

HIGHLY STABLE STARCH-KERATIN BASED BIO- PLASTIC FILM

Project report submitted in partial fulfillment of the requirement for the
degree of Master of Science

in

Microbiology

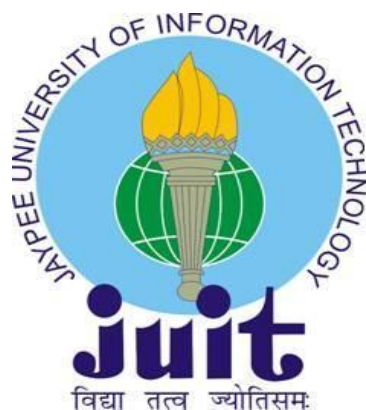
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DECLARATION

I hereby declare that the work presented in this report entitled “**Highly Stable Starch-Keratin based Bio-plastic film**” in partial fulfillment of the requirements for the award of the degree of “Masters in Microbiology” submitted in the Department of Biotechnology & Bioinformatics, “Jaypee University of Information Technology Waknaghat”, is an authentic record of my own work carried out over a period from January 2023 to May 2023 under the supervision of **Dr. Ashok Kumar Nadda** (Assistant Professor). The matter embodied in the report has not been submitted for the award of any other degree or diploma.

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SUPERVISOR'S CERTIFICATE

This is to certify that the project work titled “**Highly stable Keratin-Starch based Bioplastic film**” by **Sajid Siddiqui** during his end semester in fulfillment for the award of the degree of Master's in Microbiology of Jaypee University of Information Technology, Solan, has been carried out under my supervision. This work has not been submitted partially to any other University or Institute for the award of any degree or appreciation.

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TABLE OF CONTENTS

Content	Page No.
ABSTRACT	x
CHAPTER 1	
INTRODUCTION	
1.1 Introduction	0-1
1.2 Problem statement	2
1.3 Objectives	2
CHAPTER 2	
LITERATURE SURVEY	
2.1 Bio-based products	3-4
2.2 Bio-Plastic	4-6
2.3 Types of Bio-plastic	6
2.3.1 Starch based plastics	6
2.3.2 Plastics based on cellulose	6
2.3.3 Protein based plastics	6-7
2.3.4 Keratin and Starch based Bio-plastic	7-8
2.4 Introduction of Starch	8
2.5 Introduction of PVA	8-9
2.6 Introduction of Acetic acid	9
2.7 Introduction of Keratin	9-10
2.8 Techniques for Keratin Extraction	10-11
2.8.1 Keratin extraction by oxidation	11
2.8.2 Keratin extraction by using Microbial and Enzymatic process	11-12
2.8.3 Extraction of Keratin using Microwave radiation	12
2.8.4 Keratin Extraction by Alkaline method	12-13

CHAPTER 3	
METHODOLOGY	
3.1 Collection of Chicken feathers	14
3.2 Pre-treatment of Chicken feathers	14
3.3 Extraction of Keratin	15-16
3.4 Protocol for Keratin Extraction	16-17
3.5 Formulation of Keratin and Starch based bioplastic film	17
3.5.1 Keratin and Starch Control	17-18
3.5.2 Formulation of bio-plastic film using different concentration of Glycerol	19
3.5.3 Formulation of bio-plastic using different concentration of PVA, Starch and Keratin	20-22
CHAPTER 4	
Result and Discussion	23
4.1 Keratin yield calculation	23
4.2 Results of bio-plastic using different concentration of Glycerol	24
4.2.1 Results after using 5% Glycerol	24
4.2.2 Results after using 7.5% Glycerol	25
4.2.3 Results after using 10% Glycerol	25-27
4.3 Results of bio-plastic using different concentration of PVA, Starch and Keratin	27
4.3.1 Results of Composition 1	27-28
4.3.2 Results of Composition 2	28-29
4.3.3 Results of Composition 3	29-30
4.3.4 Results of Composition 4	30-31
4.3.5 Results of Composition 5	31-32
4.3.6 Results of Composition 6	32-33
4.4 Discussion of the results obtained	34

CHAPTER 5	
Conclusion	35
REFERENCES	36-39

List of figures

Figure number	Name of figures	Page number of figures
2.1	Used of bio-based products in different field	4
2.2	Structure of starch	8
3.1	Extraction of Keratin	19
3.2	Control of Keratin and Starch	20
3.3	bio-plastic film	
4.1	Formulation of bio-plastic film	25
4.2	by using 5% glycerol	
4.3	Formulation of bio-plastic film	26
4.4	by using 7.5% glycerol	
4.5	Formulation of bio-plastic film	27-28
4.6	by using 10% glycerol	
4.7		
4.8	Results of Composition 1	29
4.9		
4.10	Results of Composition 2	30
4.11		
4.12	Results of Composition 3	31
4.13		
4.14	Results of Composition 4	32
4.15	Results of Composition 5	33
4.16		
4.17	Results of Composition 6	34
4.18		

List of Tables

No of Tables	Name of Tables	Page of Table
2.1	Sources of Keratin	11
3.1	Composition of bio-plastic using glycerol	21
3.2	Composition of bio-plastic using PVA and keratin-starch ratio	22-23

Abstract

Nearly 390 million tonnes of synthetic polymers, such as plastics, are produced globally each year, which poses huge issues in environment, sustainable development, and human health. Despite being a sector of the world economy worth billions of dollars, plastic items and polymers derived from petrochemicals are not biodegradable, which is a serious environmental hazard. Synthetic polymers may be replaced with eco-friendly, long-lasting materials made from a variety of living things. Natural biomass sources can be used to produce biopolymers like sucrose, cotton, pectin, keratin, chitin, gelatin, and polyhydroxyalkanoates. All of these biopolymers have the physiochemical, thermal, and mechanical characteristics necessary for the development of bio-plastics that are derived from living things and are naturally biodegradable. One of the most prevalent and harmful keratin-rich waste biomasses is poultry feathers. Approximately 91% of the protein raw material is wasted when feathers are disposed of in landfills because it causes environmental damage. Keratin may be used in many different ways through chemical processing since it is hydrophilic, and biodegradable, keratin which makes up the majority of hair, hooves, horns, feathers, and wool, has a greater cysteine content (7–13%) when compared to other structural proteins.

In this work keratin Highly stable keratin-starch based bio-plastic film was composed and characterized. For this work poultry biomass (chicken feathers) were collected from M.C. Abattoir, Shimla, and then pre-treatment was done by soaking feathers in detergent and again soaked in petroleum ether for removing the fat and blood stains and dried in direct sunlight. Extraction of keratin powder was done by using alkaline hydrolysis and 56% keratin yield was obtained. Then the extracted keratin powder along with starch powder was used for formulation of bioplastic film by using different concentration of glycerol and polyvinyl alcohol and acetic acid. Several formulations were practiced and the best results were obtained by using 10% PVA with the ratio of 1:2 (Keratin-starch :PVA) solution.

The bioplastic was dissolved in water within 4 days. The formulated bioplastic can be used for various applications for example food packaging material, biomedical application.

Keywords: *Chicken feathers waste, Starch, Keratin powder, Bio-plastic, Waste management, PVA*

A type of plastic known as "bioplastics" is produced using renewable resources such as microbes, hemp, soy, maize, sugarcane, and chicken feathers. A variety of plastic waste has been created by rising human activity and rapid urbanization, which has accumulated in the ecosystem and accelerated environmental deterioration [1]. Every day, the livestock and poultry markets, slaughterhouses, and the wool sector produce millions of tons of waste biomasses high in keratin. The majority of keratin waste biomasses generated from living organisms are feathers. Many academics have shown interest in keratin waste biomasses in recent years with the aim of promoting their utilization and valorization due to the significant quantity of keratin found in these biomasses (80% in chicken feathers). One of the most prevalent structural proteins, keratin serves as the most significant biopolymer in mammals together with collagen. The secondary structure of feather keratin is composed of 11.18% random structures, 9.38% random helix, 47.19% random sheet, and 32.25% random turn [1]. It differs from many other structural proteins like elastin and collagen because it has a large quantity of cysteine residues (7%), resulting in a high Sulphur content. The keratin protein isolated from chicken feathers may be employed in a variety of biotechnological procedures, including sponges, film, and fibers, either alone or in combination with other synthetic polymers [2]. In addition, several researchers are striving to create viable materials from feather and keratin in an effort to reduce the pollution caused by feather waste and utilize it in a variety of applications. Among other things, bioplastic may be a use for feather and keratin that is disposable or bio-based and safe for the environment [1].

