Addis Ababa University
College of Technology and Built Environment
School of Chemical and Bio Engineering

Synthesis of Biodegradable Plastic Film from Waste Sheep Hair keratin and Corn Starch

By: Kewani Alemseged

Advisor: Dr. Sintayehu Nibret

A Thesis Submitted to the School of Chemical and Bio Engineering for the Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemical Engineering (Environmental Engineering)

ADDIS ABABA UNIVERSITY

ADDIS ABABA, ETHIOPIA

September, 2024

Approval Page

This is to certify that this thesis submitted by Mr. Kewani Alemseged entitled "**Synthesis of Biodegradable Plastic Film from Waste Sheep Hair keratin and Corn Starch"** for the partial fulfillment of the requirements for the degree of master of science in chemical engineering (environmental engineering stream) complies with the regulations of the university and meets the accepted standards concerning content, quality, and originality.

Approved By the Examining Board:

Chairman, School's	_____	_____
Graduate Committee	Signature	Date
Dr. Sintayehu Nibret	_____	_____
Advisor	Signature	Date
_____	_____	_____
External Examiner	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date

Declaration

I hereby declare that this research entitled "**Synthesis of Biodegradable Plastic Film from Waste Sheep Hair keratin and Corn Starch "** is based on my original work, towards the MSc degree and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the university, except where due acknowledgment has been made in text.

Abstract

This study presents the synthesis of a biodegradable plastic film from waste sheep hair keratin and corn starch. Biodegradable plastic films were prepared by first extracting keratin from waste sheep hair and mixing the keratin with corn starch in the starch to keratin ratio of 10:90, 30:70, 50:50, 70:30, and 90:10. The as-prepared biodegradable plastic films were characterized by using X-ray Diffraction (XRD), and UV-Visible spectroscopy. In addition, the effect of operating parameters (processing temperature, processing time and starch to keratin ratios) on the tensile strength and elongation at break of the plastic film were studied. The highest tensile strength (2.37 MPa) was achieved at a processing temperature of 80 °C, a processing time of 30 minutes, and a keratin-to-starch ratio of 30:70. This value is comparable to bioplastics produced from various starch types with glycerol as a plasticizer and without fillers, which typically exhibit tensile strengths ranging from 0.22 to 18.49 MPa. The biodegradability test revealed that the biofilms can be degraded within five days showing the biodegradability potential of bioplastic film from waste sheep hair and corn starch and their future application and capability of replacing fossil fuel-based plastics. The study demonstrates that the prepared bioplastics exhibit good transparency, are safe and environmentally friendly due to their biodegradability, and can be applied in household items, decorations, grocery bags, and related applications.

Keywords: Biodegradable Plastic, Keratin, Starch, Waste Sheep Hair

Acknowledgment

First and foremost, I would like to thank the almighty God who gave me the health and strength to accomplish this research work. I would like to express my gratitude to my advisor Dr. Sintayehu Nibret for his valuable guidance, encouragement, and motivation throughout this study. Moreover, I would like to thank the College of Technology and Built Environment, School of Chemical and Bioengineering laboratory assistants for their technical support. Finally, I am grateful to thank my family and friends for their continuous encouragement and endless support.

Table of Contents

List of Abbreviations

FTIR - Fourier-Transform Infrared Spectroscopy

TS - Tensile Strength

UV-Vis - Ultraviolet Visible

XRD - X-ray Diffraction

ASTM - American Society for Testing and Materials

ISO - International Organization for Standardization

BaSo4- Barrium salfate

CaCO3- calcium carbonate

CEN – European Committee for Standardization

DEHP- Diethylhexyl phthalate

GM- Genetic modification

HCL- hydrochloric acid

PHB- polyhydroxybutyrate

PHA- polyhydroxyalkanoates

PHV- polyhydroxyvalerate

PHH- polyhydroxyhexanoate

PE- PolyEthylene

PP- polypropylene

PET- polyethylene terephthalate

PLA- polylactic acid

PBS- polybutylene succinate

EB - Elongation at break

List of Tables

List of Figures

1. Introduction

1.1. Background of the Study

Leather making is one of the most widespread industries in the world. In tanneries, raw hides and/or skins go through different chemical and mechanical operations and converted into finished leathers. Hence, the tanning industry generates and releases huge amount of solid waste. Sheep hair is one of the wastes generated and not utilized in tanning industry and is usually dumped or incinerated. Due to poor solid waste management in such tanneries, some of the waste end up in water bodies and aggravate environmental pollution.

Current studies show that natural proteins are considered promising resources for the development of bio-based films as alternatives to petroleum based plastic films [1]. In contrary, waste from petroleum-based plastics impose a pollution problem on the environment by clogging water body streams, floating and shading of sun light in water bodies, requiring long degradation period and introducing micro-plastic and xenobiotic compounds to the ecosystem. Petroleum-based plastics have gained significant interest for a wide variety of applications from technological advances to the packaging sector due to their light weights, low production costs, durability, resistance against corrosion, thermal, and electrical insolation [2]. However, nearly 50 % of the plastics are employed in single-use applications, such as disposable consumer items and packaging, with food packaging accounting for the majority of plastic waste [3]. Many of these plastics are used in plastic bags or on food products, with a lifetime of mere minutes to hours, while the plastics stay in the environment for decades. By acknowledging the serious environmental and health issues attributed to petroleum plastics, and inevitable demand for their application in various industries, researchs in the development of environmentally friendly plastic, biodegradable plastics, has gained huge interest in the past decades [4-5].

To tackle the aforesaid problems different types of biodegradable plastics have been developed and some are commercialized. Compared with synthetic chemicals, natural proteins offer several advantages, such as being abundant, eco-friendly, renewable, and biodegradable. Natural biopolymers, such as polysaccharides, starch, proteins and lipids, can be found abundantly from plant and animal sources and they have great potential to be used as a feedstock for biodegradable packaging materials. One of the polysaccharides, starch, is a low-cost natural polymer that is originated from renewable resources [6]. Due to the film

forming nature of starch, it is used to make biodegradable polymers that have capabilities to replace petroleum-based packaging materials [3]. Starch is a completely biodegradable semi-crystalline polymer and a supplementary material for most plants. Along with many advantages, hydrophilicity, brittle nature and low mechanical strength limited starch-based bioplastic films in the food packaging and in many other industries [4].

Techniques such as plasticization, blends with cross linkers, and using stabilizers improve the aforementioned mechanical properties and water solubility of starch to produce competitive commercial commodities [6]. Hair keratin is a possible blend to thermoplastic starch and is a wildly available naturally tough polymer. Hair, nail, horn, feathers, beaks, claws, and hooves are considered as dead tissues, but these are mainly composed of a structural, fibrous, insoluble protein called keratin [4]. Hair keratin is unique compared to other biopolymers due to its rich cysteine content (7%–12%) [7]. Keratin is characterized for its greater mechanical stability, chemical resistance, and low solubility [7].

Figure1. Hair sample from Hodaoche tannery

Studies on biodegradable plastics from different sources of keratin showed the potential of keratin in different applications. However, compared to the most commercialized bio-plastics, keratin lacks some physical features in terms of strength and film appearance [7]. To overcome this problem, Tesfay et al. prepared a composite material from starch and feather keratin by taking advantage of physio-chemical compatibility of starch with keratin [8]. Tesfay et al. reported a thin, transparent, homogeneous and flexible film from chicken feather keratin and avocado seed extracted starch; the film show higher flexibility, white colour, homogeneous surface appearance and increased strength with the blended starch [9]. In a Tesfay et. al study, a biodegradable plastic which has been synthesized from waste sheep hair

and wool showed poor tensile strength and film appearance [8]. However, studies showed that blending of starch into keratin can improve the mechanical and physical characteristics of a bio-plastic [8]. On the other hand, a well-studied and commercialized corn starch biodegradable plastic have fulfilled the basic quality requirements, but have a limitation of being costly due to the edibility of corn starch [8]. Until now, there is no prior study on biodegradable plastic preparation from waste sheep hair extracted keratin and starch. In this study, waste sheep hair keratin and corn starch based biodegradable plastic is synthesized and characterized anticipating comparable or better physical properties than corn starch based biodegradable plastic.

