A Study of Optimization of Keratin from Waste Biomass of Human Hairs: Industrial Importance

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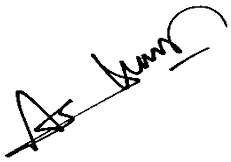
CERTIFICATE

This is to affirm that the work entitled –Keratin Characterization from Human Hair‖ submitted by Priyanjalee Bhattacharjee (161825) and Sambhavana (161829) in partial fulfillment of the requirement for the award of Bachelor of Technology Degree in Biotechnology of Jaypee University of Information Technology, Waknaghat is an authentic work carried out by them under by supervision and guidance. The matter embodied in this report is original and has not been submitted for the award of any other degree.



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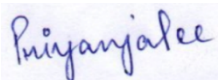
Sincerely,



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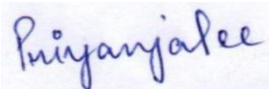
DECLARATION

We hereby confirm that the work proclaimed in the B-tech thesis entitled –KeratinCharacterizationfromHumanHairsubmittedatJaypeeUniversityof Information Technology, Waknaghat is a 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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Abstract:

Nail, fleece, quills and feet have a vital component known as keratin which offers unity to body and muscles. Keratin is made up and associated with cysteine-rich fiber shaping proteins. It is classified into Alpha-keratin and Beta-keratin. Keratin is not soluble in hot and cold water because the sulphur content in this protein is high. It has a vast number of applications in the clinical, pharmaceutical, corrective and biotechnological ventures. By permeable froth of various wipes, shapes, coatings, mats, microfibers, gels and materials which have high atomic weight, the keratinous materials can be changed. It has been utilized for bio-clinical gadgets, particularly in the field of tissue designing and controlled medication conveyance.

1. Introduction

The products from different creatures are being used lately for the purposes which are advantageous for example, medicate conveyance, prescriptions, beautifying agents and bio plastics. **Keratin** is an abundant structural protein **(Coulombe and Omary 2002). In** animals, it is associated with collagen**(McKittricketal.2012). It** is known as the most important biopolymer **(McKittricketal.2012).**The materials which are produced from organized keratinized cells are particularly made of fibrous proteins. There are different types of shape in keratin, such as, polypeptide chain shape, filament matrix shape, lamellar shape and sandwich shape which referred to as natural polymeric composites. Keratin serves many functions but protection is one of the major function. It is mainly found in epithelial cells of higher vertebrates **(Korniłłowicz-Kowalska and Bohacz 2011)**. Strength and stiffness are the main properties of keratin, and they are not soluble in polar as well as non-polar solvents. The major producers are in USA, Brazil and China (for more than 40 million tons annually). Keratin has many by-products which contain 15-18% Nitrogen, 2-5% Sulphur, 1.27% Fat, 3.20% Minerals and 90% Proteins **(Kunert 2000; Sangali and Brandelli 2000; Gessesse et al. 2003.** The cytoskeleton detail which is referred as intermediate filament is related to keratin which is made up of cysteine and are called cysteine rich proteins. Keratin is categorised in two types. The Keratins which are found in soft tissues and are rich in cysteine are known as **Alpha Keratins** while the keratins which are found in hard tissues are known as **Beta Keratins** and have high amount of Glycine and Alanine and have low amount of Cysteine, Hydroxyproline and Proline **(Guptaetal.2012)**. They are stable and mostlynot soluble in organic solvents. It is highly vulnerable to hydrolytic and oxidation reactions due to the presence of cysteine **(Schrooyen et al. 2000; Barone et al. 2006b; Endo et al. 2008)**. The hydrolysis of enzyme is less prone due to high cross-linkage by H-bonding, Disulphide bonds and hydrophobic interactions **(Korniłłowicz-Kowalska and Bohacz 2011)**. To use keratin as a biopolymer its extraction from biomass is mandatory Due to acidic keratin many useful amino acids are ruptured **(Breinl and Baudisch 1907; EarlandandKnight1956)**. Enzymatic keratin is formed by a slow process, has a higher cost and less species alteration **(Eslahietal.2013)**. The recovery of protein is low and Ionic- liquid keratin is too costly **(Idrisetal.2014; WangandCao2012)**. Purified keratin results into the regeneration of biomaterials and tissue repair (**Alsarra 2009; Ramshaw et al. 2009; Natarajan et al. 2012; Ramadassetal.2013;Kumaranetal.2016)**.Keratin from feather is 10 kda in Molecular weight **(Fraser et al. 1972; ARAI et al.1983; Ullah et al. 2011; Kamarudin et al. 2017)**. The composition of keratin from feather has alpha helix (41%) and beta sheet (38%) structure. The composition of Quill fraction has beta sheet (50%) and alpha helix (21%) **(Barone et al. 2006;**