Functional keratin protein may be removed from chicken feathers using a variety of techniques, including chemical, enzymatic, and ionic treatments.

The origins, structure, characteristics, uses, and extract of keratin will be the key topics of this report.

1.1 Problem Statement

Since they have been around for a while, synthetic polymers are widely employed in daily items. Plastics are superior polymeric materials because of their physiochemical and mechanical characteristics, which enable them to be used in the manufacture of many everyday items. Despite having a wide range of uses, plastics are environmental pollutants due to their inability to degrade. Plastics are often employed for various applications from electronic devices to heat-resistant goods because of their physical durability. Environmental damage and climate change are caused by the non-biodegradable nature of synthetic plastics made from fossil fuels. Natural areas and water reservoirs have become pollution hotspots due to plastic trash and waste. To overcome this problem keratin-based bioplastic is used in the manufacturing of products because of its biodegradable property.

1.2 Objectives

- Collection and Pre-treatment of chicken feathers.
- Keratin Extraction from Chicken feathers.
- Formulation of Keratin and Starch-based biodegradable bio-plastic film for Packaging and Biomedical applications.

2.1 Bio-based products

Bio-based goods, excluding those that are fossilized or embedded in geological formations, are those that are entirely or partially made of materials of biological origin. In other terms, we may state that a commercial or business item (other than food and feed) that's also made entirely or primarily of biosimilars or domestic based on renewable (plants, animals, marine) or forest materials is referred to as a "bio-based product." Products made from biomaterials can help the economy become more sustainable and less reliant on fossil fuels [3]. Due to its significant potential for growth, re-industrialization, and tackling social concerns, the biobased goods sector has thus been identified by several nations as a priority industry. Forecasts predict that by 2020, with an annual growth rate equal close to 20%, the percentage of sales of all chemicals that are bio-based will increase to 22% from their current level of 12.3%. The toxicity, petroleum content, and volatile organic compound content of items made from biomaterials are all reduced (VOC). Enzymes are employed in industrial operations to create basic components, detergents, pulp & paper, textiles, and other goods. Higher process efficiency may be achieved by switching from traditional chemical synthesis to fermentation and bio-catalysis, which reduces the amount of hazardous waste produced as well as the amount of energy and water used in the process. Bio-based goods, which are made from renewable raw materials like plants, can reduce CO₂ emissions and provide additional benefits like lower toxicity or innovative product qualities (like biodegradability). There are so many benefits of using bio-based products [4]-

1. Enhance national security by reducing our dependence on imported oil.
2. Generating work for the agricultural and rural sectors
3. Make workplaces safer and healthier
4. Keep the ecosystem safe and intact
5. Production of bio-plastic
6. Decrease dependence on synthetic plastic

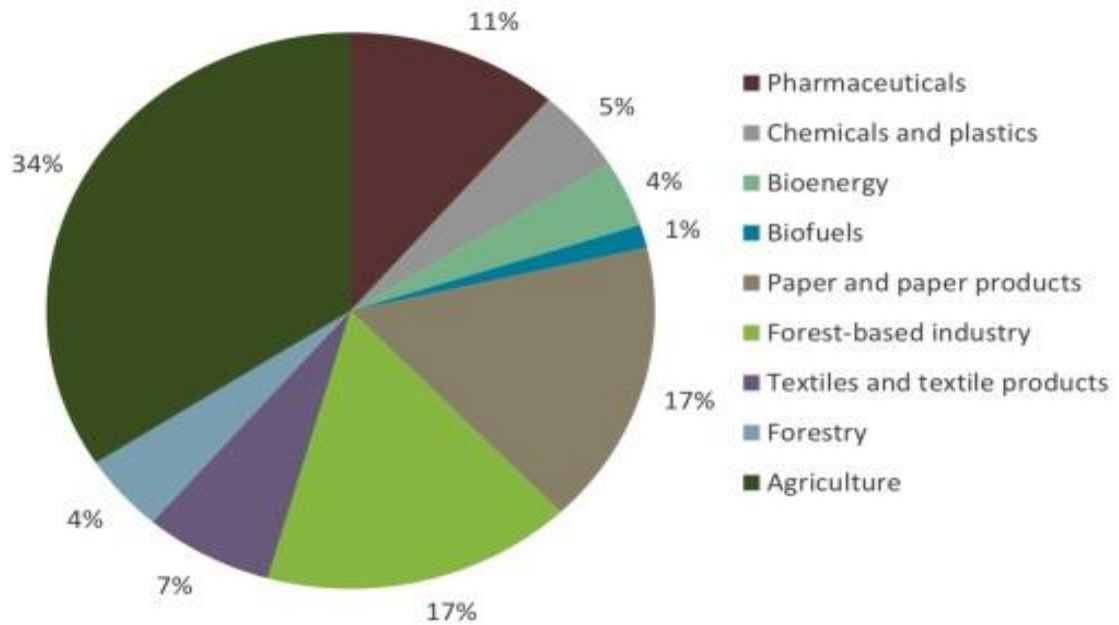


Fig.2.1

Use of bio-based products in different fields

2.2 Bio-plastic

The phrase "bioplastics" describes plastic products made from sustainable biomasses, including, but not limited to, plant fats and oils, maize starch, grain, wood shavings, dust, and salvaged food scraps. Some bioplastics are created by processing organic biopolymers like proteins and polysaccharides (including glucose, cellulose, chitin, and glycolate) that are found in nature. Others are created naturally through carbohydrate fermentation or lipids, or chemically from lipids derived from either plants or animals, such as lactic acid, and fats and oils. On the other hand, common polymers, like these fossil-fuel polymers (also known as hydrocarbon polymers), are created by petroleum or natural gas. The primary resource for the creation of sustainable polymeric material is natural polymers obtained from agricultural goods such as starch, proteins, plant oils, and cellulose [6].

The best biodegradable plastics are those that can eventually be totally broken down into smaller particles of CO₂ and H₂O. Commercial biodegradable polymers must include at least 50% organic molecules and adhere to heavy metal exposure limitations. The product must also have the capacity to decay (>90%) in 6 months under regulated environmental conditions

[7]. In order to determine the variables influencing the mechanical properties, thermal conductivity, and barriers and permeability qualities of biodegradable polymers, a number of experiments have been conducted after their synthesis. The characterization and investigation of a variety of different plastics' characteristics likewise make use of a wide range of analytical techniques. The following polymers fall into the (PHAs), (PHB), (PCL), Glucose, and Viscose are among the biodegradable polymers in this group. The creation mixes improve the matrices of the separate biodegradable polymers that can be used to overcome the disadvantages of these materials, according to studies [10].

The benefit of bioplastics is that they don't rely on fossil fuel as a raw material, which is a scarce and unequally distributed resource with political and economic ramifications. Using biomass as a raw resource and an energy source, for example, can yield some bioplastics with a carbon content that is comparable to their fossil equivalents, as per life cycle research. However, compared to the manufacture of fossil plastics, alternative bioplastics are less efficient and have a greater carbon footprint. Since resources like petroleum are just fossilized biomass, the difference separating biodegradable plastics and fossil-based polymers is mostly meaningless. As a result, the molecular structure of a plastic determines whether it is disposable or not (durable), not whether the biomass that makes up the raw material has been fossilized.

There are bio-based alternatives to polythene and polyethylene that are both long-lasting and biodegradable, such as poly (lactic acid, ethylene) succinate, and polyhydroxyalkanoates. Durable bioplastics include Bio-PET and polyethylene. To prevent plastic pollution, biopolymers must be recycled similarly to plastics made from fossil fuels; "drop-in" bioplastics (such as bio polyethylene) are compatible with current recycling processes [11].

Although the idea of biodegradation is not as simple as many people think, it may provide life pathway in some applications, such as field waste. Since various bioplastics have varying chemical backbone configurations and bioplastic susceptibility is largely reliant on the structure of the polymer, it cannot be expected that biopolymers in the environment will easily degrade. The opposite is also true; fossil fuels may be used to create biodegradable polymers.

Over than 380 million tonns plastics, or around 2% of global manufacturing, are produced in China —was made of bioplastics as of 2018. The production of fossil plastics is constantly

growing while bio-plastics are rapidly gaining market share thanks to continuous study of bio-plastics, financial support for bio-plastic companies, and increased public scrutiny of plastics made from fossil fuels [12].

2.3 Types of Bio-plastic

2.3.1-Simple bio-plastics made from maize starch are known as starch-based bioplastics.

