight of 50-100kDa. Keratin-based totally biomaterials have emerged as capability applicants for numerous biomedical and biotechnological programs because of their properties which might be intrinsic biocompatibility, biodegradability, mechanical sturdiness, and herbal abundance. Poultry industries generate a big quantity of feather waste. These feathers contain a high amount of keratin which can be bio-transformed into peptides and amino acids using microorganism to make cost delivered merchandise. Some of the waste biomass, like feathers and hair, represents one of the primary resources of waste rich in protein content with tremendous capability to be converted into fee surplus merchandise along with feed, formalizer or biofuel. This industry affords with big quantities of protein dietary supplements inside the human weight loss program, continually with the manufacturing of huge biomass that is desolate. The predominant fraction of biomass generated is in the form of feathers, blood, bone residues, hair, and meat. Feathers include approximately 90% keratin. Due to its restricted programs in industrial scale, it is been disposed of via incineration or land filling.

Feathers, being the foremost waste constituent of the chook industry, its utilization as feather biomass becomes greater important to defend the surroundings and to generate numerous precious merchandise. It is necessary to use waste biomass in an environment friendly along with greater sustainable way for the synthesis of merchandise in industrial use. These are without difficulty available, low cost, renewable, and biodegradable, reliable biopolymer feedstock which has comparable homes to polyamides.

Researchers are being done to look for new packages of feather substances, consisting of meals or compostable packaging, safe to eat movie, sponges, bio sorbents, and different composites.

Hair is a composition of proteins, lipids, water, and small traces of int constituents. All the proteins that are present in animals and humans are constructed from amino acid molecules diversifications in a polypeptide string. Protein keratin are prepared into polypeptide chains present in filaments of hair cells. Keratinase is a proteolytic enzyme that catalyzes the cleavage of keratin. Most of the keratin-containing substrates like feathers or animal hairs is either discarded or incinerated due to tough degradation procedure, resulting in higher consumption of energy and environmental pollutants. Microbial keratinase is affordable than keratinase produced conventionally and can be acquired from fungi, bacteria, and actinomycetes. `These have replaced the proteases within the leather-stationed enterprise and detergent enterprise because of their better overall performance.

Being one the difficult proteins to be digested or to be solubilized. The maximum commonplace dissolution techniques for hair are hydrolysis based on acidic, alkaline, and enzymatic. For analyzing hair, the samples which were stable, was transferred through solubilization through digestion directly into a liquid section. Small molecular solvents and hydrophobic group molecules exhibit to have a better affinity towards hair. A precise solvent directly attacks the disulfide bonds among cysteine molecules and moreover hydrates the shaft. Subsequently, the biomass of hair turns into a jelly-like mass, accompanied through drying in oven and dialysis. After drying, the use of liquid nitrogen the mass became crushed into powdered form. Thus, keratin becomes synthesized.

Hair constitutes of nearly 68-98% protein, 6% lipids, melanin, small quantities of polysaccharides, 2-4% water, and 0.25–0.8% hint elements. Proteins may be labeled in line with their shape/shapes into two sorts. Lengthy and stringy molecules having extended chains of polypeptide along one axis and resembles a globular shape is referred to as “Fibrous proteins”. These are foremost additives of each source of keratin and are predominantly natural constituents of connecting tissues such as bone, cartilage etc. When the chains of polypeptide are tightly folded into compact round or globular shapes, then they are referred to as “Globular Proteins”. Approximately each of the enzymes, antibodies, and proteins transporting blood are particularly

globular proteins. Hair, feathers, claws, horns, hooves mainly constitutes of α keratin. The amino acid that is most reactive is cysteine which is a residue in keratin. The explanation is given through the disulfide bonds presence which may be oxidized or reduced. Rearrangement of keratin structure takes placed due to reactions, which affects the physicochemical houses of hair. Keratin incorporates approximately 17% cysteine:

HOOC-CH(NH2)-CH2S-S-CH2CH(NH2)-COOH

There are three critical companies in cysteine:

1. Carboxyl corporations, acting in formation of ester or salt.
2. Amino businesses, which is a part of peptide linkages with the aid of hydrogen bonds with keratins.
3. S-S grouping that makes it easy to damages the gel-like precipitate in depilation.

The breakdown of cysteine breakdown is initiated via (-OH)alkali's, sulfides(S2-), thiol(-SH), or mixture of ions.

Keratin, known to be the critical component of feathers, hair, hooves, horns, nails etc. To be precise, there is no environmental influence and are affected at lower ratio physically or chemically (Teresa et al. 2011). Keratin is extracted with about amino ranging between90–100 (Kamarudi et al.2017). The synthetic shape of keratin as depicted α-helix and β-helix in addition to β-pleated (Lee et al. 1975). It has an excessive quantity of cysteine 8–16% of amino acids in contrast with others that helps in making intermolecular pass-links (Rouse and Van Dyke et al. 2010). The amount of cysteine residues relies upon at the keratin supply, which is varied from 7% \to 15% in feathers and wool keratin (Fraser et al. 1972; ARAI et al. 1983).

Keratin biomass is hydrolyzed with the aid of alkaline, acidic, enzymatic hydrolysis to extract keratin. Protein keratin extracted has diverse programs in different industries which includes beauty, biomedicine, pharmacy etc. Even more, as it doesn't cause any harmful effects and that is the reason why it is used in cosmetics such as crèmes, shampoo, hair products, etc. Their usage for treating skin along with human hair as predominates other applications. In retaining pores and moisture in skin, due to its existence in hair particularly in cuticle and stratum further as cosmetic interaction. In beauty merchandise its aggregates with natural polymers foe instance chitosan, collagen, fibroin acts as a core factor. Keratin exhibiting molecular weight which is higher are broadly used for skin health programs because of its versatility like film forming. Keratin protein derived from special assets had been advanced and applied as micro-scaffolds in

medication and cosmetics. Proteins are useful for keeping skin and hair healthier. Keratin derived from human hair is more biologically compatible. Degradation of waste biomass. Reduce pollution from waste biomass. Sustainable management of keratin-rich waste from the poultry industry or local market salon. An efficient method for the extraction of pure keratin of industry use. Applications of purified keratin for bioplastic synthesis, hair remedy product.

The major objectives of this study are

1. Keratin extraction from waste biomass (human hair)

2. Characterization of keratin extracted. (i.e SEM, FTIR, XRD, etc.)

3. Production of product from keratin for novel biosynthesis.

CHAPTER 2

REVIEW OF LITERATURE

2. Keratin and biomass management

The natural waste wealthy in keratin, is applied as source which is natural using strategies that are chemical or mechanical. Protein acquired with the aid of biomass. Now do not comprise of any toxic chemicals,that can be used for procuring a plethora of products. The monomeric units of keratin can penetrate within the pores and skin and hair cuticle and are capable to nourish the skin without any harsh outcomes. In the prevailing evaluated researches, various strategies for the purification and separation of keratin from the natural waste have been defined and use of natural keratin in cosmetics and pharmaceutical enterprise has additionally been explored.

2.1 Sources of Keratin

Keratin biomass is derived from livestock mentioned in the figure below. The plenteous sources of keratin are nails, hair, feather, beak, horns, hooves, scales, wool, etc. The amount of keratin in hair is about 80%, in feathers is about 90%, in wool is about 95% by weight. Keratin is biodegradable, naturally abundant, mechanically durable and biocompatible in nature.