1.2. Problem Statement

The tanning industry generates a large amount of solid waste, among which sheep hair is often discarded or incinerated without utilization. At the same time, plastic waste poses a significant environmental pollution problem. To mitigate the issues associated with petroleum-based plastics, various biodegradable plastics have been developed, some of which are already commercialized [4]. Previous studies, however, have shown limitations in the physical properties such as tensile strength and film appearance of biodegradable plastics derived from waste sheep hair and wool [2].

Starch-based bioplastics have been widely studied due to their biodegradability, abundance, and cost effectiveness; nevertheless, their practical applications are restricted by poor mechanical properties, particularly low tensile strength and water sensitivity, which limit their use in products like shopping bags [7]. To overcome these drawbacks, several strategies have been explored, including the incorporation of reinforcing agents or blending starch with other biopolymers. Among these, keratin, a fibrous protein found in hair, feathers, and wool, has gained attention for its film-forming ability and mechanical resilience. Studies have demonstrated that keratin-based films exhibit moderate tensile strength and flexibility, making keratin a promising additive or partial substitute in biodegradable plastics [4]. Furthermore, other research has shown the potential of combining keratin with polysaccharides to enhance both biodegradability and mechanical performance [9].

Limited work has investigated the direct substitution of starch with keratin extracted from leather industry waste, particularly sheep hair, without compromising the tensile strength of the resulting bio plastic. Addressing this issue provides an opportunity to valorize organic solid waste in alignment with circular economy principles. Accordingly, this research aims to develop a biodegradable plastic film for shopping bag applications by partially substituting starch with keratin extracted from waste sheep hair, using glycerol as a plasticizer, while evaluating whether this substitution can maintain or enhance the tensile strength of the material. This approach offers a sustainable, and environmentally friendly alternative to conventional single-use plastics.

1.3. Objectives

1.3.1. General Objective

The general objective of this study was to synthesize and characterize biodegradable films from waste sheep hair keratin and corn starch.

1.3.2. Specific Objectives

The Specific Objectives of this Research where:

- To extract and characterize of waste sheep hair keratin.
- To synthesis film from waste sheep hair keratin and corn starch.
- To study the mechanical and physico-chemical properties of the prepared biofilms.
- To investigate biodegradability of waste sheep hair keratin and corn starch based biodegradable film.

1.4. Significance of the Study

The tanning industry generate huge amount of solid waste. Sheep hair is one of the wastes generated and unutilized. Due to poor solid waste management some of the waste end up in water bodies and create environmental pollution. The parts which are incinerated also create air pollution burden. This study is important as it studies on the utilization of this waste to alleviate the environmental impact of the tanning industry.

On the other hand, the petroleum-based polymer plastics are non-biodegradable and found to have toxic effect on humans, animals and the environment and the majority of plastics are made from a limited resource which is unsustainable [6]. Thus, it is critical to replace fossil fuel-based plastics by a product that is biodegradable and sustainable such as by biofilms that are synthesized in this study.

Corn starch biodegradable plastics have been well studied and commercialized especially in use and through food utensils and materials. However, due to edibility of corn starch, competition with food resource and continual price increment hampered its competitiveness as raw material for biodegradable plastic. Thus, this study proposes a way of replacing corn starch by alternative raw material, waste sheep hair keratin.

1.5. Scope of the Study

This study developed biodegradable plastic films for shopping bag applications by partially substituting starch with keratin extracted from waste sheep hair. The research includes: extraction and characterization of keratin from sheep hair waste sourced from leather industry by-products; formulation of biodegradable films using starch, glycerol as a plasticizer, and varying amounts of keratin as a partial starch substitute; evaluation of the mechanical properties of the films, with particular emphasis on tensile strength, to determine whether keratin substitution maintained or improved performance; and assessment of basic biodegradability under controlled laboratory conditions. The study was limited to laboratory-scale preparation and testing, while large-scale manufacturing and commercial feasibility were beyond its scope. Although environmental impact assessment and economic analysis were not included, they are recommended for future research.

2. Literature review

2.1. Plastic and Waste Plastics

Plastics play an important role in our world and naturally occurring polymers such as rubber, waxes, resins, and horn have been used in for a variety of applications since ancient times. However, since the 19th century and with the development of petroleum based thermoplastics, a revolution in plastic industry has occurred [8]. Petroleum-based plastics, due to their light weights, low production costs, durability, resistance against corrosion, thermal, and electrical insolation, have gained interest for a wide variety of applications from technological advances to the packaging sector [9]. However, the increasing of generation and usage of petroleum-based materials in the recent decades has put an immense stress on the environment through generation of plastic waste and their accumulation in landfills [10] and oceans [4]. Nearly 50 percent of the plastics are employed in single-use applications, such as disposable consumer items and packaging, with food packaging accounting for the majority of plastic waste [11]. Many of these plastics are used in plastic bags or on food products, with a lifetime of mere minutes to hours, while the plastics stay in the environment for decades. It is estimated that the oceans are polluted with approximately 100–200 million tons of plastic waste, with 8 million tons entering the waters each year [12]. Micro-plastics generated from plastics breakdown are reaching alarming levels in the air, water, seafood, and table salt presenting a serious health issue for marine wildlife and humans [13].

Although only a low percentage of the carbon footprint is attributed to fossil-based plastic materials (4% of European greenhouse gas emissions are from using plastics); looking for alternative packaging materials with a lower carbon footprint and less emission of greenhouse gases is environmentally and economically interesting, and also helps the sustainability of the value chain [9]. Plastic packaging materials are one of the major challenges for waste management and the environment. For example, less than 30% of plastic waste generated in Europe is recovered for recycling and the majority is exported to the Southeast Asia [9]. By acknowledging the serious environmental and health issues attributed to petroleum plastics, and inevitable demand for their application in various industries, research in the development of environmental friendly plastic has gained huge interest in the past decades [10-12]. Moreover, the global economy is shifting to sustainable development strategy[14], particularly in the packaging supply chain, have accelerated the demand for shifting from

petroleum-based packaging to the bio-based plastics that are more effective, safer for human, and more eco-friendly [15].

2.2. Waste Sheep Hair

Leather making is one of the most widespread industries in the world. In tanneries, raw hides and / or skins go through different chemical and mechanical operations and converted into finished leathers. As a result, the industry is one of the polluting industries; operations during the processing of leather release different types of pollutants [11] such as generation of huge amount of liquid and solid wastes, emission of obnoxious smell because of degradation of proteins material of skin and generation of gases such as NH_3, H_2S and CO_2. There is considerable use of chemicals and water in the conversion of hides/skins into finished leather product and this generates a large amount of solid and liquid waste [16]. These wastes pollute the environment and pose threat on the health and wellbeing of human beings if they are not well managed. In comparison with the disposal of leather wastewater to damping site or incineration, the treatment of leather solid wastes is more intractable. Consequently, the best practice is valorization to other material production as an input raw material.

The leather industry produces solid organic wastes in the form of untanned (trimmings, fleshing's, splits, hair) and tanned (trimmings, splits and shavings) from raw hides and skins, semi-processed leather, as well as sludge as a result of wastewater treatment [8]. Waste sheep hair is one of organic solid waste generated during processing of leather and it is posing disposal problems due to bulk volume (low density) and requires long composting time due to slow biodegradability of hair. The tanning industry generate (75%) that will end up as solid waste. Currently most of the generated solid waste end up in open land fill [10]. From the main solid waste constituent, waste hair accounts for 2-5% as shown in table1.

Table 1: Tannery solid waste generation [8]

Type of waste	Amount (%)
Fleshing	30-50
Chrome shaving, chrome splits and buffing dust	25-30
Raw trimming	5-7
Hair	2-5
Sludge others	20-30

Even though the percentage of hair waste compared to the total solid waste generated seems small, due to the bulk volume (small density) of hair and slow biodegradability during composting, its disposal management is highly challenging in the tanning industry. The other difficulty the waste hair pose is after sun drying and during transportation and at the damping site since it is easily blown away by wind to the nearby areas and water bodies further aggravate environmental pollution problems. Thus, finding a way for utilization of this waste as a raw material in another industry is a sustainable approach for managing such wastes promoting circular economy.