Schmidt and Jayasundera 2004; Wallenberger and Weston 2003; Fraser et al. 1972).Keratin is made up of 7% cysteine. This cysteine forms S-S bond with other cysteine and forms cysteine by disulphide bridges **(ARAI et al. 1983)**. Due to keratin's low chemical reactivity, the charge of the surface becomes positive and becomes pseudo-cation reactivity .Low pH, high temperature and reducing agents increases the solubility. The keratin components are least affected by physical, chemical and environmental factors **(TeresaandJustyna2011)**. One could find alpha keratin in wool, quill, hair, horns, fingernails and hooves and Beta keratin in feathers, avian beaks and claws, reptilian claws and scales. By their high cysteine content the keratin is differentiated from elastin and collagen. With alpha helix and beta sheets, **Gama Keratins** are non- structural **(Fraser et al. 1972; Hill et al. 2010)**. Based on the physical property of keratin's sulphur, it is classified as soft and hard form **(Rizvi and Khan 2008; Zoccolaetal .2009)**. In soft keratin, the cysteine content is low,the cross-linking is weak and the resistant to other chemicals is smaller. The soft keratins are found in hair and outer layer of epidermis **(Fraser et al. 1972)**. Hard keratins are found in mammalian epidermal appendages **(Douglas et al. 1996)**. Talking about the diverse family of keratin we can include the fibrous protein like horns, wools, nails, feathers and hooves. The proteins which are formed by high amount of cysteine filament are represented by these classes of biological co-products. Arrangement of chemical and mechanical resistance it significantly influences the keratin properties. The very known property of keratin, for example, not soluble in hot and cold water assists with forestalling their absorption with proteolytic proteins, for example, some enzymes such as, pepsin, papain or trypsin **(Bohacz 2017; Staron et al. 2011; Tesfaye et al. 2017)**.Human keratin protein genome can be encoded in almost 54 genes. These genes are categorized into two types of intermediate filament. First, type A has 28 classes and type B has 2 classes**(Schweizer et al. 2006)**and a single polypeptide chain is encoded by each type of gene. Smaller and more acidic proteins belong to Type A class while larger and more alkali proteins belongs Type B class **(Schweizer et al. 2006)**.The interaction of Type A and Type B class promotes polymerization of keratin resulting into a heterodimer **(Steinert et al. 1976; Fuchs and Cleveland 1998; Kim and Coulombe 2007)**. The integrity of epithelial cell lines is maintained by these two types of classes but, on the other hand, the severe epithelial disease such as epidermolysis bullosa may cause by their improper arrangement and misfolding **(Coulombe and Lee 2012)**.

2. Keratin Structure and Classification

2.1 Alpha- and beta-keratins

The sub-atomic load of keratin is down about 10,500Da and early it is comprised of polymerized chain of amino acids **(Staron et al. 2011).** The strong holding of H-atoms among amino and carboxyl gatherings in peptide chain leads to nonstop collapsing of polypeptide chain because of Beta-keratin and Alpha-keratin structure. The size of Alpha-keratin is approximately 7-10 nm and their molecular weight is approximately between 48-68 kDa **(Wang et al. 2016, Alibardil et al, 2006)**. The size of Beta sheet is around 3-4 nm and its molecular weight is around 10-22kDa.