Fig 2.1: Various sources of keratin

The rich sources of keratin include wool, hair, horn, hooves, feather etc. Extracted from animal horns and hooves, wool, feathers, and human hairs. The food industry produces million heaps of keratin biomass. Nearly 80% of hair is fashioned of simplest protein that is keratin. Wool and hair is an extremely good instance of the difficult-keratinous cloth. Wool is an exquisite animal fiber, containing eighty-two percent keratinous content. It is broadly used in the textile

utility and is scientifically important. Studies reveal that hair and wool both exhibits commonplace functions besides wool has a huge diameter. Hair acts as an essential function for phrases of protection in opposition to dirt and pathogens (hairs in the nose). The toughest of keratinous material is in hooves and is attractive for research. They have complicated shape, plus complexities permit the wall of hoof to soak up power because the crack increases. Nails are a critical supply of α-keratin.

For developing the strategies for extraction of keratin efficiently proves to be very beneficial for sustainable control of waste. Scientists are running to increase a lot of chemical, organic, and physical techniques in addition to mixed shape for keratin. The insolubility of protein has blessings inside the enterprises to increase merchandise of medicine use, in tissue engineering, in the agriculture industry. Recently there are many advances in the field of sustainable management of biomass i.e. desolate, strategies for extraction being utilized by various researchers.

Table 2.1: Applications of Keratin extracted from different sources

Sr. No.	Keratin Sources	Appliance	References
1.	Human Hair	Medicinal Use.	Zheng et al. (2005)
		Biomaterials.	Hanna Lee et al. (2014)
		Tissue Engineering.	S.Hana et al. (2015)
		Films as substrates.	Stephan Reichl et al. (2009)
		Drug permeation.	Lusiana et al. (2011)
2.	Horns and Hoofs		
3.	Feathers	Micro and Nanofibers.	Sun et al. (2009)
		Bio-plastic Film.	Ramakrishnan et al. (2018)
		Thermo-plastic films for food packaging.	Reddy et al. (2013), Jin et al. (2011)
		Bio-Fertilizers.	Adetunji et al. (2012)
		Bio-Composites.	Flores-Hernández et al. (2014), Spiridon et al. (2012)
		2D and 3D scaffolds.	Helan Xu et al. (2014)

		Diet Supplements.	Emma M. Crum et al. (2018)
		Waste Management.	Korniłłowicz-Kowalska et al. (2011)
		Leather and Textile Processing.	Helan Xu et al. (2014)
		Elimination of Polluting Chrome Shavings, Chrome, and Dye Exhaust Liquors of Tannery	G.Ramamurthy et al. (2015)
		Hydrogels.	Ju Wang et al. (2017)
		Cosmetics	Pavel Mokrejs et al. (2017)
4.	Wool	Medical science. (Nanosheets)	Xiangyu Xu (2018)
		Biopolymer.	K. Katoh et al. (2003)
		Anti-Pilling Processing.	Ji Ru Jia et al. (2015)
		Sponges and Porous Foams.	A. Tachibanta et al. (2002)
		Nano-fibers	Hui Ma et al. (2017)
		Hydrogel	Y. Ozaki et al. (2014)

2.2 Keratinase and its sources

Keratinase is an enzyme hydrolyzing keratin, which is an insoluble protein determined in fowl feathers, human hair, animal horns, and wool. It is a protease that is produced by using keratin-degrading species i.e. Microorganism, Actinomyces, and fungi. Its important goal is to assault at the disulfide (-S-S-) bond of the keratin substrate. The important building blocks, defining residences together with the diploma of tension and hardness of corneous tissues, are specialized structural proteins named keratins due to their shielding role, the shape of these proteins could be very recalcitrant and resistant to the degradation by full-size enzymes. Keratinases has a huge utility in biotechnology which helps in the degradation of keratin wastes via non-polluting strategies. Keratinases has a capacity to dispose of hair and feathers inside the hen enterprise and it helps to enhance the dietary price of feather meal within the feed enterprise.

The 3-dimensional shape of the keratin molecule became first defined in 1959, but the number of research expanded extensively after 1990. Keratins are proof against the degradation via conventional proteases and insoluble in diluted acids, alkaline reagents, water, and natural solvents. The amino acid series and composition of keratins have an effect on their folding, houses, and capabilities of keratin filaments. Methionine and cysteine play a key function inside the formation of disulphide bonds, essential for structural stability of those molecules. Hard keratins (located in tough excrescences, such as feathers, hair, nails, and hooves) include extra disulfide bonds than smooth, flexible keratins. The amino acid sequence additionally influences the secondary shape of keratins, which can be enriched in α-helix (common for α-62 keratins) or β-sheet structures (standard for β-keratins). Keratins normally encompass three domains with extraordinary secondary systems: head domain, imperative helical area, and tail area. Head domain or N-terminal part of the protein, is a globular shape with β-turns which includes a variable number of amino acids (50-100) with a tremendous net charge. The domain names and subdomains of one keratin molecule interact with the ones of adjoining keratin molecules forming heterodimers, tetramers and, eventually, keratin fibres. Keratinases are the most effective institution of proteases with an extensive temperature and pH variety that allows the whole degradation of complicated and recalcitrant proteins.

Keratinase homes depend upon its producers i.e. serine protease. The application of keratinases is underexploited due to restrained availability of green enzymes with versatile substrate specificity. The mechanical stability of keratin and the resistance to microbial degradation are due to the tight packing of the protein chain, that is both in α-helix (hair α-keratin) or β-sheet (feather β-keratin) structures, and their linkage via cysteine bridges which have a excessive diploma of cross-linkages via disulfide bonds, hydrogen bonding, and hydrophobic interaction. Cysteine is the most reactive amino acid residue in keratin. This is explained by means of the presence of disulfide bonds that may be oxidized or reduced. There are 3 critical groups in cysteine:

- The carboxyl organizations, which act in ester or salt formation.
- The amino agencies, which might be part of the peptide linkage through the move-chain formation by using direct hydrogen bonding with other keratins.

• The sulfur to sulfur institution, that's without problems damaged to shape a gel-like mass in depilation. The breakdown of cysteine is promoted by way of alkali (OH–), sulfide(S2–), or thiols (-SH), and the mixture of those ions.

Keratinolytic degraders can be observed in various businesses of microorganisms: from fungi, actinomycetes, and bacteria.

2.2.1. Dermatophyte fungi

They are some of the maximum recognized keratin degraders. To degrade each soft and hard keratin, their virulence and pathogenicity had been related. However, biotechnological packages of these fungi have not been widely explored, because of the potential risks of infection. A comprehensive review of nearly 300 fungi species (both pathogenic and non- pathogenic) has been posted detailing their potential to degrade one-of-a-kind keratinous substrates.

2.2.2 Actinomycetes

They also are regarded to be a wealthy supply of keratinase. A wide variety of mesophilic Streptomyces and thermophilic *Streptomyces spp.* Produced keratinases that wreck down keratin. Another promising keratinase becomes isolated from *Nocardiopsis sp*. Stress TOA-1 and has been established to degrade artificial keratin substrate, as well as scrapie prion.

2.2.3 Microorganisms as a source for keratinases

They are also determined to be very important for keratinase manufacturers. From the Gram-fine class, the most outstanding and prolific of the keratin degraders are the individuals of the Bacillus genus. In unique, keratinases from *B. licheniformis* are capable of degrading feathers, wool and animal disguise. From the Gram-poor class, keratinases produced with the aid of participants of the genera *Chryseo bacterium* or *Stenotrophomonas* were extensively studied and proven to degrade feather, animal hair, wool, hoof, and horn. The capability to provide serine-kind keratinases have been tested by means of a few thermophilic anaerobic microorganisms. *Fervido bacteriumpennav orans* and *F. Islandicum* had been removed from hot springs and produced keratinases that may degrade feathers efficaciously.