2.3. Composition and Structure of Keratin

The structural units of keratin are 20 amino acids, united by varied inter and intermolecular links consisting of hydrogen, disulphide, hydrophobic, and ionic bonds, thus leading to increased mechanical strength and stability of keratin structure and keratinous materials[1]. The high stability, resistance, and insolubility of keratin are due to the network structure created by the numerous strong, covalent disulphide bonds between (-SH) groups contained in cysteine residues, within and between polypeptides of keratin [16]. The formed structure induces compactness to keratin, because of the network created by the adjacent polypeptides and sulphur–sulphur cross-links [17]. On the other hand, disulphide linkages avoid releasing some helpful amino acids and short peptides found in keratin [18].

Keratin is a fibrous protein with high content of cysteine in the amino acid sequence. Relative to other structural proteins, keratin presents a higher content of cysteine in its structure, ranging between 7–13% [19]. Besides cysteine, which is responsible for disulphide bonds that cross-link protein chains, keratin also includes important amounts of other amino acids

such as arginine, glycine, serine, proline, glutamic acid, and aspartic acid and the essential amino acids valine, leucine, and threonine. Low quantities are registered for histidine, lysine, and methionine. Glycine confers hydrophobicity, rigidity, and degradation resistance, and together with cysteine is responsible for keratins strength, low water solubility, and resistance to biological degradation, due to their presence in high levels. Glycine is present in high amounts especially in rigid keratinous materials, such as claws or beaks, where its level exceeds 28% [10].

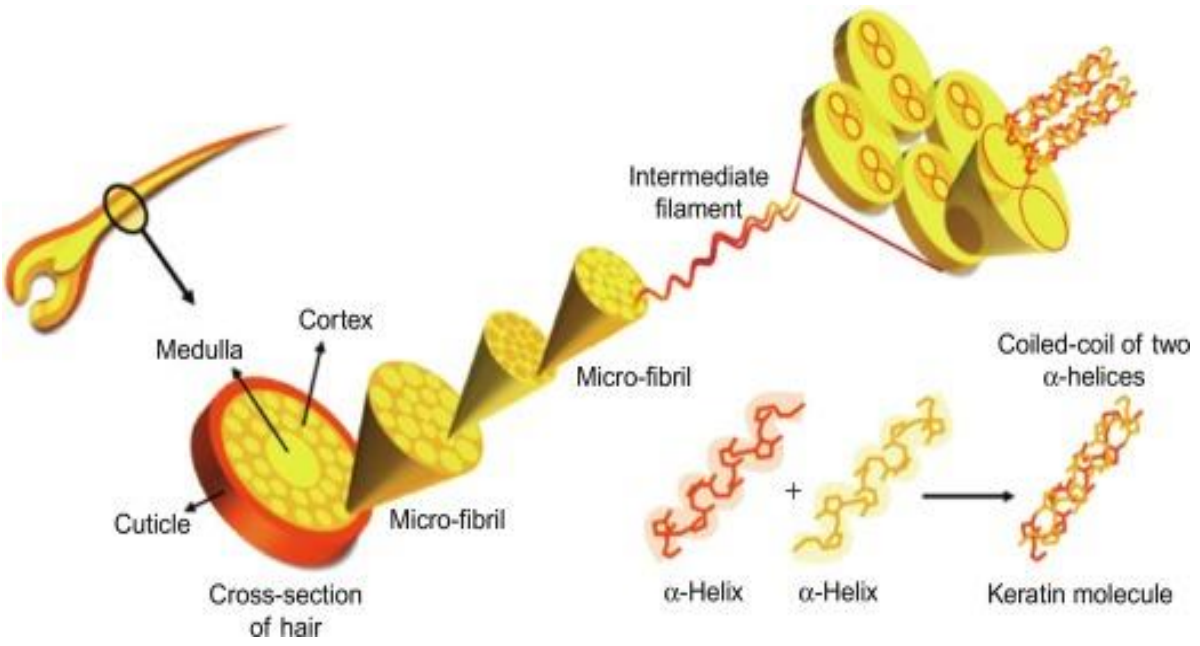


Figure 2: Hair keratin structure [10]

Keratin's solubility in water may be improved by using the heat accompanied by a reducing agent when a mild and acidic pH is present [17]. The structure of keratin is based on polypeptide chains, which can curl into helices in the α - conformation or configure into pleated sheet arrangements in the β -conformation [3]. Depending on their structure, keratins are differentiated in the literature as α-keratins and β-keratins [20]. Keratins have also been described the γ-keratins [21]. These distinct configurations are possible due to the differences registered within keratins' molecular structure and in their filaments' formation [7]. In regard to the α- and γ-keratins from hair, their special properties can be revealed only by using further advanced and laborious separation and purification methods of the crude extracts of keratin [21].

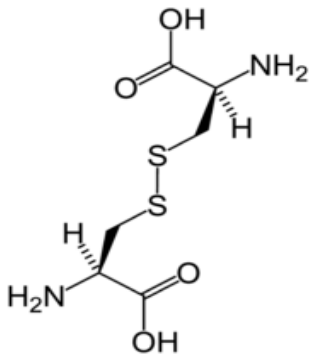


Figure 3: α- and γ- hair keratin structure [21]

2.4. Extraction of Keratin for Valorisation

Enormous amounts of keratin-containing waste (e.g., hair, wool, feather, and so on) are produced by various industries, such as textiles, food, leather, and so on. The world produces over 2.5 million tonnes of wool, greater than 40 million tons of hair, and more than 65 million tonnes of feathers every year [1–4]. This waste fur and hair, which contains about 90% keratin, remains a rich, yet unexploited bio-resource for bio-film production [5]. Disulphide bonds are the key to maintaining the structural stability of keratin molecules [22]. As such, the disruption of inter- and intra-molecular disulphide bonds in natural fur and hair is the most critical step during keratin extraction [23].

To extract keratin, highly concentrated chemicals (acid and alkali) are customarily used to destroy the inter- and intra-molecular disulphide bonds in the fur and hair, other methods include pressurised steam and thermo chemical extraction method. Conventional keratin extraction methods include methods such as acid hydrolysis, alkaline hydrolysis, sulphitolysis hydrolysis, reduction, oxidation, and enzymatic hydrolysis. All reported keratin extraction methods use chemical or enzymatic methods to cleave the disulphide bonds. The original ordered structure of the keratin is destroyed and a randomly disordered structure is formed as the fur and hair disintegrate during keratin extraction. In general, as the amount of chemical reagent increases, the degree of destruction of non-covalent bonds increases, which then improves the extraction efficiency of keratin.

Studies showed that various methods have been applied to extract keratin from keratinous waste. For instance, chemical hydrolysis of keratinous waste using strong acids can damage the keratin and destroy some of its amino acids [11] while supercritical water and high steam flash explosion treatments resulted in the disintegration of keratin [12]. On the other hand,

reduction, oxidation, and enzymatic hydrolysis can break disulfide bonds, which in the process leave the peptide bonds undamaged [13–15]. The oxidation method is time-consuming, requiring large quantities of oxidizing agents while the enzymatic method is also time-consuming in addition to being costlier and yielding very less keratin content.

The reducing agents such as sulfites, bisulfites, 2-mercaptoethanol, thioglycolic acid, and dithiothreitol are widely used for keratin extraction. These reducing agents produce free cysteine residues, and the resultant cysteine comprising derivatives generated are referred to as keratins. The cellular-binding motifs, such as LDV (leucine-aspartic acid-valine) and RGD (arginine- glycine-aspartic acid) [16, 17], are present in the keratin. They mimic the native extracellular matrix (ECM). Therefore, ensuring that bioactivity in the keratin is kept intact could be useful for the fabrication of biomaterials that may assist in bioplastic film preparation. Balaraman Madhan et al. investigated the optimization of keratin extraction from waste sheep hair and showed that thermochemical hydrolysis method is cheaper and gives improved keratin yield with intact structure [12].

2.5. Biodegradable Plastic Materials

Biodegradable plastic materials are commonly generated from sustainable natural resources or from the by-products of food and agricultural products and, based on the raw material used, can be categorized into three main groups. These non-toxic polymers exhibit considerable mechanical and barrier properties, and can be biodegradable and compostable making them excellent candidate material for food and agricultural applications [24].

Even though different types of biodegradable plastic materials are developed and under research, the commercialized ones include polylactic acid (PLA), Polyhydroxyalkanoate (PHA), starch based bioplastics, and others.