2.3.2-Cellulose-Based bio-plastics: Made from cellulose ester and cellulose derivatives.

2.3.3-Bioplastics made from protein sources such milk, casein, keratin, and wheat gluten.

2.3.1 Starch-based plastics

With nearly 50% of the market share for bio-plastics, thermoplastic starch is the bio-plastic that is most commonly utilized. Humidity may be absorbed by pure starch. By varying the proportions of these additions, the qualities of the resulting bio-plastic, sometimes referred to as "thermoplastic starch," meet certain requirements are a few common polymer processing methods that may be utilized to convert starch into bio-plastic. The amylose/amylopectin ratio has a significant impact on the characteristics of starch bio-plastic. Superior mechanical qualities are often the product of high-amylose starch. The increased gelatinization and greater melting viscosity of high-amylose starch, however, make it less processable Starch-based bioplastics are routinely coupled with biodegradable polyesters to produce carbohydrate-protein, flours, or white rice adipate-co-terephthalate [13].

2.3.2 Plastics based on cellulose

The derivatives of celluloid, such as cellulose acetate and nitrocellulose, make up the majority of cellulose bio-plastics. When heavily altered, cellulose can turn thermoplastic. The pricey and infrequently used cellulose acetate is an illustration of this. However, since they are less hydrophilic than starch, cellulosic fibers a starch additive can enhance their automatic qualities, susceptibility to gases, and water opposition [20].

2.3.3 Protein-based plastics

Proteins from various sources can be used to create bioplastics. As potential starting points for various biodegradable polymers, wheat gluten and casein, for instance, have interesting features.

Another potential source of bioplastic is soy protein. For more than a century, soy protein has been employed in the manufacture of plastic. For instance, the original Ford automobile's body panels were composed of plastic derived from soy. Because of their moisture sensitivity and expensive price, soy protein-based polymers present challenges [21]. The cost and water sensitivity are therefore increased by creating soy protein blends with various biodegradable polyesters that are currently on the market. Due to growing environmental concerns, there is a growing need for bio-based materials that are safe and sustainable, which has forced substitution with readily accessible organic byproducts. Recently, byproducts from various animal source have been put to good use in the creation of bioplastic, drugs, and cosmetics. One of the most abundant proteins is Keratin present in the environment in the form of chicken feathers. For various applications, they can also be produced as hydrogels, films, and sponges [21].

Hairs, horn, fibers, feathers, and nails are primarily made of keratin. The structure and characteristics of these biopolymers are highly variable. Wools, hairs, nails, feathers, and horns are the most abundant sources of keratin because they are the least used sources of protein. Wool fibers are multi-cell structures with a high Sulphur content in general. The top five nations producing wool worldwide are Argentina, New Zealand, China, Australia, and Iran. Annual production of wool exceeds 2.5 million tons, and that of feathers exceeds 65 million tons [22].

2.3.4 Keratin and starch-based bio-plastic

The keratin protein isolated from chicken feathers may be utilized in a variety of biotechnological applications, including sponges, films, and fibers. It can also be combined with the other biopolymers to create new polymers.

Starch has been viewed as a viable raw material in the search for fully biodegradable polymers since it is a natural polymer, is inexpensive, renewable, infinitely available, and biodegradable. As a result, numerous efforts have been made to create starch-based polymers in order to preserve petrochemical resources and lessen their negative effects on the environment. Because it is environmentally friendly and biodegradable, bioplastic is a significant usage of keratin and starch [23].

Bio-based plastics have the potential to solve issues with sustainability and environmental problems brought on by the creation and disposal with synthetic plastics [24]. In this study I

formulated the bioplastic by the help of keratin and starch powder mixed with glycerol which is used as a plasticizer to increase the plasticity of the film and PVA (Polyvinyl Alcohol) is used to increase the stability and strength of the film. Cross linker used in this film is Acetic acid which helps in the cross linking of bonds between keratin and starch and it also provide coating to plasticizer and also helps starch molecules to stay disrupted after heating.

2.4 Starch

Starch is a tasteless, fluffy white powder that is not insoluble in alcohol, cold water, or other solvents but is soluble in them nonetheless. Starch is a polysaccharide made of 1,4 linkages between glucose monomers. The starch molecule has the formula $(C_6H_{10}O_5)_n$. Starch is made up of extensive strands of joined sugar molecules. The branching form of starch is called amylopectin, while the most basic form is a polymer with a linear structure amylose. Most often, plants employ starch to help in energy storage. Animals diets frequently include starch as a source of sugar. This enzyme, which breaks down starch for energy and is present in saliva or the pancreas, is required by animals in order to break down starch. Starch is the most ubiquitous polysaccharide in nature and is the one that is most usually associated with biomass derived from plants. The simple monosaccharide glucose is joined by 1-4 links to form the polymer that makes up this polysaccharide [25]. A simple structure of the starch is shown below-

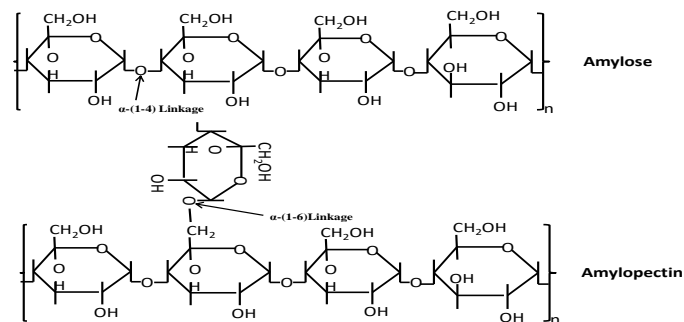


Fig. 2.2 Structure of Starch

2.5 PVA (Polyvinyl Alcohol)

The hydroxyl groups (-OH) in the structure of polyvinyl alcohol (PVA), a biodegradable synthetic substance, enable it to create intramolecular and intermolecular

hydrogen bonds to thermoplastic starch, strengthening the mixture's structural stability. PVA has been extensively employed in the materials sector due to its benefits of strong conglutination, good film formation, and high heat stability. Because it is nontoxic, water soluble, tasteless, odorless, and is impermeable to oil and fat, PVA is also frequently utilized in the packaging sector [30].

2.6 Acetic acid

Cross linker used in this film is Acetic acid which helps in the cross linking of bonds between keratin and starch and it also provide coating to plasticizer and also helps starch molecules to stay disrupted after heating.

2.7 Keratin

The family of fibrous proteins known as scleroproteins includes the structural protein keratin. Vertebrate species contain a keratin called alpha-keratin (-keratin). For vertebrate, it is the primary component of their outer layer of skin, scale, hair, fingernails, feathers, horns, and claws. Keratin also provides protection for epithelial cells against damage or stress. Keratin is very insoluble both in water and chemical solvents [32]. Reptiles, birds, amphibians, and mammals all have unmineralized epidermal appendages made of tough intermediate filaments, which are formed when bundles of keratin monomers assemble into bundles. The osteoderm of armadillos and the horns of cattle and rhinoceroses are two examples of tissues that have been strengthened by excessive keratinization.

There are two kinds of keratin: the primitive, soft forms that are present across all vertebrates and the tougher, evolved ones that are present exclusively in sauropsids (reptile and bird) [32]. Although the creation of the protein might well have done separately of a process in vertebrates, spider silk is categorized as keratin. All vertebrates have alpha-keratins (-keratins). the hair, horns, nails, claws, and hooves of mammalian species, as well as the threads of hagfish slime. keratinocytes produce a lot of keratin filaments, or keratinized proteins. They are generally found in epithelial cells [34]. Only sauropsids, which include all extant reptiles and birds, have the tougher beta-keratins (keratins). They may be found in wings, beak, and claw in birds as well as in certain reptile shells (testudines, including tortoises, turtles, and terrapins), nails, scales, and claws. The majority of this keratins are created in beta sheet. Whales to eat through filtering have keratin-based baleen plates. Recent

research has revealed that, both genetically and structurally, sauropsid -keratins differ significantly from -keratins [34].

Israel Hanukoglu and Elaine Fuchs identified the first keratin sequences. These sequences demonstrated the presence of class I and class II keratin, two distinct yet related keratin families. Based on their examination of the primary structures of such keratins along with other intermediate filament proteins[34].