Table 2.2: Amino acids composition of different sources of keratin

S. No.	Source of Keratin	Amino Acids	Reference(s)
1.	Chicken feathers	Aspartic acid Serine Proline Tryptophan	Sangali and Brandelli (2000) and Saravanan Kumar and Dhurai(2012)
2.	Human hairs	Aspartic acid Threonine Half cystine Isoleucine Phenylalanine	Cannan and Levy (1950), Robbins and Kelly(1970) and Miranda-Vilela et al. (2014)
3.	Human nails	Alanine Isoleucine Threonine Arginine Half cystine	Greaves and Moll (1976), Mavis and John (1976) and Marshall and Glipie (1977)

2.3 History of Keratin

The earliest use of keratin comes from Li Shi-Zhen, who turned into a Chinese herbalist who makes use of it as medication. He wrote a group of approximately 800 books which was popularly known as Ben Cao Gang Mu. He documented his work which was published after his death in 1956. In the 18th Century while proteins have been referred to as "albuminoids", various proteins have been discovered. Then, the term "Keratin" was coined. It came from the Greek phrase "Kera" which means that horn, used to describe cloth that is made of hard tissues for example hooves and horns. Researchers have been facing a hassle with this protein due to solubility strategies that were useless, making it exclusive from different proteins. This hassle turned into solved throughout the period of World War I when John Hoffmeier from America documented a protocol for keratin extraction the use of lime. With improvements in the studies location, within the length from the 1900s to Forties, a plethora of protocols turned into developed to extract keratin based on its houses. Scientists and researchers started out open discussions and seminars for keratin studies. Extensive research was performed that led to the renaissance in keratin studies. In later years, monumental efforts had been made to broaden a dependable technique to transform extracted keratin into films, gels, powders, foams and so on. Exponential increase for keratin derived materials resulted in the formation of new companies, so one can commercialize the use of keratin. The esteemed organizations had been Croda International from the UK, Rita Corporation from United States of America, Seiwa Kasei Ltd. From Japan. The earlier research revealed that keratin powders may be mixed with other compounds, this brought about the emergence of a new studies region which in addition continues the increase within the discipline of keratin. The maximum mentioned topics of keratin research are Wound Healing, Drug Delivery, Tissue Engineering, Cosmetics, Medical Devices are growing base for keratin information.

2.4 Production and purification of Keratinases

2.4.1 In submerged fermentation

Keratinases are predominantly extracellular when it's far grown on keratinous substrates. In most instances, keratin serves as the inducer. However, to induce enzyme manufacturing, non-keratin substrates like soy meal is used. In this, two steps were assumed to be worried in keratinolysis: sulfitolysis that's the discount of disulfide bonds and proteolysis. Because of the form

of organisms and the methods of cultivation, it is difficult to examine the production condition for keratinase. Furthermore, carbohydrates together with glucose (easy sugar) were stated to suppress the synthesis of keratinase because of catabolite repression and then again, complicated sugars like starch have shown to beautify the synthesis of keratin

2.4.2 In solid state fermentation

Substrates inclusive of feathers, hair, horn, and sugarcane bagasse have used an inducer for the manufacturing of keratinases beneath solid state fermentation. In a study carried out, an endophytic keratinolytic pressure of Penicillium sp. reported having significantly produce keratinase under solid state fermentation, using distinctive agricultural and chicken waste.

2.4.3 Activity improvement by mutagenesis

To enhance the production and assets of keratinases, there are manipulations of keratinolytic organisms by means of bodily and chemical mutation. A triggered mutant i.e. Bacillus subtilis by way of the use of N-methyl-N'-nitro-N-nitrosoguanidine reported to produce keratinolytic activity of about 2.5 times that of the wild-type strain.

Fig. 2.2: Schemed mechanism of keratin degradation

Yamamura et al. (2002), in his research work, proposed a mechanism for extracellular protein degradation by a bacterium in keeping with that a mixture of two styles of animate thing proteins i.e. chemical process (proteolytic) and disulfide bond reducing are needed for complete reaction or hydrolysis of keratin. Firstly, the disulphide bond in keratin is attacked by disulphide reductase-like protein. Then, it yields the partially chopped protein which is served as a substrate for protease.

*2.*5 Purification methods of Keratinases

For the higher keratinase manufacturing underneath most efficient situations, the purification is an imperative element for technical packages to facilitate the performance for action of keratinases. In some instances, purified keratinases of several sizes were recorded with a range of molecular weight from 27 to 200 kDa by specific strains of bacteria and fungi. However, there has been a recuperation of keratinase with a molecular weight of 440 kDa. Purified enzymes can be acquired via the use of one-of-a-kind methodologies. The maximum not unusual strategy which is precipitation to purify the enzymes further followed through column type of chromatography. Using the feather-degrading bacterium, ammonium sulphate precipitation was accompanied through ion-alternate (DEAE-Sepharose) and gel filtration (Sephadex G-seventy five), Keratinase of 35 kDa was purified. It becomes determined to have thermotolerant and additionally confirmed excessive unique interest.

Keratinase exhibiting weight of 41 ±1 kDa and activity below the accurate situations with pH 9.0 and 50°C become remoted from *Bacillus megaterium*. This enzyme analysed to have an energetic website online and which changed into inhibited by using PMSF. With the help of immunoprecipitation also, purification of keratinase may be achieved, when there is appropriate availability of anti-keratinase antibody. Similarly, the immuno chromatography approach also can be carried out the usage of the anti-keratinase antibody for the effective and efficient purification of keratinase.

Table2.3: Biochemical characteristics of keratinolytic microorganisms

Bacteria	**Class of enzyme**	**Molecular weight kDa**	**pH optima**	**Temperature optimal (°C)**	**References**
Bacillus licheniformis PWD-1	Serine	33	7.5	50	Lin et al. (1992)
Bacillus spp.	Metallo	134	7	40	Lee et al. (2002)
Bacillus cereus DCOW	Serine	80	8.5	50	Ghosh et al.(2008)
Bacillus subtilis RM-01	Serine	20.1	9	45	Rai et al. (2009)
Actinomaduraviridilutea DZ50	Protease	9.54	11.0	80	Elhoula et al. (2016)
*Streptomyces brevicaulis*45	Serine	40	7.8	40	Malviya et al. (1992)
Streptomyces pactum	Serine	30	7–10	45	Syed et al. (2009)
Hermoanaerobacter Riessen and *kerationophilus*	Serine	135	8	85	Antranikian (2001)
Aspergillusoryzae	Metallo	60	8	50	Hassan(2004)
Aspergillus fumigates	Serine	–	9	45	Santos et al. (1996)
Paecilomycesmarquandii	Serine	33	8.0	60–65	Gradisar et al. (2005)
Trichodermaatrvoviride F6	Serine	21	8–9	50	Cao et al. (2008)
Candida parapsilosis			7.4	37	Duarte et al. (2011)

2.6 Biochemical properties of keratinases

The majority of keratinases which have been reported are said to be monomeric enzymes with a one of a kind range of molecular weights. The keratinase produced with the aid of Bacillus pumilus A1 has the bottom molecular weight, whereas, the highest molecular weight of keratinase become produced by means of K.Rosea. Although much less, not unusual, multi-merickeratinases have additionally been removed in a number of microorganisms. Keratinase from fungal isolates of *Coccidiodesimmitis* produced seven awesome polypeptides ranging from 15 kDa to 65 kDa, S. Brevicaulis and Penicillium spp. Morsy1 both produced fractions while purified with the aid of SDS-PAGE that had been 24–45 kDa and 19–40 kDa, respectively.