2.5.1. Commercialized Biodegradable Plastic

a. Polylactic acid (PLA)

Aliphatic polyester from lactic acid or lactase monomers is one of the most studied and commercialized biodegradable polymers with various applications in the industrial packaging sector. PLA is manufactured by the controlled formation of carbohydrates from different bio-sources such as corn starch or sugarcane. PLA films have a good mechanical and barrier

properties but due to the slow degradation rate in mild home composting conditions and their high dependency on hydrolysis for complete degradation, they need industrial composting conditions to be degraded [25] Moreover, PLA production is associated with a high water footprint ($0.248 m^3/kg$) mainly due to maize cultivation. Utilization of edible and nutritive plants such as corn, cassava, sugarcane, and sugar beet pulp as raw material for production of PLA are another drawback attributed to the industrial production of PLA.

b. Polyhydroxyalkanoate (PHA)

Polyhydroxyalkanoate (PHA) is another bio-based polymer with good mechanical and barrier properties which is completely biodegradable. PHA is manufactured from microbial fermentation of carbon sources and nutrients under nutritional stress [26]. Nevertheless, the high price of manufacturing PHA is limiting its application in the packaging sector; therefore, it is mainly used in high value-added products in pharmaceutical or medical applications [26].

c. Biodegradable plastic from natural biopolymers

Natural biopolymers, such as polysaccharides, starch, proteins and lipids, can be found abundantly from plant and animal sources and they have great potential to be used as feed stock for biodegradable packaging material. Polysaccharides are the most abundant natural polymers in nature. Starch, cellulose, and its derivatives, pectin, chitosan, alginate, carrageenan, pollutant, and gellan gum are some of the polysaccharides that have been extensively studied for producing biodegradable films. Polysaccharide films are associated with a low toxicity, mechanical stability, oil and lipids barrier properties, and selective permeability against oxygen transfer. Application of polysaccharides in edible films and coatings has been extensively studied [23]. A characteristic of these films is that they are commonly brittle and hydrophilic, which reduces their attractiveness for food packaging. The source of polysaccharide, the degree of substitution in cellulose, the degree of modification in starch, chemical groups attached to monosaccharides, and the molecular weight of polysaccharides are the most important factors influencing the properties of these films.

d. Biodegradable plastic from starch

Starch is a low cost polysaccharides polymer that is originated from renewable resources and it has abundant resources in nature [6]. Due to the film forming nature of starch, it is used to

make biodegradable polymers that have capabilities to replace petroleum-based packaging materials in future [27]. Along with many advantages, hydrophilicity, brittle nature and low mechanical strength limited the use of the film in the food packaging and in many other areas. To deal with these problems, there are many options to eradicate these hindrances. Starch is a completely biodegradable semi-crystalline polymer and a supplementary material for most plants. However, to improve the mechanical properties and water solubility of starches have led to the development of proposed techniques such as plasticization, blends of crosslinks and stabilizers to produce competitive commercial commodities [28].

In order to optimize the overall properties of such blends, starch can be blended with low molecular mass plasticizers such as glycerol, glucose, sorbitol, urea and ethylene glycol [23]. And the addition of plasticizers conduct to thermoplastic starch, [29], which is characterized by the spontaneous de-structuration of the semi-crystalline structure of starch and the formation of hydrogen bonds between the plasticizer and the starch [30].

Depending on the type of the plasticizer blended with the starch, the final properties of the thermoplastic starch differ. In general, plasticizers produce an increase in flexibility, extensibility, and fluidity by reducing the strong intermolecular chain interactions. The latest advancements and studies show that addition of glycerol as plasticizer and adding acetic or citric acid without changing the pH from neutral, a commercially viable thermoplastic starch can be produced. Additionally, plasticized starch is a very hydrophilic material and need enhancement in physical properties [31]. Recent advances in starch blending such as blending a thermoplastic starch with biodegradable polymers is one of the most recent advancements mainly in food packaging applications [24].

2.5.2. Biodegradable Composite Plastic Material from Keratin and Starch

a. Biodegradable plastic from keratin

Hair, nail, horn, feathers, beaks, claws, and hooves are considered as dead tissues, but these are mainly composed of a structural, fibrous, insoluble protein called keratin [4]. Slaughterhouses, breeding centres, textile mills, poultry farms and tannerys produce more than 5 million tons of keratin biomass every year [5]. Utilization of this waste for generating value-added products is an efficient method to alleviate pollution. Keratin belongs to the family of intermediate filaments (IFs) and has been observed to be a part of the cytoskeleton

in eukaryotes [8]. Keratin is unique compared to other biopolymers due to its rich cysteine (7%–12%) content [9]. Keratin is characteristic for its greater mechanical stability, chemical resistance, and low solubility. These properties are due to the presence of hydrogen bonds, compact micro fibrils, high sulphur content, and numerous disulphide crosslinks between the two cysteine amino acid residues on the separate polypeptide chains [10].

Various methods have been applied to extract keratin from keratinous waste. Chemical hydrolysis of keratinous waste using strong acids would damage the keratin and destroy some of its amino acids [11]. Therefore, thermochemical hydrolysis is usually used, as it not only improves the yield of keratin but also ensures that its structure is intact. Supercritical water and high steam flash explosion treatments resulted in the disintegration of keratin [12].

Research has shown that keratin can be used for manufacturing sponges and scaffolds, films, fibres, tissue engineering, and regenerative medicine due to its biodegradable, bio-compatible, and biological properties. Different studies have shown that keratin films are commonly too weak and mixing glycerol in the keratin solution leads to more translucent films with improved mechanical properties [32]. Yin et al. reported that keratin films, due to their good mechanical properties and also pH-responsive behaviour, can be used as an excellent candidate for producing controllable drug-released applications in biomedical fields [33]. The study of the effects of physical and chemical treatments on feather keratin films, by Poole and Church, showed that the drying condition has the greatest influence on the properties of the film [33].

Different chemical and physical treatments such as cross-linking by glycerol, formaldehyde or glutaraldehyde or soaking the films in isopropyl alcohol or adding a weak acid (acetic or citric) improved the physical properties of keratin films [3]. Keratin has also been used as biodegradable scaffolds for example in tissue engineering [1] or developing 3D-printing responsive materials [36]. Studies have been conducted on manufacturing keratin films from different animal co-products. The study showed that keratin films from sheep wool were transparent, a barrier to UV-rays, and exhibited considerable thermal stability up to 200 ^{0}C with no inherent thermal transition.

Further thermal cross-linking with glycerol and formaldehyde improved the mechanical properties of the film [33]. Edible cross-linked keratin films from poultry feathers with dialdehyde carboxymethyl cellulose (DCMC) were prepared by Dou et al. (2020) and the

physical analysis showed excellent transparency and UV-barrier properties for the films. Cross-linking enhanced the water barrier and solubility of the films, while the tensile strength and water solubility reduced [34]. Ding et al. (2020) introduced citric acid as a nontoxic and natural cross-linker to prepare feather keratin nano fibres via electro spinning method. The results showed that using citric acid increased the thermal stability of keratin nano fibres and cross-linking significantly increased the mechanical properties (tensile strength and elongation at break) of the films compared to the non-treated fibres. The study confirmed the possibility of using citric acid as a natural cross-linker to increase the physical properties of keratin nano fibres and their possible use in a variety of applications such as biodegradable packaging [35].

In another study, the effect of 1, 8-octanediol as a plasticizer on the reduced and native keratin films from duck feather was evaluated [36]. The results showed that the addition of plasticizer enhanced the mechanical and water resistance of films. Using formaldehyde as a cross-linker reduced the tensile strength of films while the elongation at break improved. However, using formaldehydes due to its toxicity restricts the application of the films in food packaging and biomedicine.

A much current study on waste sheep hair from tannery to synthesize a broken bone scaffolding adding (1.9%) of acetic or citric acid and glycerol as plasticizer showed best physical properties [3]. The study also showed that chemical hydrolysis of keratinous waste using strong acids would damage the keratin and destroy some of its amino acids and supercritical water and high steam flash explosion treatments resulted in the disintegration of keratin [12]. Therefore, thermochemical hydrolysis it not only improves the yield of keratin but also ensures that its structure is intact [3].

b. Composite biodegradable plastic from keratin and starch

Many studies on the developing of biodegradable plastic from different sources of keratin have showed the potential applicability of the material for different use. However, compared to the most commercialized bioplastic, keratin lacks some physical structures in terms of strength and appearance of the film. To overcome this problem different studies have been reported on synthesis of keratin with other material to form a composite material [32]. One of the studies being keratin-starch composite due to the compatibility of the physio-chemical property of starch with keratin. Tesfay et al. reported that a thin, transparent, homogeneous

and flexible film were obtained from chicken father keratin and avocado seed extracted starch. The film shows higher flexibility, was white in colour, homogeneous in surface appearance and increased its strength as the starch blended [32].