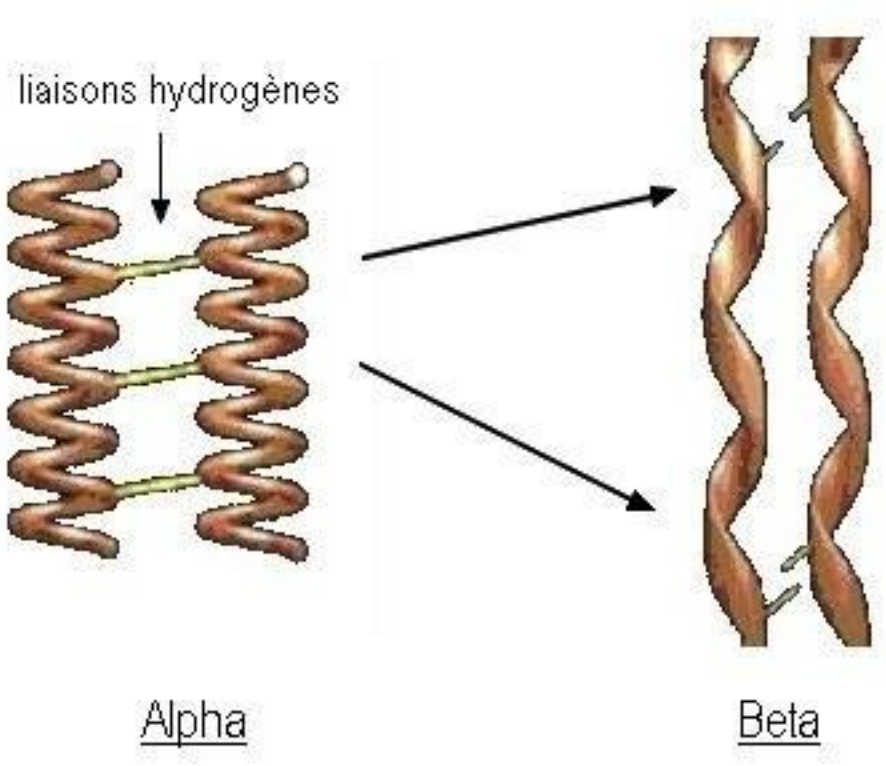


FIG: Structure of Alpha-keratin and Beta-keratin

Table: Characterization of Alpha-keratin and Beta-keratin

CHARACTERIZATION	ALPHA-KERATIN	BETA-KERATIN
Bonding	Intra-molecular H-bonding	Inter-molecular H-bonding
Size	7-10 nm	3-4 nm
Structural features	Intermediate filament matrix	Amorphous matrix
Molecular weight	40-68 kDa	10-22 kDa
Examples	Wool, horns, hairs, nails, and hooves	Avian beaks, feathers, claws, reptilian epidermis, and claws
Stiffness	High	Low

3. Sources of keratin

80% of human hair is shaped of keratin. It gives adaptability, quality, sturdiness and usefulness to hair as various conformations **(Velascoetal.2009).**

FIG: Sources of keratin from feather, hair, nail, horn, hoof and beak.

3.1 Hair, Feather, Wool and Beaks

To prevent feather from getting sloppy, keratin (Beta-form) protein is used to provide mechanical support to the feather. Birds beaks are characterized into two forms (1) Long and Thin (2) Short and Thick **(Wang et al. 2016)**. As fleece are the acceptable creature fiber and with it hair is the fantastic case of hard-keratinous material.

3.2 Epidermis of Testudines and Nails

Turtles and reptiles are addressed by the social affair testudines. Alpha and Beta keratins are rich in turtles (dalla valle et al. 2013). In hard-shelled turtle the alpha-keratins/beta-keratin content is higher in carapace. Nails are the huge wellspring of alpha-keratin and covers the completion of the fingers and toes. During the assessment under x-shaft diffraction plan these proteins structures show twisted paws and alpha-type configuration (bear and rugo 1951). The important feature of primates is fingernails, (hamrick 1998) because they utilize their nails during battling, scratching and opening of something.