Table 1.1 showing sources of keratin based on their type

Alpha-keratin	Beta-keratin
Wools	Bird beaks
Quills	Claws
Hair(humans and animals)	Reptilian claw
Horns	scale
Fingernails(humans and animals)	
Hoves stretum corneum	
Epidermis	

2.8 Techniques for Keratin Extraction

Keratin must be removed from the biomass in order to be used as a biopolymer. There have been various attempts in recent years to extract keratin using mechanical, enzymatic, and chemical processes[34]. There are several methods for removing keratin from the sources of keratin, this include-

2.8.1 Keratin extraction by oxidation

2.8.2. Keratin extraction technique using microbial and enzymatic processes

2.8.3. Extraction of keratin using microwave radiation.

2.8.4. Alkaline keratin extraction technique

2.8.1 Keratin extraction by oxidation

Earland et al. reported the early research on oxidation-based keratin extraction. For keratin extraction, he employed HCl, mild ammonia, and 2% peracetic acid. The oxidation technique was primarily used to remove keratin from wool and hair. The wool was not entirely dissolved during the extraction procedure, and insoluble beta-keratin was found in all studies. Alpha-keratin is the part of keratin that is totally soluble. Additionally, prolonged beta-disulfide keratin has been found to be less soluble than alpha-form. In another investigation by Weston, it was discovered that wool treated into 2% peracetic acid during 30 hours caused disulfide links to oxidise to sulfonate groups. Later, to validate these findings, Strasheim and Buijs carried out an infrared study and discovered cystine monoxide, dioxide, and sulfonate groups. Therefore, the disulfite to sulfonate conversion was at the heart of all these oxidation events. The partial oxidation of cystine to cysteic acid by peracetic acid or the loss of certain aminoacyls are the two main disadvantages of the oxidation approach for keratin extraction in comparison to other methods. Simmonds et al. noted that after wool was exposed to performic acid, it lost threonine, serine, tyrosine, histidine, and phenylalanine while nitrogen content rose. Tyrosine and phenylalanine were slowly restored by Smith and Stockell with peroxide of hydrogen (9:1 v/v) with performic acid (87%) [34].

2.8.2. Keratin extraction technique using microbial and enzymatic processes

These techniques are utilised in the biotechnology and food sectors to degrade keratin-rich materials like feathers and wool using microorganisms and enzymes. Because they operate as a catalyst and as a green, environmentally acceptable method for hydrolyzing keratin, enzymes are frequently used at a commercial basis. In comparison to other frequently

employed chemical techniques, this technology required less energy and had fewer stringent treatment requirements.

Certain bacteria produce keratinases, which are microbial proteases with the ability to hydrolyze keratin. Keratinases are mostly utilised in the cleaning of wool, the textile and leather industries, the treatment of sewage system obstructions, etc. Gram-positive bacteria are the most often studied bacteria for the breakdown of beta-keratin based compounds. The components made of alpha-keratin can be broken down by prokaryotes and keratinophilic fungi. The keratin can be totally disintegrated by certain *Bacillus* genus bacteria as well as keratinophilic fungi and Actinomycetes. *Bacillus licheniformis* strains have reportedly been shown to be capable of breaking down the proteins in feathers. In the literature, species of the genera *Chrysosporium* and *Dermatophytes* are described as keratinolytic fungus. These keratinocyte fungi are cost-efficient, mesophilic, and ecologically benign choices for keratin breakdown [34].

2.8.3. Extraction of keratin using microwave radiation.

The primary function of microwave radiation in this technique is to heat the solution, which shortens the processing time. The extraction yield that was about 60% was achieved by Zoccola et al. using microwave irradiation of power ranging from the range of 150-570 W at an ambient temperature for 180 °C for 7 min. The microwave procedure appeared to be quicker than the traditional steam processing method. The main disadvantage of this method is the considerable loss of amino acids like cysteine from 9.4 mol% to roughly 0.5 mol% in the final wool keratin sample after about 90 minutes of treatment. When compared to the uneven, non-uniform heating encountered in traditional heating, Chen et al. claim that the use using microwave heating approach significantly reduced the activation energy needed for keratin extraction. Due to the complicated keratin structure, there is currently a lack of knowledge on the interaction between electromagnetic radiation with the wool matrix. It was asserted that the hydrolysis of ester groups reduces the activation energy needed [34].

2.8.4 Alkaline keratin extraction technique

Due to possibility for sulphur nucleus splitting and the breakdown of cystine residue upon exposure, hot alkali solutions with a high concentration have the ability to dissolve feathers. The primary limitations of this technology, which prevent its widespread commercial

application, include the significant volume of alkali chemicals employed during procedure and damage to keratin's structure. Strong alkali solutions can be used to lower the quantity of NaOH needed for keratin extraction. When 1% sodium sulphide was given into NaOH solution (0.065 N). they saw faster degradation of feathers in around 30 minutes as opposed to NaOH solution. However, the leftover feathers from this reaction of NaOH with sodium sulphide had a significant sulphur content. Wool and feather keratin extracts contain 11–17% and 7% cysteine, respectively. Major amino acids like cysteine, which are easily broken by the presence of highly alkaline solutions, must be preserved throughout the entire extraction process. The main by-products of cysteine breakdown are oxalic and pyruvic acids [34].

They noticed the protein recovery yield following alkali treatment. They said that the technique resulted in the loss of half the initial material. He next exposed feathers to 0.1 N NaOH sol. For about fifteen minutes at ninety degrees Celsius, and he discovered that the end product had a different amino acid composition from the typical composition in a untreated feather sample. This approach produced Keratin with low levels of cysteine, serine, and arginine and high levels of lysine, methionine, and glutamic acid. Jiang Tao has suggested a two-step alkali reduction technique to recover keratin from hair samples. He initially used 0.1 mol/L, Na₂SO₃, SDS, with urea to treat the chosen samples for 5 hours. The beta sheet and alpha helix structures of the obtained keratin were well-preserved. In other words, the utilization of keratin on a big industrial scale is restricted by the severe chemical conditions during the alkaline treatment procedure [35].

In this report we use Alkaline method for Keratin Extraction because this method is more efficient, easy to do and cost effective in comparison with other extraction methods.

3.1- Collection of chicken feathers

Chicken feathers are collected from slaughter house and then we pre-treated them before use.

3.2-Pre-treatment of chicken feathers

Chicken feathers were soaked in detergent (labdet) with water at least for 24 hours and then these chicken feathers were boiled at 90 °C for 2-3 hours to remove the blood stains, fats, and grease from them and then these feathers were Again soaked in petroleum ether or hydrogen peroxide for removing remaining fat and other residues and then Wash the feathers 2-3 times with normal water and then Dry them for 2-3 hours in direct sunlight or in the incubator for 2-3 hours at 40 °C and in last Properly pack in plastic seal bags and store them for further analysis.

3.3-Extraction of Keratin-

- **Material and Method:**
- **Method used:** Alkaline hydrolysis.
- **Sample size:** 25 gm
- **Volume:** (0.5 M in 1L)
- **Raw material:** Chicken feathers
- **Chemical used:** Sodium hydroxide pellets, Hydrochloric acid, distilled water, petroleum ether (used to remove fat and blood stains from feathers during cleaning processes).
- **Glassware used:** Beakers, conical flask, plastic sieves, glass rod, weighing balance.
- **Instruments used:** Mechanical overhead stirrer, pH meter, fume hood, incubator, lyophilizer (for freeze drying), grinder, centrifuge.

Sodium hydroxide pellets

2 gram of sodium hydroxide pellets are used which is dissolved in 1 Litre of water in the concentration 0.5 M NaOH (sodium hydroxide) in 1 Litre distilled water. This work as a reducing agent, it degrades the feathers at a high rate.

It can be calculated by-

$$\text{Molarity} = \frac{\text{number of moles of solute}}{\text{volume of solution (in litre)}}$$

2N Hydrochloric acid

20 ml Hydrochloric acid is used which is work as a precipitating agent it precipitates the keratin molecules which are present in the NaOH solution. It can be prepared by mixing 834 ml of deionized water and 166ml of concentrated 12N HCl.

It can be calculated by-

$$\text{Normality} = \frac{\text{number of gram equivalent of solute} \times 1000}{\text{volume of solution in litre}}$$

3.4- Protocol for Keratin extraction

25 gram of pre-treated chicken feathers are chopped in small size and then these chopped feathers are dissolved in 1L of NaOH solution by mechanical overhead stirrer for 2 hours at 500 rpm and then continuous shaking is perform for 6 hour at 40 °C at 150 rpm in incubator, after incubation filter the solution with the help of plastic sieve to remove the undissolved residues after filtration pure dissolved lysate is obtained, to get the keratin in powder form the precipitation of solution is done by the help of 2N (HCl), Precipitation is done under fume hood with (2N HCl) by maintain pH up to 5 pH with the help of pH meter. Precipitates are washed 3 times with distilled water to remove the sodium hydroxide and hydrochloric acid, and then Centrifuge it to obtain the keratin pellets, then these pellets are Freeze dry at -55 °C for 24 hours by using the lyophilizer to obtain the keratin powder after keratin extraction from chicken feathers store the keratin powder at 4 °C for further analysis.