Olarewaju et. el. reported fabrication of a bio-composite film using waste chicken feather keratin and starch extracted from turmeric. The results showed the physical attributes of the bio-composite film, such as surface smoothness and significant increment of the tensile strength with increasing keratin content, while transparency and solubility significantly decreased with increasing keratin level. In this case, keratin extracted from feather incorporating with starch showed a good improvement in the physical and mechanical properties [12].

Prior study on keratin and starch based biodegradable plastic preparation mostly done on feather keratin. Based on the aforementioned studies, it can be speculated that the blending of corn starch into keratin can result in improvement of the mechanical and physical characteristics of the bioplastic to be obtained from keratin-corn starch bio plastic [32]. In addition, corn starch based bioplastic showed better quality than other starchs due to its higher amylose/amylopectin ratio since amylose affects the degree of crystallinity and entanglement of the product [37].

2.6. Factors affecting Bio plastic Synthesis

Materials used for food packaging, are required to possess a low permeability to water and exhibit acceptable mechanical strength and flexibility. However, biodegradable films obtained from natural polymers tend to have lower mechanical and barrier properties compared to those produced using synthetic polymers, thus limiting their industrial applications [37]. Protein and polysaccharide films, due to their hydrophilic nature, exhibit a higher permeability to water than synthetic films and generally have weaker and less flexible structure.

The characteristics of the polymers, employed as building blocks, dictate their potential application in packaging sector, as different products demand specific properties to guarantee their safety. Mechanical properties, barrier characterization, colour and optical properties, water solubility, biodegradability, and sealing properties are the main criteria of packaging polymers in industrial production. Mechanical properties, such as tensile strength and

flexibility, are important attributes of flexible packaging materials, influencing the barrier properties, integrity and product's attractiveness. These are commonly influenced by the source of the biopolymer, manufacturing methods, thickness of the film, and the relative humidity of the surrounding. One of the major drawbacks of bio-based films is their weaker mechanical properties compared to the synthetic polymers. Incorporation of plasticizers such as glycerol, sorbitol, or poly-ethylene glycol, are suggested to increase the mobility of these biopolymer chains by positioning between their molecules [37].

Chemical modifications, enzymatic cross-linking, physical treatments (irradiation or heat treatment), incorporation of different compounds such as essential oils or nanoparticles are other proposed methods to improve the mechanical strength and flexibility of biodegradable films [38]. Water barrier and gas barrier properties are important parameters for evaluation of food packaging materials as they can give information about the chemical structure, free volume between molecules in the film matrix, water affinity, crystallinity, and cross-linking of polymers. Mechanical and barrier properties of bio-based polymers are directly linked to their microstructure properties [34]. Polysaccharide and protein films are generally excellent gas barriers with a high tendency for water vapour permeability. Chemical and enzymatic cross-linking, irradiation, combining with different polymers, and incorporation of nanoparticles are the suggested methods to improve the water barrier properties of biodegradable films.

Due to the relatively high water vapour permeability of natural biodegradable polymers, such as proteins and polysaccharides, they are best used for short-term applications as moisture barriers, or can be useful in other applications such as modified atmospheric packaging (MAP) of fruits, vegetables, dairy products like cheese, and fermented foods like fish and meat where high water vapour permeability is required [38]. Colour and optical properties of packaging films directly affect the consumer's preferences, and can also help to protect the product against UV rays [10]. Generally, consumers prefer packaging with a higher transparency and less colour alteration as it gives a more realistic view of the product. However, some products are sensitive to the light and require less transparent packaging to visible light and UV in order to prevent oxidation reactions [34].

Water solubility is an important factor influencing the integrity of the packaging material which has been in contact with water and that has a direct effect on food quality. In general, biopolymers have a high solubility in water restricting their application in packaging,

especially for products with high moisture content. Chemical structure modifications or increasing the surface hydrophobicity of bio-based films, are the suggested methods to reduce water solubility of these bio-polymers. Biodegradation is another important parameter which show the eco-friendliness of the material, it is the change observed in polymeric materials by breaking down the structure and converting them into carbon dioxide, water, methane, inorganic materials, and biomass during contact with enzymes, or microorganisms such as bacteria, fungi, and/or algae. For successful application in industrial packaging the film must demonstrate good sealing properties. This can be limited due to the lower strength and elastic properties of the biodegradable films, compared to petroleum-based plastics [39].

Thermal sealing is the most commonly used technique in the packaging industry with the melted polymers forming a firm seal due to the inter-diffusion and entanglement of polymers from both layers [40]. Use of natural organic adhesives such as starch or dextrin, sugar-based glues or gelatine solutions are also proposed for non-heat sealing of biodegradable and edible films.

2.7. Possible Application Area of Keratin and Starch Composite Material

High-quality keratin films can be obtained by extraction from duck feathers by using urea and L-cysteine. The L-cysteine reinforced keratin films obtained through drying at room temperature showed good characteristics for biomedical applications [1]. Another interesting application of keratin is to use it for materials that allow drug delivery. For example, Posati et al. have prepared keratin-based films by using nano sized ZnAl hydrotalcite-type material in which intercalated diclofenac in anionic form. The obtained hybrid films supported a controlled drug delivery for wound healing and indicated the possibility to use these films for tissue engineering applications [37].

There is a huge potential of application of bioplastics composite material in the area of food utensils used as plates, spout, cups and food packaging due to the ability to improve the mechanical strength and heat resistance properties [12]. However, in current stage, lower strength of bioplastics from the required standard compared to petroleum based plastics are hindering bio-plastics applicability for long term food packaging [17]. Large market is available for sustainable eco-friendly grocery bag. Considering the characteristics of starch and sheep hair keratin it is preferable to use it as grocery store bag holder. Hence, more researches and development of novel bio- plastic materials is vital.

3. Materials and Methods

3.1. Materials

3.1.1. Raw materials and chemicals

The raw material used for the synthesis of biodegradable film from waste sheep hair keratin and corn starch was collected locally. Untanned solid wastes and waste sheep hair were collected from local tannery in Mojo town. All chemicals used in this study were analytical grade and they were used as received without any further purification. Chemicals such as sodium hydroxide (99.9%), sulfuric acid (98%) hydrogen peroxide (30%), and potassium hydroxide (90%), and iodine crystal (99.9 %) were used for raw materials preparation and synthesis of the biodegradable films.

3.1.2. Apparatus and Equipment

Buckets were used for soaking the waste sheep hair and a conical flask with controlled heating system was used for cooking during keratin extraction. A mixer was used for mixing the base solution and hair during hydrolysis while Petridis as a mold was used to form the final bioplastic film. In addition, Erlenmeyer flask, volumetric flask, measuring cylinder, beakers, vials, pipettes, thermometer, suction funnel, weighing balance, pH meter, moisture analyzer, plastic sheet, cutting tools, hose, spade, mesh wire for screening, and photo camera were used for the synthesis and characterization of bioplastic films throughout this study.

3.2. Methods

3.2.1. Waste sheep hair precursor preparation

First, a known amount of waste sheep hair was immersed in a mixture of liquid detergent and hydrogen peroxide (1%) for 2 h to remove any dirt and residues and was washed in a drum for 30 min. Then, it was washed three times with tap water to remove the surface-bound salts and other dirt. After that, the washed waste sheep hair was exposed to sunlight to obtain the dried form, which was used as the raw material for this study. Finally, the dried waste sheep hair was cut into small pieces using scissors and kept in sealed plastic bags [4].