Actinomycetous isolates of *Streptomyces sp.* Strain 16 produced a keratinase that become Keratinase produced via *B. Licheniformis* PWD-1 is as it should be studied and there has been the determination of entire nucleotide sequence of the coding and flanking areas of the keratinase structure gene, kerA. Although many microorganisms are able to produce keratinase and lots of have been sequenced. In evaluation, *B.subtilis* regarded to be the right host for keratinase production in a comparative take a look at the use of *Escherichia coli, B.subtilis* and *Pichia pastoris* used as a cloning host to specific the keratinase gene from *B. licheniformis* BBE11-1.

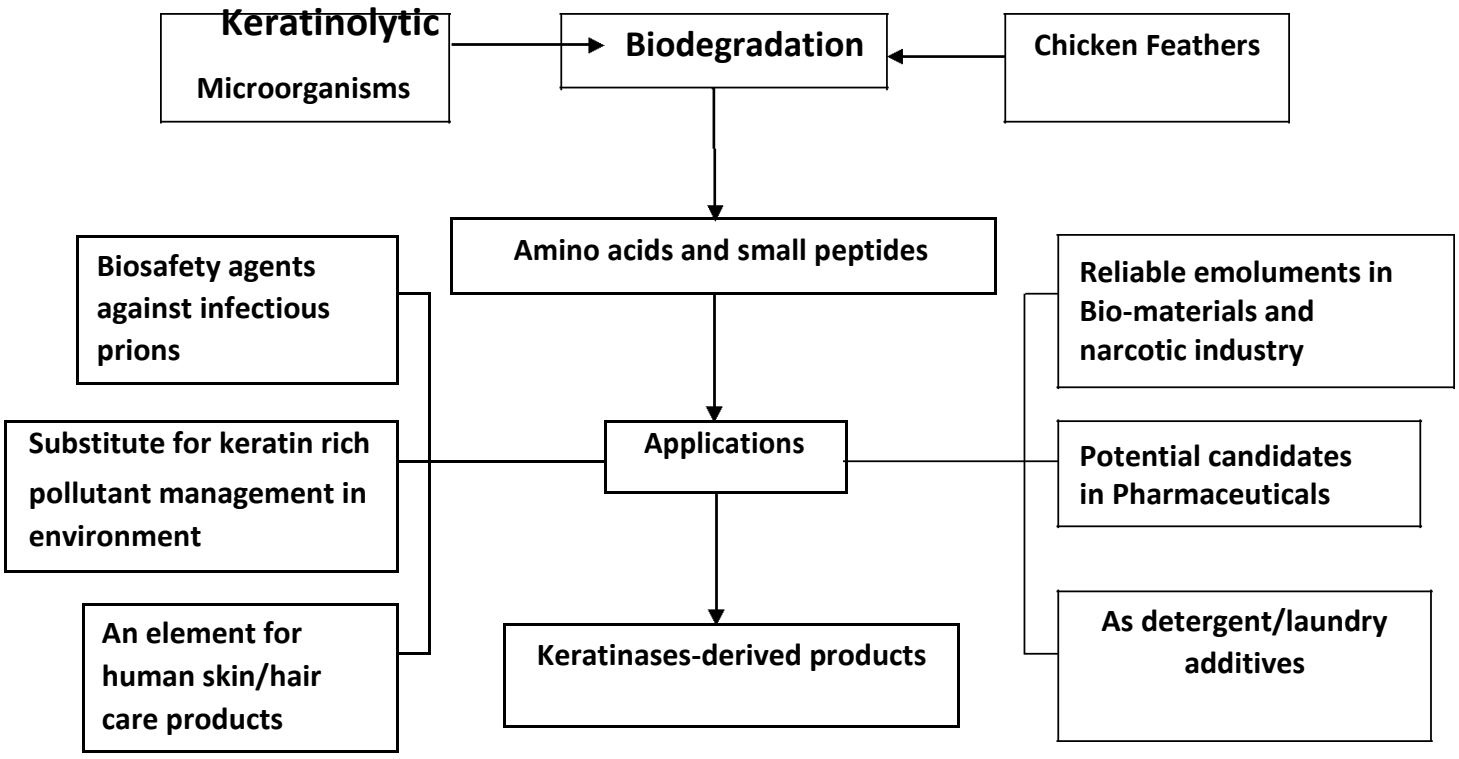


Fig.2.3: Illustrative representation of implied applications of microbial keratinases

2.7Applications of keratinase

2.7.1 Keratin waste management

Several microbial strains possess a very remarkable feather-degrading ability which could be beneficial in waste management of biomass. Wastes which are generated in the meat industry such as collagen, elastin, keratin, and prion proteins were effectively degraded by the usage of keratinolytic enzyme.

2.7.2 Animal feed production

The hydrothermal treatment reduces the nutritional value, as it destroys certain essential amino acids such as methionine, lysine, histidine and tryptophan, and the inability to release some amino acids from the keratins. The use of keratinase in degrading feather is more advantageous than microbial degradation, as it helps to avoid the possibility of exposure of the users to organisms that could be pathogenic.

2.7.3 Production of nitrogen fertilizer/biofertilizer

Being a laggard for the release of nitrogen fertilizer, feather meal produced from the recycling of keratinous wastes is still applicable. A keratinase-producing strain of B. subtilis demonstrated plant growth-promoting and broad-spectrum antimicrobial activities, as its outcome in the form of indol acetic acid (IAA) and antifungal activities during keratinase production.

2.7.4 Detergent industry

The keratinolytic proteases elevates the value of proteolytic enzymes in regulation of detergent. They have potential to degrade insoluble keratin. They show properties like stability, activity at peak, ample range of temperature and pH, also the stability in the presence of surfactants, oxidizing and bleaching, chelating agents and conjugative compatibility with plethora of detergents.

2.7.5 Cosmetic and pharmaceutical applications

In medication and beautification industries, these non-collagenolytic keratinases are promising and reliable. They have been described as an ingredient in depilatory formulation procedures for shaving and skin lightening agents. Some crude keratinases have been shown to improve hair virtue such as texture, thickness, colour, softness, and strength; thus could be applied as hair care product. Keratinases are capable of skin peeling to remove acne, which occurs through the blockage of the sebaceous gland by keratins.

2.7.6. Textile industry

In textile industries, keratinases have the capability to modulate silk and wool which indicated their potential application. The treatment for wool and polyester fabrics with crude keratinases i.e. Pseudomonas strain have shown that the resistance to shrinking and tensile strength were improvised by the usage of enzyme.

2.7.7 Leather processing industry

In the dehairing process, mixtures of proteases, lipases, and carbohydrates are used as biocatalysts. Keratinolytic proteases without collagenolytic and elastinolytic activities are good for the dehairing process as the integrity and quality of the hides are guaranteed.

Dehairing by keratinolytic proteases is said to be the finest preference because it is basically removal of hair from the skin without causing destruction to the skin and hair fragments. This process is easy along with inexpensive. In addition it also safeguards the release of toxicities effluent in the surrounding.