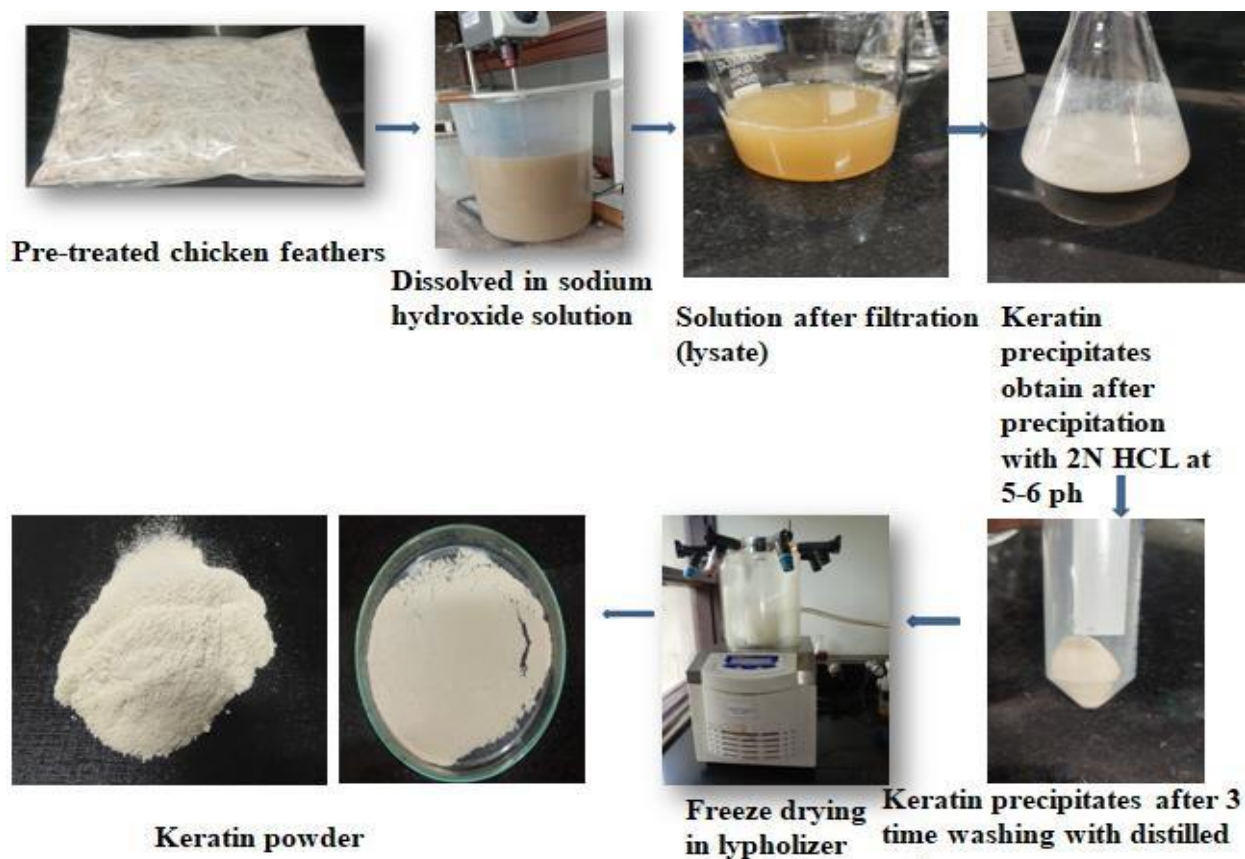


Fig.3.1

Figure showing Extraction of Keratin

3.5 Formulation of keratin and starch-based bio-plastic film-

Material Required-

Chemicals required- Keratin and Starch powder, Glycerol, PVA (Polyvinyl Alcohol), Acetic acid, Sodium hydroxide pellets, and Distilled water.

Instruments used- Magnetic stirrer, Thermometer, Hot air oven, Weighing balance.

Glassware's used- Beakers, Measuring cylinder, Petri plates, Conical flask, Glass rod.

3.5.1. Keratin and Starch Control

Starch control

Starch powder was dissolved in D.W. along with 10% glycerol and 5% acetic acid, later on which was placed on Magnetic stirrer for 1 hr at 60⁰ C at 600 rpm.

After 1 hr the mixture was poured in a petri plate and placed in Hot air oven for 24 Hour. The results was observed after 24 hour as shown in figure below-

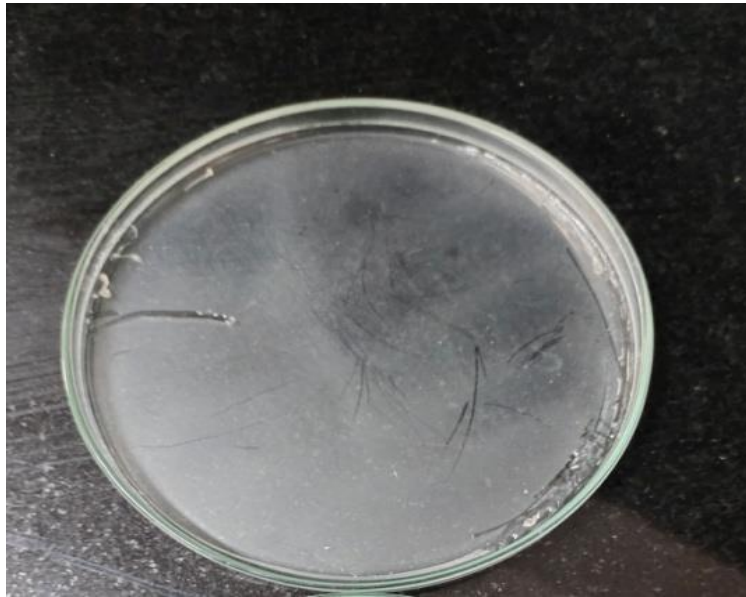


Figure-3.2 showing control of starch bio-plastic film

Keratin Control

Keratin powder was dissolved in 0.5 M NaOH solution, along with 10% glycerol and 5% acetic acid, later on which was placed on Magnetic stirrer for 1 hr at 60⁰ C at 600 rpm.

After 1 hr the mixture was poured in a petri plate and placed in Hot air oven for 24 Hour. The results were observed after 24 hour as shown in figure below-



Fig.3.3 showing control of keratin bio-plastic film

3.5.2 Formulation of bio-plastic film using different concentration of Glycerol-

Procedure:

Keratin powder is dissolved in NaOH solution (0.1M) and starch powder is dissolved in D.W respectively, and then combined using a magnetic stirrer at 60⁰ C for 20 min at 600 rpm. Different concentration and different amount of glycerol solution is now added with keratin starch(1;1) solution, and it is heated at 70⁰ C for 10 minutes. Petri plates are filled with the prepared solution, which is then incubated overnight at room temperature. After 24 hours the mixture checked to make sure it is fully dried.

Table 3.1 showing composition of Bio-plastic using Glycerol-

Keratin + starch	Glycerol		Result
	Concentration (%)	Volume(ml)	
1:1	5	5	Fragile shown in fig.4.1
		10	Very Thin sheet is formed shown in fig.4.2
	7.5	5	Fragile thin sheet is formed shown in fig.4.3
		10	No result shown in fig.4.4
	10	5	Thin sheet is formed with very low stability shown in fig.4.5
		7.5	No result shown in fig.4.6
		10	Thin sheet is formed with moderate stability shown in fig.4.7

3.5.3 Formulation of bio-plastic film using different concentration of PVA, Starch, and Keratin-

10-gram keratin powder is dissolved in NaOH solution (0.5M) and 10gram starch powder is dissolved in D.W respectively, and different amount of glycerol(10%), 10% PVA (Polyvinyl alcohol), and 10% Acetic acid is used for the formation of bio-plastic film. All these solutions are mixed in different amounts by the help of Magnetic stirrer at 600 rpm for 1 hour continuous stirring at 60⁰ C.

After the formation of different solutions these mixtures are poured into Petri plates for film formation different codes is given to every composition and incubated at room temperature (25⁰C) for 48 hours, and results were observed after 48 hours.