3.2.2. Extraction of Keratin from Waste Sheep Hair

First, 0.5 M sodium metabisulfite (Na_2S) aqueous solution was prepared in 1L distilled water. Then, 70g of dried waste sheep hair was soaked in the 0.5 M Na2S solution for 5 hours. The

soaked waste sheep hair was then heated at 50° C for a period of 2 h with mechanical agitator to mix the hair and produce a complete hydrolyseate. The hydrolysate was filtered with 50 µm nylon mesh and cotton cloth. Unhydrolysed hair and impurities were removed using a filter paper. The hydrolysate was then centrifuged with 5000rpm for 5 minutes to remove the unwanted impurities and debris. The pH of the hydrolysate solution was brought to 2.5 to precipitate out the keratin from the solution using 1M HCl. The solution was then kept undisturbed for 3 h in a decanter to precipitate out the keratin forming white aggregates. Later, the keratin was dried at 45° C for 2 h and keratin flakes were obtained. Finally, the keratin flakes were crashed to get the powder form that was sealed and packed in plastic zip bag. The keratin yield was determined by the ratio between the weight of the dried keratin and the initial weight of dried waste sheep hair using equation 1 [4].

$$\text{Keratin Yield} = \frac{\text{Extracted kertin(g)}}{\text{Initial Hair weight (g)}} \times 100 \quad \text{...} \quad (1)$$

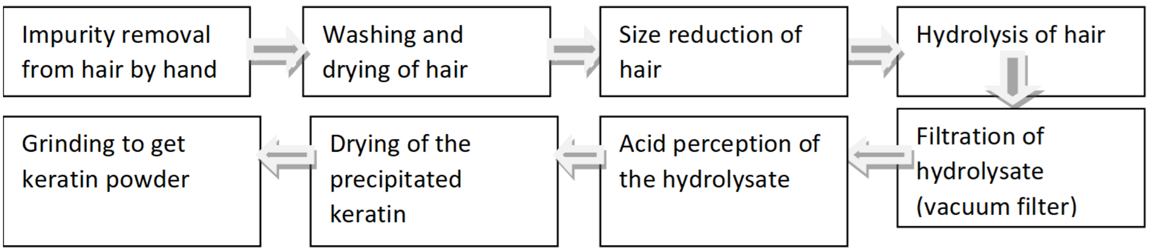


Figure 4: Process flow diagram of keratin extraction from waste sheep hair

3.2.3. Preparation of Biodegradable Plastic from Waste Sheep Hair Derived Keratin and Corn Starch

An aqueous starch solution was prepared by adding 5 g starch powder to 100 g deionized water (5% w/w) under constant stirring at 70 °C for 30 min. A 7 g of the as prepared keratin powder was added to 100 ml of 0.1 M NaOH and heated at 60 °C for 20 minutes with constant stirring with a magnetic bar. Various ratios of keratin to starch solutions10:90, 30:70, 50:50, 70:30, and 90:10 were used. And 1.9 % glycerol and 1 % acetic acid were added to the keratin starch solutions. Glycerol is a common plasticizer used in the preparation of bioplastic films. The mixtures were heated at different temperatures of 70, 80, 90 °C for 20, 30, and 40 minutes with continuous magnetic stirring to prepare keratin-starch blend

dispersion. The prepared keratin-starch dispersion was sonicated for 5 min to remove bubbles and form homogeneous dispersion that was poured into glass petri dishes (15 cm diameter) and was dried in a vacuum oven at 60 °C for 24 h. After drying, the films were peeled from the petri dishes and conditioned at a relative humidity of 65 ± 2% and a temperature of 20 ± 2°C for 24 h for further analysis [35].

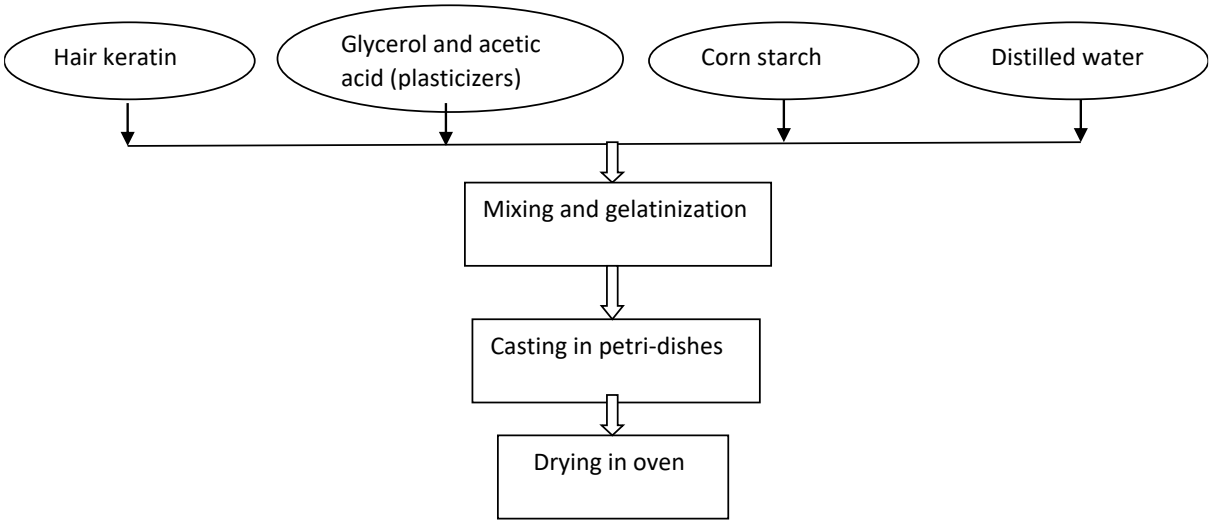


Figure 5: Preparation of biodegradable plastic from waste sheep hair keratin and corn-starch

3.2.4. Experimental condition

The effects of contact time, temperature, and mixing ratio during bioplastic film formation on the response variables, tensile strength and elongation at break , were studied by systematically testing all possible combinations of the selected experimental conditions, as shown in Table 2. The variation of operating parameters and experimental conditions is summarized in Table 5(Annex). A total of 45 experiments were conducted to examine the interaction effects of the three operating parameters on tensile strength and elongation at break, as presented in Table 5(Annex). Each experimental run was performed in duplicate, and the average values of the response variables, tensile strength and elongation at break, were calculated.

Table 2: Experimental Conditions

Parameter	Symbol	Unit	Condition/Value
Contact time	t	minutes	20, 30, and 40
Temperature	T	^{o}C	70, 80, and 90
Mixing ratio	MR	%	10:90, 30:70, 50:50, 70:30, and 90:10

3.3 Material Characterization

3.3.1 Fourier Transform Infrared Spectroscopy Characterization of Keratin and the biodegradable plastic

FTIR technique was used to identify the extracted keratin and the bioplastic interaction among the starch, plasticizer and acetic acid. IR Spectra of both crossed and the control where taken using FT-IR (Shimadzu-8400, Japan) in the range 400–4000 cm-1 with a resolution of 4 cm-1 by the accumulation of 32 scans. Before taking spectra of specimens, they were conditioned at RH 53 ± 1% for 24 h.

3.3.2 Mechanical property of biofilm

Mechanical properties of the films were evaluated in a texture analyser (TA.XT2, Stable Micro Systems, Surrey, UK) equipped with tensile test attachments, using two replicates. Tensile strength and elongation-at-break of the films were measured with the help of Texture analyser with a load cell of 5 kg and a crosshead speed of 0.5 mm/s. The test specimens consist strips of uniform width and length (1.5 cm × 6.0 cm), corresponding to the recommended size (ASTM D882) [16]. The distance between the grips was 4.0 cm. Tensile strength and elongation at break of the films were calculated from the initial linear part of the stress-strain curve of the specimen and percent change of the original length of the specimen between the grips of the texture analyser, respectively, according to a standard method (Farhan and Mohd 2017) [16]. All specimens were put in a desiccator for conditioning at 58 ± 1% RH at 25 ± 1 _C for 72 h. These tests were run immediately after removing the specimens from the desiccators, to minimize adsorption/desorption of water by them.

Conditioned specimens were mounted between the grips with an initial grips distance of 50 mm. Tensile strength and elongation were determined by applying following Equation (2) and (3).

$$(TS) = \frac{F}{A} \qquad (2)$$

$$EA\ (\%) = \left(\frac{X_1 - X_0}{X_1}\right) \times 100 \qquad (3)$$

Where F is the maximum force at break point, A is the cross-sectional area of specimen (Thickness, Width), X1 is the initial gaps in grips and Xo is the length of the specimen at break point. Three replicates were used to analyse for each formulation of the film [16].