2.8 Industrial applications of Keratin

In the 16^{th} century, Li Shi-Zhen, a Chinese herbalist used keratin for medical applications. He used it to treat wounds and blood clots, which were known as Crinis Carbonisatus, a substance which was formulated of ground ash extracted from pyrolyzed human hair. There are no harmful compounds in keratin reported yet, therefore this led to substantial production of keratin derived products in the market. There are countless applications of keratin in tissue regeneration, wound dressing, pharmaceuticals, hemostat, implant filter, bio sorbent, rubber, keratin hydrolysate, cosmetology, etc. The uses of keratin in biomedical sciences, pharmaceuticals and in industries are discussed below:

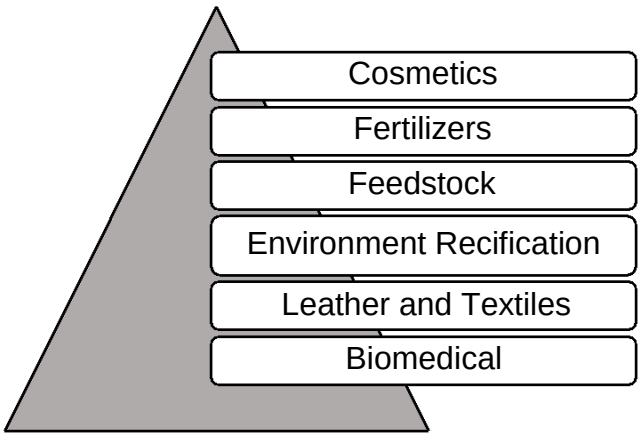


Fig 2.4: Representation based on Industrial prospects of Keratin

2.8.1 Uses in Cosmetic Industry

There is a prodigious application of keratin in the cosmetic industry. The benefits of natural keratin favor its use in hair, skin and nail care products. In hair care products, its role is to replace

lost proteins due to strong chemical treatment and heat styling by increasing hair elasticity and strengthening hair and its health. In skin care products, it adds elasticity and glows to the skin by reducing wrinkles. In nail care products, it adds elasticity to nails and helps in healthier nail growth. It is basically biologically compatible and products are beneficial for use.

2.8.2 Uses in Fertilizers industry

Keratin, being a good nitrogenous source is used for preparing biofertilizers. A process of breaking down of insoluble feathers into water-soluble keratin and then, taking a step ahead by nitrogen availability to crops for optimum growth and elimination of pathogenic disease effects. There is a production of keratinolytic enzymes by bacteria and fungi which helps in the degradation of waste keratin biomass. This organic keratin could be the starting point for research in organic hydroponics, organic fertilizers, and greenhouse applications.

2.8.3 Uses as Feedstock

Keratin extracted from chicken feathers when treated with lime, contained abundant amino acids and polypeptides. That is why could be used in feedstock due to similarity with soybean and cottonseed proteins. Thus, feed containing keratin hydrolysate has elevated nutritional value, growth rate, and feed efficiencies.

2.8.4 Uses in Environment Rectification

Waste biomass was the poultry industry could be used to produce novel products. It was stated by *Poole et al.*in the year 2009 that fibers attained from keratin were environmentally safe, inexhaustible and ecological in nature. Thus keratin based derivative products have potential to upheaval the bio-based material market. The solution to waste management by utilizing waste biomass to produce a novel bio-product which acquires the property of being biodegradable, biocompatible and mechanically durable.

2.8.5 Uses in Leather and Textile Processing

For the processing in the leather and textile industry, various processes such as the production of fibers, yarn, and imbibition of different properties such as water repellent, anti-

wrinkling, and softness. Keratin being pocket-friendly exhibit cohesion and abrasion resistance which plays an important role in improving tensile strength and weaving performance. Keratin paces the potential to replace PVC, which is too expensive.

2.8.6 Uses in Biomedical Area

There are certain cellular binding motifs present in natural keratin that plays an important role in the cellular attachment (*Marshall et al. 1991*). It has a characteristic of self-congregate and to polymerize. Thus it's applications in the biomedical area are in wound healing, protein film and fiber formation, scaffold (2D or 3D) for tissue engineering (*Xu et al. 2014*), nanoparticle, nanofiber controlling drug delivery. In clinical diagnosis, keratin augments human mesenchymal stem cell (*Hartrianti et al. 2015*).

2.9 Keratin Products in Market

Keratin is one of the fibrous protein utilized in the manufacture of personal cleansing products which are mainly for nails, skin, and hair. Due to stylistic changes in society, the demand for self-care and cosmetics are exponentially increasing with each passing day. Cosmetic and hygiene products constitute the largest fragment of about 50% in global share.

Fig 2.5: Various Keratin derived products.

2.9.1 Certified Keratin products in the market

There is a wide range of products available in the market, out of which the highest proportion is for hair care products that constitute about 75%. The main concern for keratin hair products is to reduce fizziness, restore strength, elasticity, and softness of hair. Keratin vanishes the effects of heat styling and harmful chemicals that have resulted in damage of hair. Keratin flat iron is also available in the market on which keratin is infused that helps in making hair shiny and healthy. Some famous keratin products readily accessible are listed below:

- **4 Hair:** Hair straightening product.
- **Bella:** It is an advanced technology of Incobella Group.
- **Brazilian blow out**: It shows long-lasting results against frizziness.
- **Cadiveu:** It is a cocoa-based combined with keratin and panthenol, which moisturizes hair.
- **Natura Keratin:** A certified organic natural keratin.
- **LBD:** It is a Kera Green Keratin which repair, rejuvenate and moisturize the scalp.
- **Pure NV:** It uses the highest grade of keratin for reducing curls in hair.
- **Brazilian Tech Keratin Iron Rod:** Its plate material is ceramic infused with keratin titanium which makes it effective for home styling.
- **Arlak Hair Wealth Tablets:** A hair supplement that helps in improving hair growth rate and maintaining the volume of hair.
- **KèRASTASE Nutrients:** A nutritive supplement for healthy scalp and hair growth.
- **Keratin Research Brazilian Keratin Treatment:** Composed of argan oil, coconut oil, and protein to maintain hair moisture and prevent breakage.
- **It's a 10 Haircare Miracle Leave-in Plus Keratin:** Quick-fix treatment which is pocket-friendly. A little spray goes a long way to remove frizz and flyaways.
- **Paul Mitchell Awapuhi Keratin Intensive Treatment**: Contains Keratriplex that rebuild and reinforce cuticle. It is Paraben-free.
- **TRESemme Expert Selection Heat Activated Keratin**: Contains hydrolyzed keratin protein, silicones, and polymers. It shows longevity and moisturizes the scalp.

Many other products are available in the market for consumers by increasing production capacity.

2.10 Future of Keratin Products

With the rising demand for cosmetics and necessity products, it is presumed that there would be substantial growth in the Global Keratin Market. There would be a plethora of embellishments in technological areas to increase economy and meet the needs of the customers. It is noticed that KeraGreen would have a positive impact on society, resulting to hit the market with elevations in the economy. Growth in keratin derived products specifically from Asian countries including India, China, and Japan would impel the business in the coming decade. Top Keratin companies such as Rejuvenol, Scherdiva, Keratin Express, Keraplast take into account 70-80% of the global market. They use market tactics by utilizing various combinations and approaches to regulate scenario, so as to create an impact and expect developments in the market. In the forthcoming years, there would be a boost in market dynamics, trends and competitive behavior.