Table 3.2 showing composition of the bio-plastic film using keratin-Starch and PVA

Composition code	Keratin (ml)	Starch (ml)	Glycerol (10%) (ml)	PVA (10%) (ml)	Acetic acid (1%) (ml)	Results	Solubility time (In Days)
Composition 1	15	15	10	30	10	Thick film is formed with high stability shown in fig.4.8	6 days shown in fig.4.9
Composition 2	15	15	10	20	10	Thick film is formed with rough surface shown in fig.4.10	4 days shown in fig.4.11

Composition 3	15	15	10	10	10	Thin film is formed with smooth surface and high elasticity in fig.4.12	2 days shown in fig.4.13
Composition 4	15	15	10	7.5	10	No result a slimy solution is formed shown in fig.4.14	No result
Composition 5	20	10	10	10	5	Thin and hard film is formed shown in fig.4.15	4 days shown in fig.4.16

Composition 6	10	20	10	10	10	Thin sheet is formed with good elasticity and moderate stability shown in fig.4.17	1.5 days shown in fig.4.18
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4.1 Keratin yield calculation

Total keratin obtained after freeze drying = 14 g from 25 g of chicken feather sample.

$$\text{Yield \%} = \frac{\text{weight of keratin} \times 100}{\text{the initial weight of a sample}}$$

$$= \frac{14 \times 100}{25}$$

$$\text{Yield \%} = 56 \%$$

The amount of keratin which has been extracted from 25 g of chicken feathers is only 14 g which is the 56% yield. The decrease in yield is because of the long incubation after the mixing of feathers in NaOH, which causes the degradation of keratin and the yield decreases.

In previous studies the yield of keratin obtained after using alkaline hydrolysis method was 60% (Sandleen Feroz et al) [1]. Similarly in this study the same composition was used for keratin extraction and the yield obtained was 56%. The decrease in yield was due to excess of heat during extraction process.

4.2 Results of bio-plastic film after using different concentration of Glycerol-

4.2.1 Results after using 5% glycerol-

After using 5% glycerol in different amounts the results obtained are not satisfactory when 5 ml of glycerol is used there is no sheet formation, small pieces are obtained which were very brittle and a powdery form of mixture is also formed shown in fig.4.1.

After increasing the amount of glycerol from 5ml to 10ml the film is formed which was very thin and easily breakable, as shown in fig.4.2.

When the obtained results are compared with the previous studies the results are not satisfactory. When Singha *et.al.* use 30% glycerol the bio-plastic formed was flexible and having high tensile strength [15].

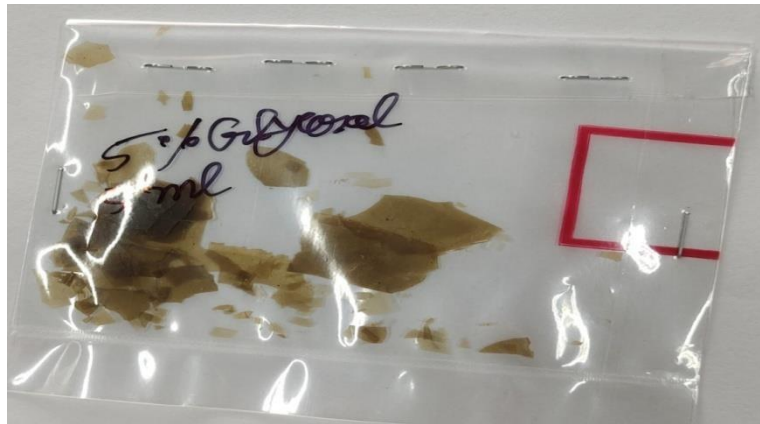


Fig.4.1



Fig.4.2

Formulation of Keratin-starch bio-plastic film using 5% glycerol

4.2.2 Results after using 7.5% glycerol-

After using 7.5% glycerol in different amounts of 5 ml and 10 ml the results obtained are not good enough. when 5 ml of glycerol is used there is a very small sheet is formed which don't have any smoothness in its surface and the sheet is easily breakable there is no stability in it, as shown in fig. 4.3.

When 10 ml of glycerol is used there is no any sheet formation a sticky solution was obtained, Shown in fig.4.4.

When the obtained results are compared with the previous studies the results are not satisfactory. When Singha *et.al.* use 30% glycerol the bio-plastic formed was flexible and having high tensile strength [15].

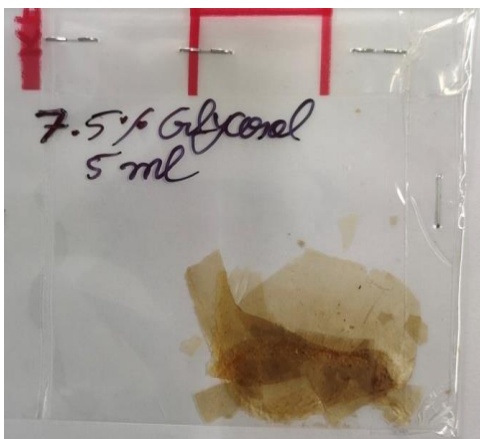


Fig .4.3



Fig.4.4

Formulation of Keratin-starch bio-plastic film using 7. 5% glycerol

4.2.3 Results after using 10% glycerol-

After using 10% glycerol in different amount of 5 ml, 7.5 ml and 10 ml the results obtained are not acceptable. When 5 ml of glycerol is mixed with the mixture of keratin-starch solution

thin sheet is formed but the sheet formed is easily breakable having 0% stability and easily breakable, shown in fig.4.5

When 7.5 ml amount of glycerol there is no sheet formation has been observed, a thin, but easily breakable structure is formed which cannot be called a sheet, shown in fig.4.6.

When 10 ml amount of 10% glycerol is used a thin sheet is formed having smooth surface and plastic properties like stretchability and having moderate stability, shown in fig 4.7.

When the obtained results are compared with the previous studies the results are not satisfactory. When Singha *et al* use 30% glycerol the bio-plastic formed was flexible and having high tensile strength [15].

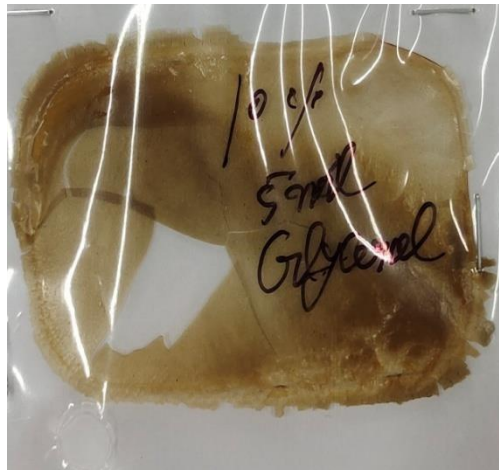


Fig.4.5



Fig.4.6



Fig.4.7

Formulation of Keratin-starch bio-plastic film using 10% glycerol

4.3 Results of bio-plastic film using different concentration of PVA, Starch, and Keratin-

For the formulation of bio-plastic film optimization of PVA, Starch and Keratin has been done along with 1% Acetic acid and 10% Glycerol, different composition has been formed and code is given to every composition i.e. “Composition code 1,2,3,4,5 and 6” shown in Table 3.2.

4.3.1 Results of Composition 1

In this formulation keratin and starch is used in 1:1 and 10% PVA is added in equal amount of Keratin-Starch solution for so that it can form the intermolecular hydrogen bonds with starch and Keratin, thereby increasing the integrity of the mixture. 1% Acetic acid is used to increase the cross-linking between the bonds of starch and keratin.

After incubation of 48 hours results were observed and thick film is formed with high stability shown in fig. 4.8.

When solubility test was done with 0.3 gram of film sample and 100 ml of tap water, it has been seen that it was completely dissolved in water after 6 days as shown in fig.4.9.

When the obtained results are compared with the work done by Yao *et al* there was difference in structure and color appearance. In this study structure appeared to be smooth and flexible whereas the bio-plastic appeared to be rough and dark in previous studies [37].