3.3.3 Biofilm Solubility

The film solubility (%) in water is defined as the ratio of water-soluble solids, after 24 h immersion in water, to the initial solids content [8]. The biofilm specimen for this test was cut with an area of 1.5 × 1.5 cm^2 and, after conditioning, was weighed and placed in Erlenmeyer flasks with 20 ml of distilled water, which was sealed with para film and kept at 25 ºC for 24 h and it was dried at 105 ºC till constant weight was achieved. On the other hand, a control biofilm that was kept at ambient temperature was dried at 105 ºC till constant weight was achieved and the mass was determined. At least three replicates of each film was tested and film solubility was calculated as the average value of the measurements. Biofilm solubility of the keratin/starch was determined according to the standard method [41] and film solubility was calculated using equation 4 [41].

$$Solibility\ (\%) = \left(\frac{W_1 - W_0}{W_1}\right) \times 100 \qquad (4)$$

Where W0 is the weight of the dried specimen before water immersion and W1 is the dry weight of the insoluble specimen after immersion.

3.3.4. Biofilm Thickness

Keratin-corn starch films thickness was measured using a digital micrometre with an accuracy of ± 0.001 mm (Mitutoyo, Japan). The thickness of each film was evaluated at three different positions and then average of thickness was taken.

3.3.5. Moisture Content (MC) of biofilm

The moisture content of the films was measured in terms of weight loss. A sample of specimens with 2*2 cm dimension were each film and dried in a hot oven at a temperature of 105 ^{O}C for 24 hours. The dried sample was constantly reweighed at 24 hours' time interval until a constant weight (W1) was obtained. The difference in weight was recorded and the moisture content (MC) was calculated from the standard as loss in weight on drying divided by the initial weight of carbon multiplied by 100 as shown in equation 5 [42].

$$MC\% = (\frac{W_1 - W_2}{W_1}) \times 100 \ldots\ldots (5)$$

Where W_2 is the mass of the pre-dried specimen and W_1 is the mass of the dried specimen.

3.8.5 Moisture Absorption

Moisture intake (MI) was evaluated according to standard procedure [42]. Specimens with dimension 2 * 2 cm were cut from the film. Prior to evaluation the moisture absorption capacity, all specimens were dried in hot air oven at 105 ± 1 _C for 24 h and weighed [42]. Subsequently, dried specimens were transferred into three desiccators containing saturated solution such as sodium chloride, copper sulphate and potassium sulphate for maintaining different relative humidity and then all desiccators were kept at 25 ± 1 _C in an incubator. The gain in weight of all the specimens was recorded until the constant weight achieved. Calculation of water intake was evaluated according to equation 6:

$$\text{Moisture absorption } (\%) = (\frac{W_1 - W_0}{W_1}) \times 100 \ldots\ldots (6)$$

Where W_0 is the dry weight of the specimen and W_f is the equilibrated weight of specimen. All the results were evaluated in triplicate.

3.8.6. Biodegradability of Film

The biodegradability test of the keratin-starch biofilms in soil was studied following the method of Pavin et al.[4] A weighed amount of the keratin-starch biofilms was completely buried inside the soil for a period of 12 days and at every three-day interval, the bio-composite film was exhumed and weighed. In a typical experiment, specimens with a dimension of 10 cm by10 cm were sliced from the films. Then, specimens were buried below 2 cm in steel trays containing bio-compost fertilizer. Then, all samples holders were placed in an incubator with humidity (RH of 75%) at 25 ± 1C. To check the degradation rate in terms of weight loss of specimens were recorded after removing the specimens from the medium at different intervals, dried them at 50 ± 1 _C for 24 h and weighed.

3.8.7. Optical Characterization by UV-Vis Spectroscopy

The degree of transparency or opaqueness of the bio film was measured with the aid of a UV–visible spectrophotometer (Jenway 7305, UK). Ultraviolet and visible light spectra (UV-Vis) were recorded in the wavelength range of 800–200 nm. Films were placed across the beam and spectra was recorded. Films opacity was defined as the absorbance at 600 nm divided by the film thickness (as measured with a digital micrometre) [43].

3.8.8. XRD-of the Biofilm

XRD of all samples were analysed with an X-ray diffractometer 2500 (Rigaku, Tokyo, Japan) with a speed of scattering at 0.02(θ) s^{-1} over a range of angles between 5 and 60^0 (2θ). The operating current and voltage were fixed at 35 mA and 40 kV, respectively. The outcomes from the XRD test include relative crystallinity (Xc), crystalline area (Ic), and amorphous area (Io) [4].

4. Results and Discussion

4.1. Keratin Extraction and Yield

The yield of keratin depends on several factors, including the solvent type, extraction temperature and duration, and the type and condition of the hair. In this study, a low-temperature alkaline medium was used to minimize degradation of the keratin protein and its disulfide bonds [3]. Preserving the native keratin structure is important for producing bio plastic films with improved mechanical properties and light transparency. Keratin yield was calculated using Equation 1 (Section 3.2.2). An average of 49.7 g (dry weight) of keratin was obtained from 70 g of dried raw sheep hair, corresponding to a 71 % yield as shown in Table 4. This result is comparable to the 73 % yield reported for keratin extracted from human hair using urea/SDS/2-mercaptoethanol methods [4]. In this study, an additional filtration step with filter paper was employed to obtain a cleaner keratin extract, which may explain the slight variation in yield. Punam et al. reported a 73 % yield 7.28g of keratin from 10 g of sheep hair [4], confirming that keratin extraction yield can vary with both method and sample size. Therefore, yields should be compared only when obtained under similar conditions. The keratin yield in this study, 71 %, is slightly lower than the keratin yield (86 %) from tannery sheep-hair waste under stronger alkaline and higher-temperature conditions [34]. However, the keratin yield in this study is higher than the keratin obtained via gentle extraction of wool or hair (usually between 30 to 50 %) when conditions are chosen to preserve high-molecular-weight fractions [30]. These comparisons show that the current method achieves a high yield while employing milder conditions than many conventional protocols.

Studies consistently link extraction variables temperature, alkali concentration, and duration to both yield and protein integrity [4]. Higher temperature or stronger alkali typically raises solubilization but can cleave disulfide bonds and degrade keratin peptides, reducing molecular weight and film-forming ability [1]. Studies on wool and human hair confirm that mild alkaline conditions (around 65–80 °C with moderate NaOH) balance these effects, whereas harsher treatments give slightly higher yields at the expense of structural quality [1]. The additional filtration step used here likely improved purity but contributed to the small yield difference compared with more aggressive extractions.

Preserving keratin’s native disulfide crosslinks is critical for downstream applications such as bioplastic films, because high-molecular-weight keratin produces films with superior tensile strength, elasticity, and light transparency [4]. By maintaining low temperature and moderate

alkali strength, the present method limits structural damage while still delivering a yield comparable to or exceeding many reported values. Overall, these findings indicate that the low-temperature alkaline approach is both scalable and efficient, producing keratin of high quality and competitive yield for use in sustainable bioplastic production.

Table 3. Yield of Keratin from Sheep Hair

Hair sample (g)	Average weight (g)	Average weight (%)	Reference
70	49.7	71	This study
10	7.28	73	[4]

4.2. Fourier Transform Infrared Spectroscopy Analysis of Extracted Keratin

FTIR was employed to study the composition of waste sheep hair-based keratin with a wavenumber of 4000-400 cm^{-1} as shown in Figure 1. The peak at 3271.41 cm^{-1} which is in the range of 3000-3500 cm^{-1} corresponds to the stretching vibration of OH and /or amine N-H groups. The peak at 2920.35 cm^{-1} which is near to 2900 cm^{-1} corresponds to the presence of C-H stretching vibration [8]. The amide bands are the key features in keratin analysis [9] where the peak at 1519.97 cm^{-1} which is in the range of 1500-1560 cm^{-1} corresponds to amide II group and the peak at 1633.78 cm^{-1} which is in the range of 1600-1700 cm^{-1} corresponds to the presence of amide I. On the hand, the peak at 2850.91 cm^{-1} which is in the range of 2800-300 cm^{-1} is attributed to the presence of C=C in-plane vibrations [15, 16]. Peaks at 1022.32 and 1172.77 cm^{-1}, which are located in the range between 1000-1200 cm^{-1} represent S=O stretching vibration bond [16]. The presence of the functional groups mentioned above in the respective wavenumbers implies the presence of keratin and the successful extraction of keratin from waste sheep hair.