CHAPTER 3

MATERIAL AND METHODS

3.1 Materials and Methods

3.1.1 Chemicals

Human hair was collected from a hairdressing salon in Waknaghat, Solan, Himachal Pradesh. Sodium Hydroxide (NaOH) used to deliquesce hair was purchased from SRLchem (Sisco Research and Laboratory Chemical manufacturers, Mumbai, India). Hydrochloric acid from LobaChemie used to precipitate hair sediments. Detergent was used to wash out dirt from hair obtained from the salon. Ethanol (90%) was used to sterilize washed hair. Milli-Q water was used to prepare a solution for dissolution of hair. Liquid Nitrogen (liq. N2) was acquired from the laboratory of Jaypee University of Information Technology, Waknaghat, Solan, H.P. to crush segregates of hair.

For the preparation of bioplastic film glycerol, cellulose, pectin, gum Arabic, starch was used.

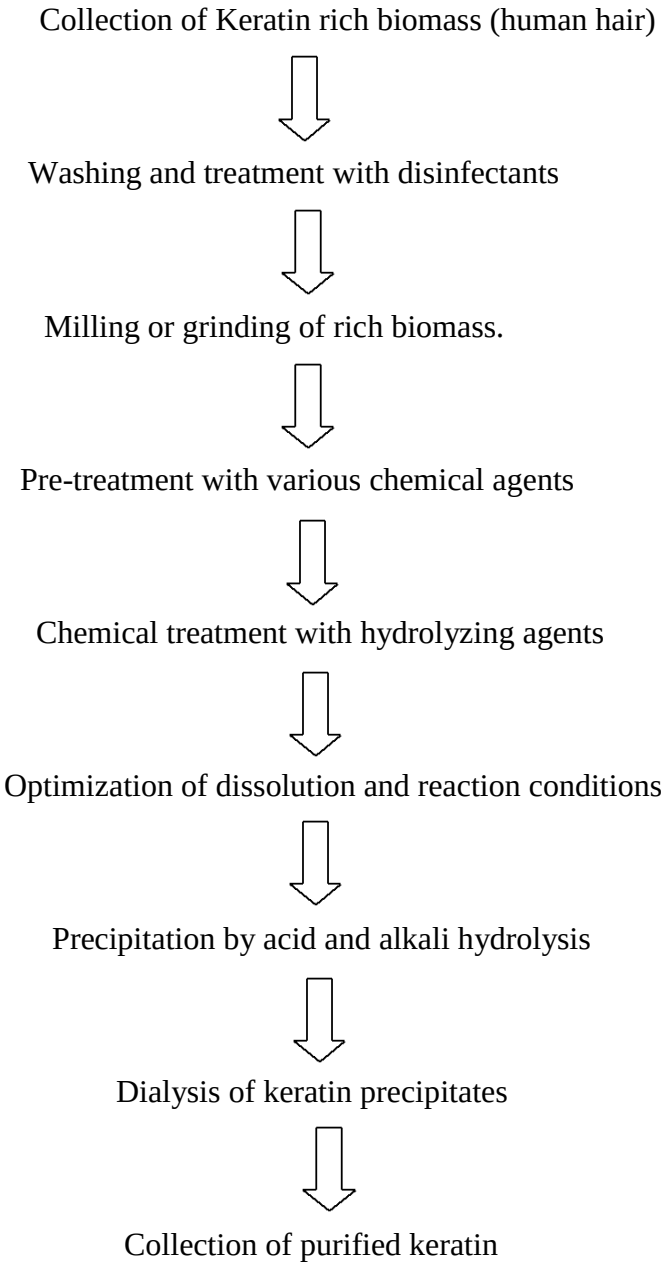
Fig. 3.1: Human hair collected from a hairdressing salon **3.1.2 Methodology**

Waste biomass was gathered from a local hair dressing salon. Then, the focal point changed into some better strategies for keratin production and degradation. Keratin extraction can be performed using alkaline, or acidic hydrolysis. Extraction along with manufacturing, then two methods especially depend on kind complementary factors:

(1) Chemical substances, vital for extraction of specific cellular proteins from the cell, tissue or organ.

(2) Optimum conditions, vital it is as it offers the appropriate help for stability of protein.

The methodology we followed is as follows:



3.2 Optimization of reaction conditions

3.2.1 Screening of reducing agent

The dissolution of keratin rich biomass was done by alkaline reagents. The hair biomass was washed and pretreated with disinfectants to make free of any pathogens and microorganisms. The dried hair biomass was dissolved in selected chemical agents NaOH, SDS, chloroform, urea, ethanol, thiourea, Tris-HCl and β-mercaptoethanol at different concentrations. The hydrolytic reaction was performed for 0-48 h under shaking at room temperature. The best dissolving agent was selected for further experiments.

3.2.2 Optimization of reaction temperature

The dried hair biomass was dissolved in selected chemical agents NaOH, SDS, chloroform, urea, ethanol, thiourea, Tris-HCl and β-mercaptoethanol at different concentrations then, was subjected at different temperatures ranging from 4°C, 20°C, 37°C and 50°C.to check the optimum temperature for dissolution of hair.

3.2.3 Optimization of reaction time

The hair biomass was dissolved in selected chemical agents NaOH, SDS, chloroform, urea, ethanol, thiourea, Tris-HCl and β-mercaptoethanol at different concentrations then, was subjected at different temperatures for different time durations (i.e. 1h, 2h, 3h, 4h, 12h, 24h) to optimize the best time for dissolution of hair.

3.2.3 Optimization of Concentration of reducing agent

The hydrolytic reaction was performed for 0-48 h under shaking at room temperature, with chemical chosen for different concentration (i.e. 0.1%, 0.2%, 0.6%, 0.8%,1%, 2%, 4%, 8%, 10%) to check the best dissolving concentration for further experiments.

3.2.4 Optimization of drying keratin hydrolysate

To get rid of the moisture from the hydrolysate obtained. We optimized drying method using oven, lyophilization, drying in sunlight, drying at room temperature. The best method was chosen.

3.3 Dialysis of Keratin Hydrolysate

Dialysis membrane of CelluSep taken about 10-12cm. Membrane was filled with the hydrolysate and tied with thread from both ends. Dialysis was performed for 2-3h at room temperature using dH_2O to remove unwanted salts from keratin hydrolysate.

3.4 Salt-free Keratin Hydrolysate for liquid N2 crushing

The salt-free hydrolysate was dried to remove moisture and were crushed using liquid nitrogen in mortar and pestle to get a powdered form of extracted keratin.

3.5 Yield Calculation of final volume

$$\text{Yield\%} = (\text{Final yield} / \text{Initial volume}) * 100$$

Yield percentage was calculated using the above formula.

3.6 Characterization of extracted Keratin

3.6.1 Scanning Electron Microscopy

A beam of targeted excessive-strength electrons generate diffusion to create alerts at the surface of solid specimens. The outcomes from electron-sample interactions screened for the sample together with secondary characteristics such as texture, composition, shape and orientation of substances.

3.6.2 X-Ray Diffraction

A speedy analytical approach which is used for identification of phase. Crystalline and Amorphous nature offers statistics on cell dimensions. The analyzed material is finely, homogenized, and bulk composition is determined.

3.7 Synthesis of bioplastic film

3.7.1 Pectin-Keratin composed biofilm

Pectin was chosen for the synthesis of bioplastic film, as it has potential of being biodegradable and biocompatible. To synthesize bioplastic film 0.18gPectin, 800mg Keratin, 200 mg Glycerol was taken in a small beaker followed by magnetic stirring. After that, it was spread in an aluminum channel placed over a glass plate.