4. Industrial and Clinical Applications of Keratin

It is extremely hard to degenerate keratin and their expulsion may provoke terrible impact on the earth. Therefore, research should proceed by focusing on the use of keratin waste (Bagewadi et al. 2018; Bohacz 2017; Karthikeyan et al. 2007). India has vast creatures and peoples, which show yearly: 82 million goatskins, 30 million sheepskins, and 28 million buffalo conceal (Karthikeyan et al. 2007). 8–10% weight of chicken is a direct result of their tuft and approximately immense countless measures of plume are made each year (Karthikeyan et al. 2007). Other than this, a colossal measure of horns, hairs, hooves, and tufts are wasted every year (Onifadeetal.1998). In past few years, these wastes are used in industry for the elimination of some (keratin) proteins which are not soluble and are noteworthy. To reduce this serious threat, these biowastes are used to convert them into beneficial structure. An extensive industrial application is that, keratin is found in polyvinyl alcohol fibers,forexample; utilized as absorbents for harmful/risky substances like generous particles similarly as formaldehyde gas (Rouse and Van Dyke 2010).

4.1 Alkali and Hydrothermal Treatment

The process of this treatment depends on a steam pressure of 10–15 psi with or without high temperature (80–140°C) in the presence of acidic solutions (Hydrochloric which is corrosive, Sulphuric which is destructive and that is just a glimpse of something larger) (Eggum 1970) or salt course of action (Sodium Hydroxide, Potassium Hydroxide, Sodium Carbonate and more) (Gousterova et al. 2003; Papadopoulos et al. 1985). Merging destructive and corrosive at the foaming temperature for around 2-3 h could open disulfide augmentations of the keratin structure and other structures such as water- dissolvable polypeptides and oligopeptides or sometimes hardly any amino acids. The huge motivation behind this methodology is the corruption of amino acids and polypeptide chains at high temperature that impacts the feeding improvement regards.

4.2 Use of Keratin in Leather Industries

Keratin is a biopolymer which have many functional properties, and these properties make (turn on the pathways of)the materials which are formed by keratin use at present day level. Efficient improvement of the fragment structure of keratin has natural waste things, participate in recuperation of standard assets and helpers in keeping up debasement free condition(Niculescu et al. 2016). Wool is the keratinous material having outstandingly defined structure and mechanical properties (Wang et al. 2016 b). Wool fiber contains approximately 82% of protein and significant level of cysteine, around 17% close by humble number of lipid wax and polysaccharides (Rippon and Lewis 2013). All of these properties together made the keratin significant in calfskin undertakings(2007).

4.3 Keratin in Medical Industry

The transport is an incredible strategy which incorporates the methodology of association of drugs, their absorption, and relationship with the goal site. For some medicine applications, drugs are arranged because it have the alternative properties as therapeutic effect or prescription adequacy with less toxicity (Nelson et al. 2002; Parveen and Sahoo 2008)

Such shows were designed for the production of keratin films loaded with drugs (Fujiietal.2004; Vasconcelos et al. 2010). So, a methodology was made using nano based PEG- actuated keratin for the steady transport to treat harmful development (Li et al. 2012).Keratins have bio-protectant which are complex and strong assistant properties. They show practically identical properties to collagen like they are not soluble, and have high flexibility, etc. These properties speak to the essentialness of keratin in clinical methodology. Midway and periphery devours are the consequence of patient organization with respect to multiple dressing. Infection, wound, injury, and outside injuries can hurt the skin and can increase the brisk in epithelial cells, so it is important to protect and restrict the skin from these type of major (unique) hurts (Loan et al. 2016).Skin is the periphery layer of the body that ensures about each living creature from serious threat to the regulation of homeostasis during troublesome conditions.. Credit and co-bundle, point by point means that "keragel and keramatrix" (keratin-based things) can be used during hurting of dermis and epidermis layers (Loanetal.2016).

5. Keratinous Biomasses Availability

As indicated by the EC guideline, the side-effects of the creatures are partitioned into three classifications dependent on their hazard examination:

(I) Category I: the reaction of animals in which the proximity of threats for individuals are possible and confirmed, for instance, limited chemicals and/or substances, and/or environment pollutants;

(II) Category II: animal by-products containing pathogens and/or chemicals (drugs) above the allowed limit;

(III) Category III: animal by-products originated from healthy and non-contaminated animals.