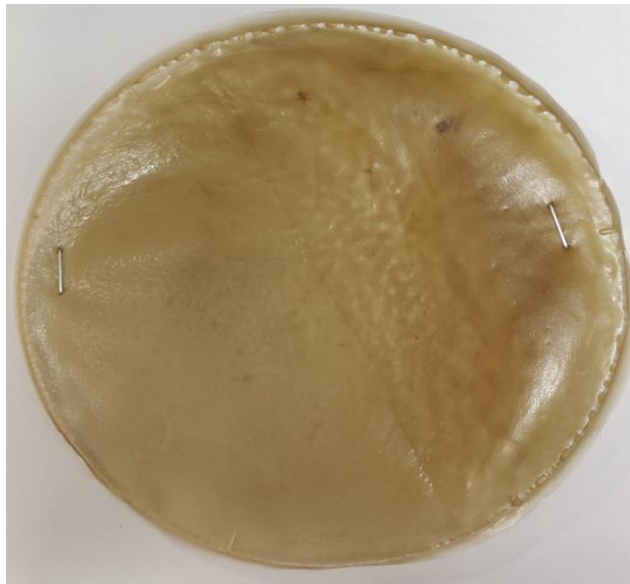


Fig. 4.8

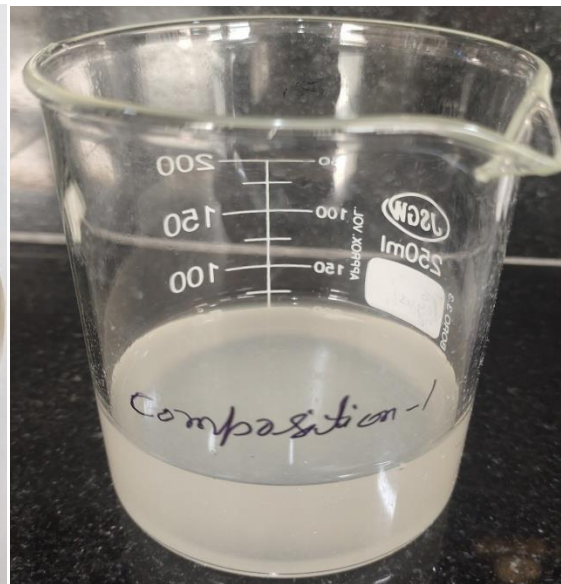


Fig. 4.9

Results of composition 1

4.3.2 Results of Composition 2

In this formulation keratin and starch is used in 1:1 and 10% PVA is added in 1:2(Keratin-Starch: PVA) amount of Keratin-Starch solution for so that it can form the intermolecular hydrogen bonds with starch and Keratin, thereby increasing the integrity of the mixture.

1% Acetic acid is used to increase the cross-linking between the bonds of starch and keratin.

After incubation of 48 hours results were observed Thick film is formed with rough surface shown in fig.4.10.

When solubility test was done with 0.3 gram of film sample and 100 ml of tap water, it has been seen that it was completely dissolved in water after 4 days as shown in fig.4.11.

When the obtained results are compared with the work done by Yao *et al* there was difference in structure and color appearance and the solubility is high. In this study structure appeared to

be smooth and flexible whereas the bio-plastic appeared to be rough and dark in previous studies and there is difference in solubility of film was also seen the obtained film have solubility period of 4 days while in the work done by Yao *et al* the solubility period was 7 days [37].



Fig. 4.10

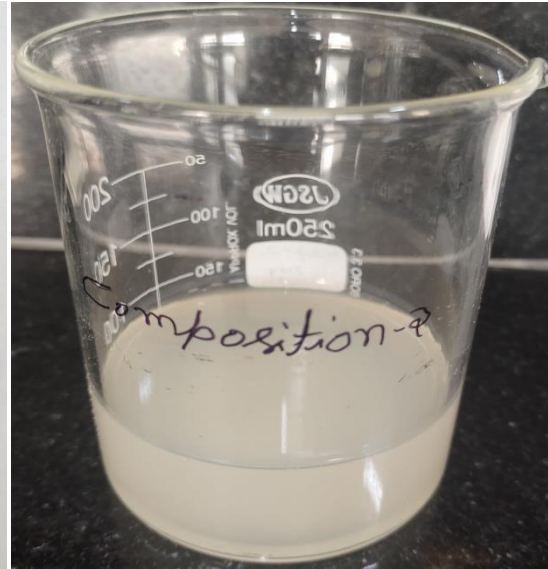


Fig. 4.11

Results of composition 2

4.3.3 Results of Composition 3

In this formulation keratin and starch is used in 1:1 and 10% PVA is added in 1:3(Keratin-Starch: PVA) amount of Keratin-Starch solution for so that it can form the intermolecular hydrogen bonds with starch and Keratin, thereby increasing the integrity of the mixture.

1% Acetic acid is used to increase the cross-linking between the bonds of starch and keratin.

After incubation of 48 hours results were observed thin film was formed with smooth surface and high elasticity as shown in fig.4.12.

When solubility test was done with 0.3 gram of film sample and 100 ml of tap water, it has been seen that it was completely dissolved in water after 2 days as shown in fig.4.13.

When the obtained results are compared with the work done by Yao *et al* there was difference in structure and color appearance. In this study structure appeared to be smooth and flexible whereas the bio-plastic appeared to be rough and dark but there is difference in the

degradability of the bio-plastic film. The film was degraded in 2 days while in the study of Yao et al the film was degrade in 7 days [37].

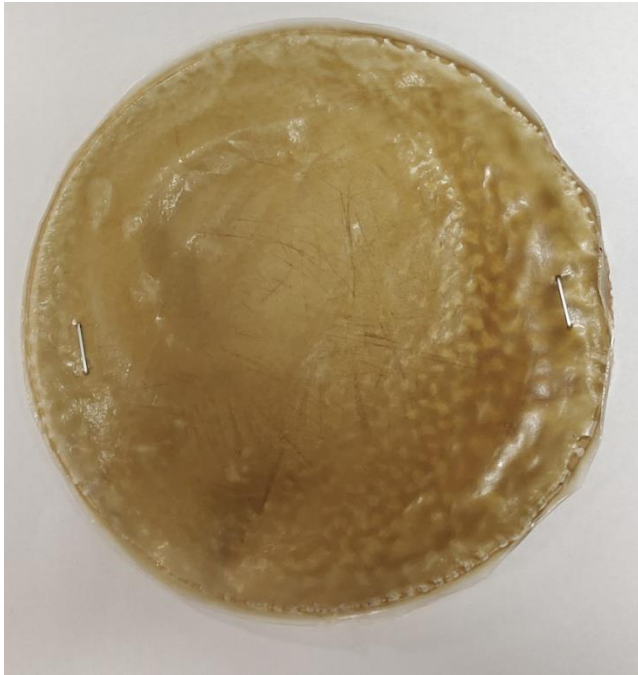


Fig. 4.12

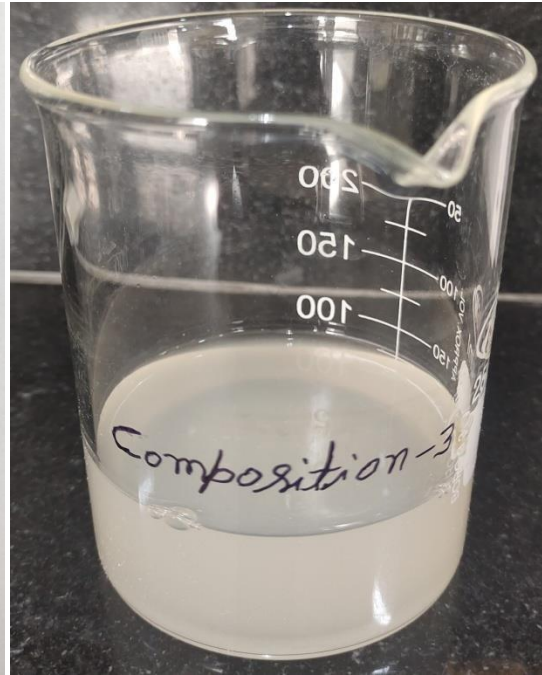


Fig. 4.13

Results of composition 3

4.3.4 Results of Composition 4

In this formulation keratin and starch is used in 1:1 and 10% PVA is added in 1:4(Keratin-Starch: PVA) amount of Keratin-Starch solution for so that it can form the intermolecular hydrogen bonds with starch and Keratin, thereby increasing the integrity of the mixture.

1% Acetic acid is used to increase the cross-linking between the bonds of starch and keratin.

After incubation of 48 hours results were observed and there is no any formation of bio-plastic film and a slimy solution is formed shown in fig.4.14.