3.7.1 Cellulose-Keratin composed biofilm

We choose cellulose for the synthesis of bioplastic film as it is an abundant renewable polymer. To synthesize bioplastic film, 0.18g cellulose, 0.8 g Keratin, 200 mg Glycerol was taken in a small beaker followed by magnetic stirring. After that, it was spread in an aluminum channel placed over a glass plate.

3.7.1 Starch-Keratin composed biofilm

Starch was chosen for the synthesis of bioplastic film because it is cheap, renewable and abundant. To synthesize bioplastic film using starch, 0.18g starch, 800mg Keratin, 20mg Glycine was taken in a small beaker followed by magnetic stirring. After that, it was spread in an aluminum channel placed over a glass plate.

3.7.1 Gum arabic-Keratin composed biofilm

Gum Arabic being a natural tree gum exudate has properties to enrich surface of the bioplastic film that is why it was chosen.To synthesize bioplastic film 0.18g gum arabic, 0.8 g Keratin, 0.16g Glycine was taken in a small beaker followed by magnetic stirring. After that, it was spread in an aluminum channel placed over a glass plate.

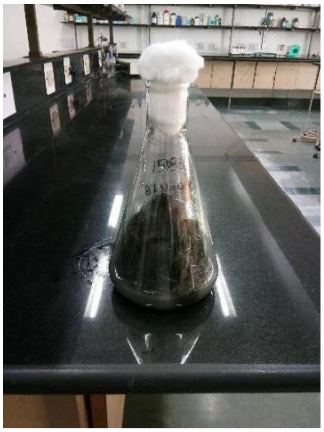


Fig 3.2: Dissolution of hair in alkaline solution.

Fig 3.3: Precipitation using Conc. HCl.

Fig 3.4: Dialysis of Keratin precipitates.

Fig 3.5: Keratin precipitates kept for drying in an oven.

Fig 3.6: Crushing using Liquid Nitrogen.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1.1 Screening of reducing agent

The hydrolytic reaction was performed for 0-48 h under shaking at room temperature. The best dissolving agent was NaOH which was selected for further experiments.

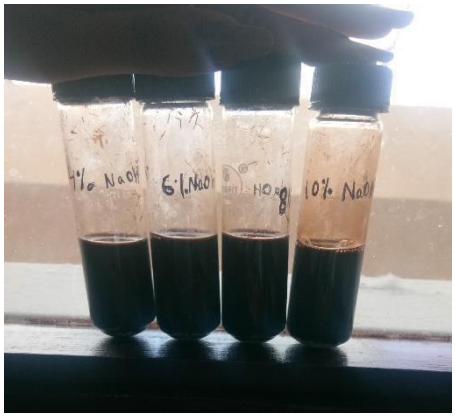


Fig 4.1: NaOH showed the best dissolution of hair

4.2.2 Optimization of reaction temperature

We found that the optimum temperature for dissolution of hair is 37°C. We took four different temperatures i.e. 4°C, 20°C, 50°C, 37°C respectively. Amongst them, best optimum temperature for dissolution was 37°C.

Table 4.1: Temperature optimization for dilution

Sr.No.	Temperature(°C)	2h	4h	6h	8h	12h	24h	48h
1.	4	No	No	No	No	No	No	No
2.	20	No	No	No	No	Slight	Yes	Yes
3.	37	No	Yes	Yes	Yes	Yes	Yes	Yes
4.	50	Slight	Yes	Yes	Yes	Yes	Yes	Yes

4.2.3 Optimization of reaction time

Dissolution of hair was highly observed within 2-3h. During the dissolution of hair with different time durations, hair was dissolved within 2-3 h.

Table 4.2: Time optimization for dilution

Sr. No.	Time (hrs)	4°C	20°C	50°C	37°C
1.	**2**	**No**	**No**	**Slight**	**Yes**
2.	**4**	**No**	**No**	**Yes**	**Yes**
3.	**6**	**No**	**No**	**Yes**	**Yes**
4.	**12**	**No**	**No**	**Yes**	**Yes**
5.	**24**	**No**	**Slight**	**Yes**	**Yes**

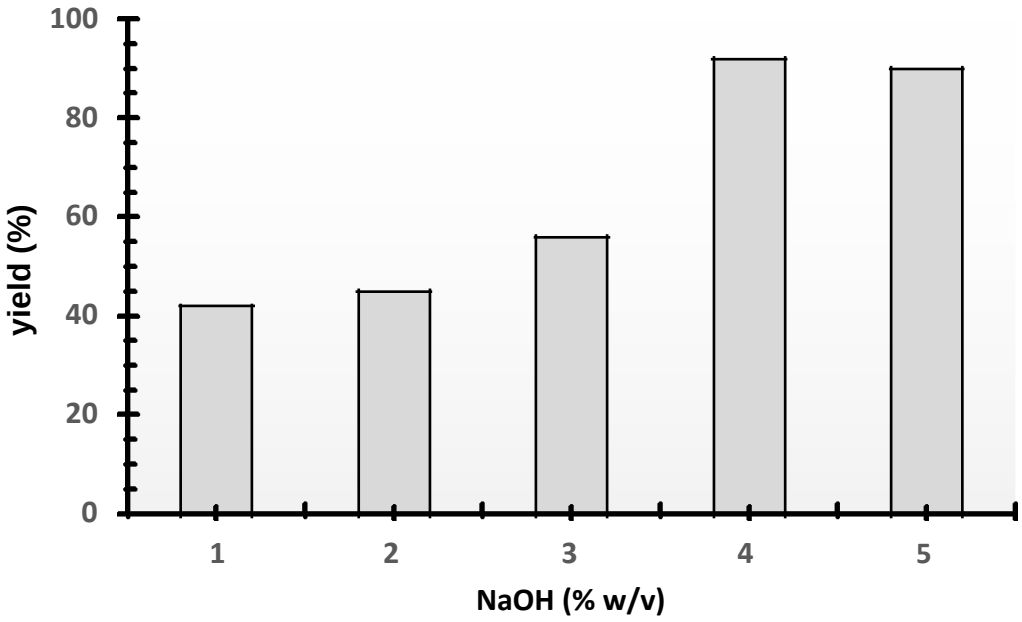
4.2.4 Optimization of Concentration of reducing agent

Best dissolution of the hair, chemical is chosen was analyzed at the concentration (i.e. 8%).

Fig 4.2: Best dissolution at 37°C of 8% NaOH

Table 4.3: Yield % vs Concentration % of NaOH

Sr. No.	NaOH (%)	Yield (%)
1.	2	42
2.	4	45
3.	6	56
4.	8	92
5.	10	90



Graph 4.1: Effect of concentration on the yield of Keratin

4.2.5 Drying keratin hydrolysate

We optimized drying method using an oven for about 2h.

4.3 Dialysis of Keratin Hydrolysate

Dialysis is the separation of particles in a liquid on the basis of differences in their ability to pass through a membrane and because of this, dialyzed keratin hydrolysate was obtained.

4.4 Salt-free Keratin Hydrolysate for liquid Nitrogen crushing

The salt-free hydrolysate was crushed using liquid nitrogen because of its cryogenic property, powdered form of extracted keratin was obtained.

Fig 4.3: Extracted keratin

4.5 Yield Calculation of final volume

Yield% = (Final yield / Initial volume) * 100

Yield percentage was calculated using the above formula, which was found out to be 83.416%.