The biomasses of keratin can enter all of these arrangements, yet all around they are considered as order II or III. At present, a large number of colossal measures of biomasses including keratin(s) are produced by food industry annually and, by textiles companies at low extent; therefore, their safe evacuation presents that has certified the biological issues has to be gone with novel mechanical game plans prepared to deal with the expense of significant things (Jayathilakan et al. 2012; Tesfaye et al. 2017). Moreover, in light of scenes of jail ailments previously, the application of food industry is, the food products produced from keratin which are used in feeding has been lately subjected to serious restrictions legally in most of the foreign countries, therefore, the improvement of novel change systems for keratin- based biomasses has gotten fundamental. The biomasses of keratin is majorly produced in United States, Brazil and China and altogether the wastes from keratin produced is more than 40 million tonnes per year (Sharma and Gupta 2016).

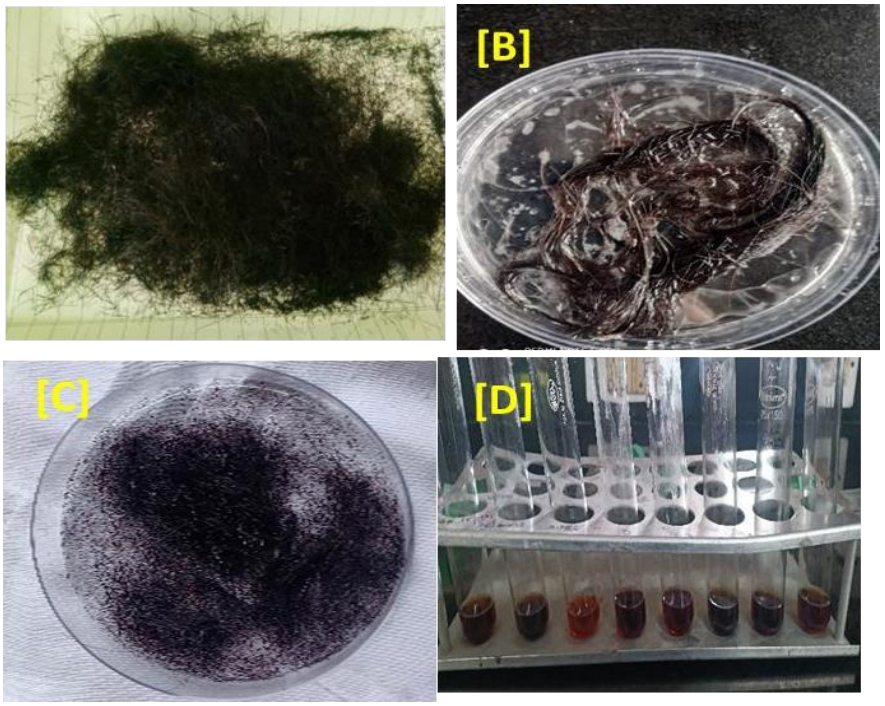
These common wastes can be utilized as a trademark source to remove the keratin to be used in business applications (Tesfaye et al. 2017). The leading group of waste wool is a problem related to sheep farming and butchery (Zoccola et al. 2015). The fleece which we get from sheep breeds is categorized into carpet grade (<50 μm fibre width) and coarse kind of wool (>50 μm fibre estimation) (Holkar et al. 2016). Coarse downy isn't sensible for making floor covers and clothing and subsequently the market value is very low. Indeed, the wool production cost is very high than the cost of shearing, sheep uphold, land renting, etc. Accordingly, sheep wool of coarse assessment has been considered as a futile consequence of sheep developing, which is or were misguidedly threw over the land for expending. Due

to regular defilement and threats of spreading irresistible disease, for instance, Bacillus anthracis (Gousterova et al. 2005), valorization developments of coarse wool for the formation of continuously noteworthy things reliant on keratin are consistently searched for.