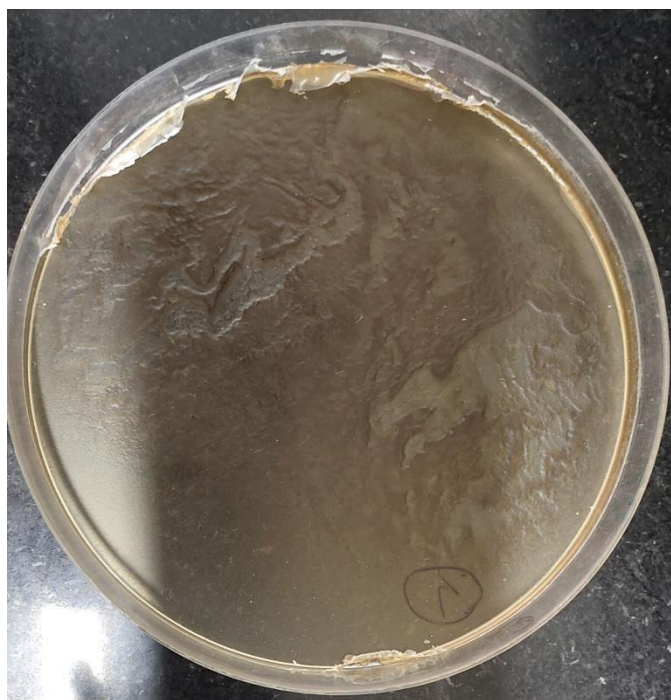


Fig.4.14

Results of composition 4

4.3.5 Results of Composition 5

In this formulation keratin and starch is used in 2:1 ratio and 10% PVA is added in 1:3 (Keratin- Starch: PVA) amount of Keratin-Starch solution for so that it can form the intermolecular hydrogen bonds with starch and Keratin, thereby increasing the integrity of the mixture. 1% Acetic acid is used to increase the cross-linking between the bonds of starch and keratin.

After incubation of 48 hours results were observed Thin and hard film is formed with rough surface and high flexibility shown in fig.4.15.

When solubility test was done with 0.3 gram of film sample and 100 ml of tap water, it has been seen that it was completely dissolved in water after 4 days as shown in fig.4.16.

When the obtained results are compared with the work done by Yao *et al* there was difference in structure and color appearance and the solubility is high. In this study structure appeared to be thick and flexible whereas the bio-plastic appeared to be rough and dark and in previous

studies there is difference in solubility of film was also seen the obtained film have solubility period of 4 days while in the work done by Yao *et al* the solubility period was 7 days [37].



Fig.4.15

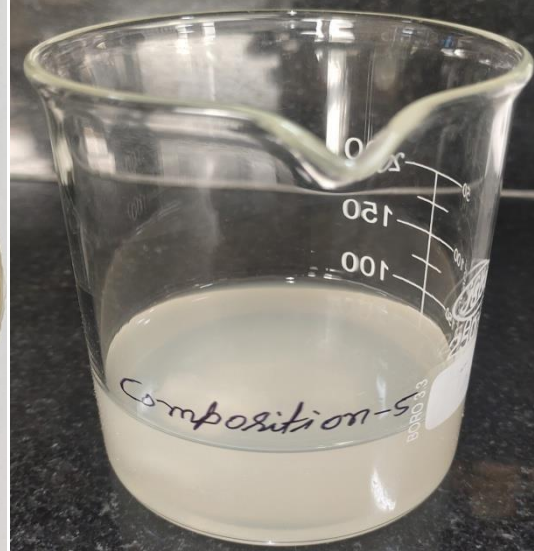


Fig.4.16

Results of composition 5

4.3.6 Results of Composition 6

In this formulation keratin and starch is used in 1:2 ratio and 10% PVA is added in 1:3 (Keratin- Starch: PVA) amount of Keratin-Starch solution for so that it can form the intermolecular hydrogen bonds with starch and Keratin, thereby increasing the integrity of the mixture. 1% Acetic acid is used to increase the cross-linking between the bonds of starch and keratin.

After incubation of 48 hours results were observed and a thin film is formed with smooth surface and high flexibility as shown in fig.4.17.

When solubility test was done with 0.3 gram of film sample and 100 ml of tap water, it has been seen that it was completely dissolved in water after 1.5 days as shown in fig.4.18.

When the obtained results are compared with the work done by Yao *et al* there was difference in structure and color appearance and the solubility is high. In this study structure appeared to

be thick and flexible whereas the bio-plastic appeared to be rough and dark and in previous studies there is difference in solubility of film was also seen the obtained film have solubility period of 4 days while in the work done by Yao *et al* the solubility period was 7 days [37].



Fig. 4.17

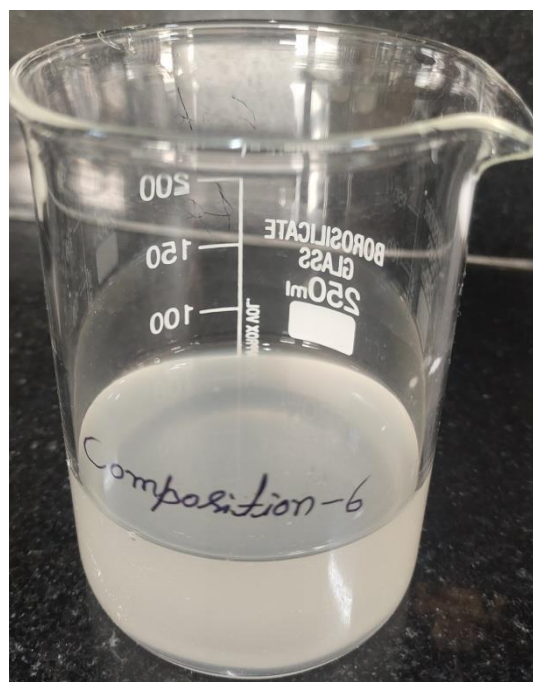


Fig. 4.18

Results of composition 6

Discussion

All keratin/starch composite films were thin, homogenous, transparent, and bendable. Their coloration ranged from yellow to white. The yellow colour of the films increased with an increase in keratin concentration. Initial findings showed that the films made of keratin and starch without plasticizer were brittle at dry conditions and soft at high levels of humidity. The films mechanical characteristics were enhanced. Glycerol, PVA and Acetic acid is added to the blended solution to increase the keratin/starch mix film's brittleness. Glycerol was shown to reduce internal hydrogen bonding between polymer chains, increasing the flexibility, homogeneity, and transparency of the film. They had no obvious holes or flaws and looked to have smooth surfaces. As the starch content rises, these films exhibit greater flexibility, a whiter colour, a homogenous surface appearance, and sticky properties while peeling the film from the plate. When several solutions of PVA and keratin-starch were combined, a delicate film was created. All of the films were simple to remove from the dish. However, both films that included PVA and films that did not contain PVA had distinct looks on the two sides of the film. Due to the polymeric arrangement during solvent evaporation, the film side facing the mould plate was shiny while the opposite side was dull.

When the formulated bio-plastic films were compared with the previous work done by Yao Dou *et al* [37] and Tamrat Tesfaye [40] it has been observed that the formulated plastic having PVA in equal concentration shows good results and in comparison to previous work the formulated plastic is not stable in comparison to the previous work done by Yao Dou *et al* [37] and Tamrat Tesfaye [40].

The development of techniques for such effective keratin extraction using chicken feathers resolve show to be highly advantageous for the long-term management of massive waste. For keratin extraction, researchers are striving to create a variety of chemical, microbial, and physical approaches alone as well as in combination.

Keratin extracted from leftover chicken feathers and waste starch were both successfully used to create biofilm. Additionally, the outcomes of the biofilm research showed that PVA containing biofilm outperformed those without PVA. In light of this, the study demonstrated that starch, a substance that is widely present in most underutilized wild tubers, and keratin in feathers, a frequent environmental contaminant, could be combined to create bio-plastics.. This might take the place of synthetic polymers, which have proved damaging to the environment. In general, the proportion of PVA, Keratin, and Starch in a mixture system can be changed to modify the properties of the films.

Keratin was found to improve the quality aspects of starch-based composite, such as the amount of moisture, opacity and transparency, as well as water solubility in the bio composite films, which were observed to increase the acceptability of the bio-composite for food packaging. In addition, it demonstrated advantageous mechanical characteristics, including high elongation and tensile strength at break, which supported its stability. The bio composite film's exceptional ability to degrade is evidence of its environmental friendliness. The keratin-starch bio-composite generally exhibited properties similar to those of nondegradable polymers. As a result, it might present a workable, environmentally responsible, and long-term solution to the problem of environmental solid waste accumulation. These protein-based films may be used in a variety of industries, including coatings, packaging, bioplastics, and regenerative medicine. The biomedical sector gains from the creation of pharmaceutical products for use in tissue engineering or the agricultural industry employing insoluble proteins.

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