4.6 Characterization of extracted Keratin

4.6.1 Scanning Electron Microscopy imaging of keratin powder

The SEM images derived from electron interactions after screening the sample together with secondary morphology such as texture, chemical composition, shape and orientation of substances at 50µm and 100µm showed smooth, micropores and irregular structures. SEM images for keratin extracted showed that keratin resembles like small particle form or aggregates of dust which was magnified to ×10,000. (Kamarudin et al. 2017).To observe the morphological structures of hair extracted keratin by scanning electron microscopy. The sectional view showed irregular, flat and fibrous structures which indicated that a number of fibers were being projected in that region. (Fujii et al. 2013)

a.) b.)

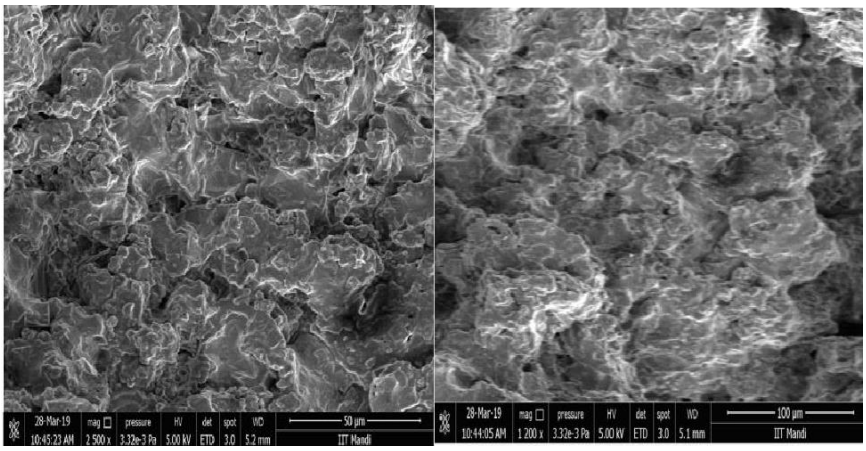


Fig 4.4: Scanning microscopic images of keratin powder obtained from human hair

a.) 50µm b.)100µm

4.6.2 X-Ray Diffraction XRD analysis of extracted keratin powder

The XRD patterns of human hair and keratin obtained using 2α Bragg angles which were scanned for a range of 5–80°, using 0.02°step size and 1.0 s per step scan speed. The fig. showed slightly peaks which indicated the crystalline characteristics were observed at an angle 27°, 32°, 47°, when compared with the keratin powder extracted from chicken feather showed strong crystalline characteristics. (Ramakrishnan et al. 2018). XRD patterns of human hair and keratin from rabbit hair showed diffraction peaks at 9° and 20°, further peaks disappeared due to destruction in dissolution process as described and there was an increase in diffraction intensity of β-sheet structures that revealed changes in keratin crystallites (Wang et al. 2018). Therefore, we conclude that the nature is crystalline of extracted keratin powder from human hair.

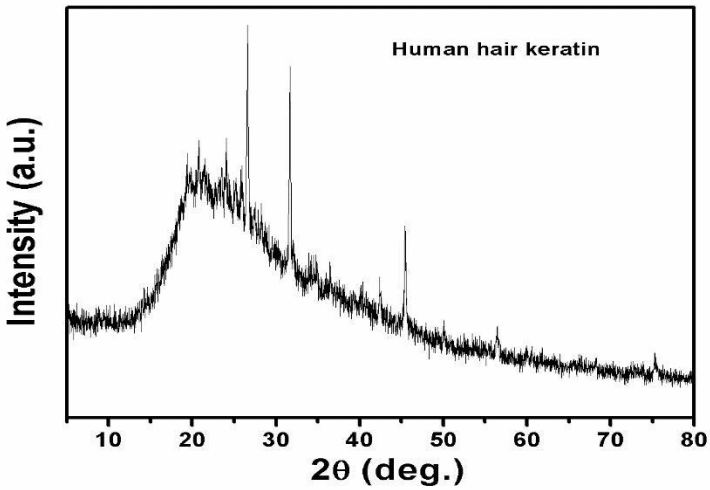


Fig 4.5: X-Ray Diffraction images of keratin powder obtained from human hair

4.7 Synthesis of bioplastic film

In earlier studies bioplastic film was fabricated using microcrystalline cellulose due to its property of being a nano-filler and it determines the industrial utility of film synthesized. Although in various researches keratin showed its fragility (Aluigi et al. 2008) (Yin et al. 2013) which helped in measuring mechanical properties of film. Glycerol provided flexibility when added at lower concentrations that was then analyzed or characterization of bioplastics produced (Ramakrishnan et al. 2018). Gum Arabic due to natural origin and waxy in nature could provide with flexibility. The main issue was to strengthen the film which opened gateway for production of bioplastics from waste biomass. We prepared bioplastic film using cellulose, pectin, gum Arabic and starch due to properties as mentioned above. The mixture of components were kept for 12-14h and films were observed for their structure and binding. Out of which, better results were observed for Glycerol, Pectin, and cellulose.

Fig 4.6: Bioplastic Films using glycerol, pectin, starch, cellulose

Conclusion

Knowledge of biochemical properties improves the knowledge that is needed to discover their cost. Applications of keratin is a growing fashion which traverses commercial biotechnology, with packages in bioenergy, nano-biotechnology, waste recycling and control, bioremediation, leather, and fabric industries, food and feed era, personal care merchandise, scientific and pharmaceutical applications, agriculture, bio-catalysis amongst others. The procedure of growing extra efficient techniques for the production and detection of keratin will quicken its utility to industries and environmental waste management. It is noticed that in recent years, a wide range of advancements in the field of the keratin research area. Extraction of Keratin from waste biomass and its production into novel biosynthesis has boosted up the pharmaceutical as well as the cosmetic industry.

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PUBLICATIONS

1. Publication at 3rd Himachal Pradesh Science Congress. Theme: Rural Upliftment through science and Technology, IIT Mandi , OCTOBER 22-23,2018.

Abstract (Agricultural and Horticulture sciences), Page 11.

"Extraction of Keratin from waste human hair and its characterization."

EXTRACTION OF KERATIN FROM WASTE HUMAN HAIRS AND ITS CHARACTERIZATION

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Keratin is basically a protein that is found in human hair, skin etc. It has unique biological and chemical properties such as bio compatibility and high tensile strength. Keratin is highly stable and biodegradable biopolymer which it can be used in various industrial applications. There are two main types of keratin proteins, namely alpha-keratin and beta-keratin. Keratin is insoluble in aqueous media or double distilled water and resistant to the attack of proteolytic enzymes. In the present study human hairs were used as a source of keratin substrate. The waste human hairs were collected from local market salons of Distt. Solan, Himachal Pradesh. The collected hairs were thoroughly washed with detergent and rinsed with distilled water followed by drying it in the incubator oven at 50°C for 24 h. Thereafter, hairs were chopped into small pieces and dissolved in alkaline solution of NaOH at selected concentrations of 2%, 4%, 6%, 8%, and 10% for 24h. The human hair hydrolysate was subjected to precipitation with concentrated HCl under vigorous stirring on a magnetic stirrer. The precipitated keratin were collected by centrifugation at 12000 rpm for 20-30 min. The precipitates were further dialyzed with distilled water and lyophilized to obtain the pure keratin powder. The total yield of the extracted keratin was approximately 63% w/w. The extracted keratin can be used to various industrial applications.

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