6.Methodology for Keratin Extraction

6.1 Optimization of concentration of NaOH

Sample of human hair was taken and then washed with detergent and ethanol. Then the sample was sun dried. Sample was cut into small pieces and weighed 0.8 gm. Then different concentration of NaOH was taken such as (0.025%, 0.05%, 0.075%, 0.1%, 0.3%, 0.6%, 0.9% and 1.2%). Then sample was immersed in 10 ml of NaOH solution. For 15 minutes each tubes were centrifuged at 12000 rpm. The dry weight of the pellet was taken after discarding the supernatant. And then the best concentration was observed by calculating the yield.



[A] Hair sample was collected.

[B] Washed with detergent and ethanol.

[C] Hair dried and chopped into pieces.

[D]Hair (0.1 gm) was dissolved in different concentration ofNaOH.

6.2 Optimization of temperature

Sample of human hair was taken and then washed with detergent and ethanol. Then the sample was sun dried. Sample was cut into small pieces and weighed 0.4 gm. The best optimized concentration of NaOH was 0.075% and was taken in four test tubes. Then the sample was immersed in 10 ml of NaOH solution. Then the test tubes were kept at different temperature such as (30, 40, 50 and 60°C). For 15 minutes each tubes were centrifuged at 12000 rpm. The dry weight of the pellet was taken after discarding the supernatant. Then the best temperature was observed by calculating the yield.

6.3 Optimization of pH

Sample of human hair was taken and then washed with detergent and ethanol. Then the sample was sun dried. Sample was cut into small pieces and weighed 0.5 gm. The best optimized concentrations 0.075% of NaOH were taken on test tubes and different pH was set such as (5, 6, 7, 8 and 9). Then the sample was immersed in 10 ml of NaOH solution and kept at 60°C, the best optimized temperature. The optimization of pH is still in process.

7. Results

In this method, we observed that the best concentration in which the keratin is extracted is **0.075**and the best temperature in which the hair is dissolved and the keratin is extracted is **60°C**and the pH is still in process.

7.1 Optimization of concentration of NaOH

S. no	Concentration (%)	Weight of hair (mg)	Wt of keratin (mg)	Yield(%)
1.	**0.025**	**100**	**69**	**69**
2.	**0.05**	**100**	**88**	**88**
3.	**0.075**	**100**	**96**	**96**
4.	**0.1**	**100**	**64**	**64**
5.	**0.3**	**100**	**4**	**4**
6.	**0.6**	**100**	**55**	**55**
7.	**0.9**	**100**	**41**	**41**
8.	**1.2**	**100**	**92**	**92**

7.2 Optimization of temperature

S. no	Concentration (%)	Temperature	Wt of hair (mg)	Wt of keratin (mg)	Yield (%)
1.	**0.075**	**30**	**100**	**11**	**11**
2.	**0.075**	**40**	**100**	**15**	**15**
3.	**0.075**	**50**	**100**	**15**	**15**
4.	**0.075**	**60**	**100**	**45**	**45**

8. DISCUSSION

Present study results indicate that there is a variation in keratin characterization from the human hair when compared with the previous studies. It may be due to the keratin yield. For our research, we have taken Sodium Hydroxide solution because it was easily available in the laboratory. We could have taken Sodium Sulphite, Urea and Mercaptoethanol based on the previous studies **(Sinkiewicz et al. 2017), but** it was not available at that time in the lab. From previous study, the keratin yield using Sodium Sulphite was 84% but using NaOH, the yield is 96%. For optimization of temperature our yield is 45% which is less than the yield came in previous studies at 60°C **(Nakamura et al.2002)**

9. CONCLUSION

For economical administration of immense waste building up the systems for proficient extraction of keratin is exceptionally gainful. For pharmaceutical use just as in rural industry, the insoluble protein has numerous points of interest to create items. The hardest hotspot for assurance, taking care of and connection is given by keratinous materials. By engineered treatment of keratin, hair harm can be fixed. For advancement of new structure for the human advantage, keratinous material has differing basic and practical properties. Keratin protein have a few applications in tissue designing, tranquilize conveyance, water depuration, air cleaning, material getting done with, bundling just as in manures and creature feed. Keratin protein can be utilized for applications in cowhide ventures, medical procedure and fixing and some more. For as of late referred applications, the materials which are produced from keratin are used to make some kind of powders, humbler extension/nanoparticles, films, hydrogels, sponges and nanofibrous membranes.

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