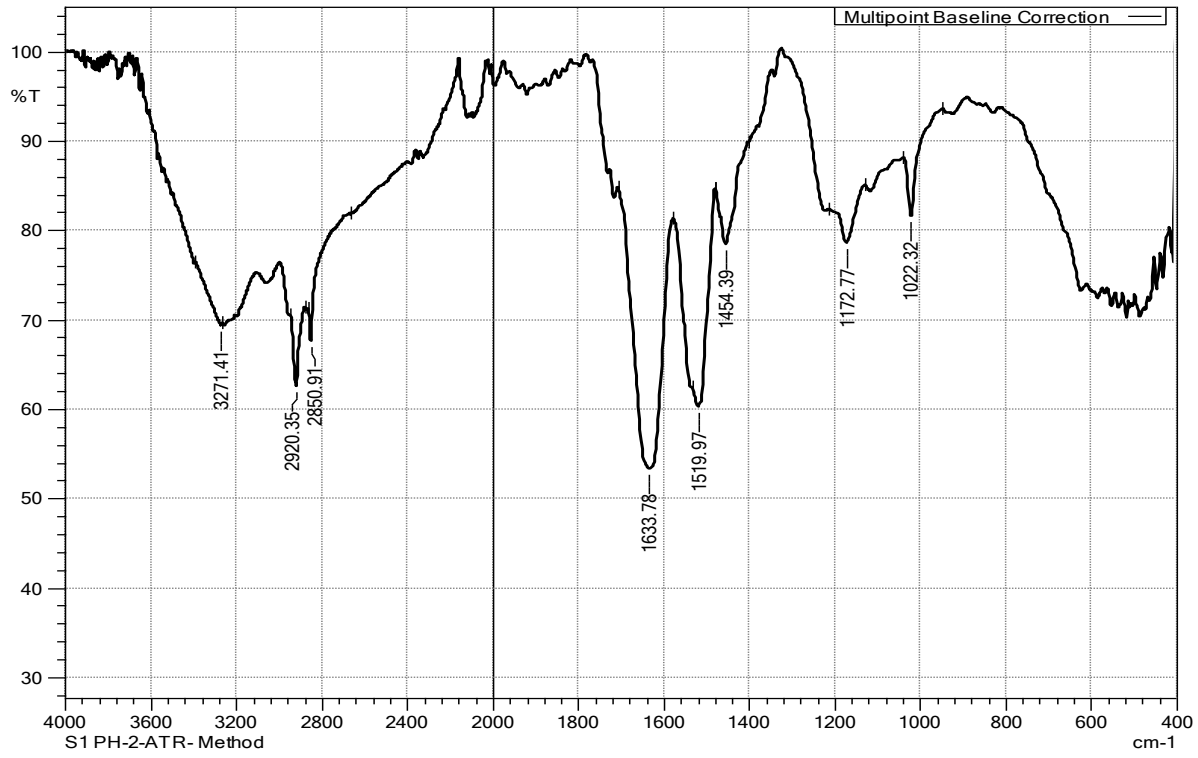


Figure 6: FTIR Spectrum of Extracted Keratin from Waste Sheep Hair

4.3 Mechanical Properties of Bioplastic Film

The mechanical properties of biodegradable films, namely tensile strength (TS) and elongation at break (EB) were evaluated to determine the influence of processing temperature, processing time, and keratin-to-starch mixing ratio. The experimental data are summarized in Table 4. Among the tested conditions, the highest performance was achieved for the biofilm prepared at 80 °C, 30 min, and a 30:70 keratin-to-starch ratio (coded S22 as per Table 4), which exhibited a tensile strength of 2.37 Mpa and an elongation at break of 32.99%. The mechanical properties of the biofilm are comparable to results reported in previous studies. For instance, Abotbina et al. obtained a tensile strength of 2.24 MPa and elongation of 33.30 % in starch-based bioplastics plasticized with glycerol [34], highlighting the consistency of the present findings with established starch–protein film systems. The mechanical properties of the keratin–starch films can be explained by the crosslinking interactions between keratin and starch chains. Reactions occur between functional groups of both polymers, such as esterification between carboxylic acid groups of a crosslinking agent and the hydroxyl groups on starch, or covalent interactions with the amino groups of keratins. In this study, acetic acid functioned as a crosslinking modifier, capable of forming covalent bonds between the biopolymer chains, thus stabilizing the network and altering its

mechanical and physical behavior. Additionally, acetic acid can act as a reaction terminator, partially cleaving glycoside bonds in starch into shorter chains, which facilitates better miscibility and flexibility within the composite matrix [44]. The schematic reactions are illustrated in Figure B-2 Specifically the interaction between starch and glycerol is shown in Figure B-2(a), where plasticization reduces intermolecular hydrogen bonding and increases chain mobility, enhancing flexibility [44]. Meanwhile, the reaction between starch and acetic acid is shown in Figure B-2, demonstrating how covalent modifications promote crosslinking and alter the rigidity of the film. Together, these molecular interactions underpin the observed mechanical behavior, confirming that both processing parameters and chemical modifications significantly contribute to the performance of keratin–starch bio plastics [44].

An appropriate amount of acetic acid was added to induce gelation, thereby producing a more homogeneous film-forming solution. However, excessive addition of acetic acid was found to hinder gelatinization, resulting in a watery solution that was more difficult to cast into uniform films. These observations are consistent with previous reports showing that the balance of cross linker and plasticizer concentrations strongly influences the film's process ability and final mechanical performance. Bio plastics prepared from various starch sources with glycerol as a plasticizer but without reinforcing fillers typically exhibit tensile strengths in the range of 0.22–18.49 MPa [43]. Mechanical performance within this broad range is determined by factors such as starch type, formulation, drying method, plasticizer ratio, reinforcement, and the addition of fillers like fibers or Nano clays [43]. For example, films plasticized with higher glycerol content generally show increased flexibility but lower tensile strength, whereas reinforcement with Nano cellulose or protein-based additives enhances both strength and elongation.

Relevant testing standards for thin plastic films include ASTM D882 standard test method for tensile properties of thin plastic sheeting and ISO 527-3 Plastics: Determination of Tensile Properties of Thin Films, both of which provide standardized procedures for measuring tensile strength and elongation. While no strict regulatory minimum tensile strength exists for starch-based grocery bags, practical benchmarks emphasize their ability to carry 4–5 kg of goods without tearing [43]. Key comparative studies indicate that starch-keratin bioplastics fabricated via mold-casting with glycerol as a plasticizer typically achieve tensile strengths between 0.8 and 3 MPa [44]. The tensile strength obtained in this study, 2.37 MPa, falls within this range and is more than three times the reported minimum. More advanced methods, such as dry casting combined with extrusion and post-heat treatment, have achieved

significantly higher values; for instance, Chin et al. reported tensile strengths of 14 Mpa under optimized thermal conditions [44]. These comparisons highlight that while the present films perform within the expected range for starch–protein systems, there remains potential for improvement through processing optimization and reinforcement strategies.

Table4. Effect of Operating Parameters on Mechanical Properties of bio plastic samples

No.	Temp.	Time	Percent keratin to starch ratio	T_A MPa	E_A %
S_1	70	20	10:90	1.65	28.21
S_2	70	20	30:70	1.68	27.06
S_3	70	20	50:50	1.64	27.01
S_4	70	20	70:30	1.64	26.61
S_5	70	20	90:10	1.60	26.24
S_6	70	30	10:90	1.74	28.91
S_7	70	30	30:70	1.74	28.91
S_8	70	30	50:50	1.75	27.42
S_9	70	30	70:30	1.78	27.71
S_{10}	70	30	90:10	1.78	26.54
S_{11}	70	40	10:90	1.75	30.51
S_{12}	70	40	30:70	1.74	29.51
S_{13}	70	40	50:50	1.84	29.11
S_{14}	70	40	70:30	1.84	29.13
S_{15}	70	40	90:10	1.84	27.95
S_{16}	80	20	10:90	1.85	30.66
S_{17}	80	20	30:70	1.90	30.61
S_{18}	80	20	50:50	1.90	29.91
S_{19}	80	20	70:30	1.94	29.41
S_{20}	80	20	90:10	1.93	28.54
S_{21}	80	30	10:90	2.34	32.25
S_{22}	80	30	30:70	2.37	32.99
S_{23}	80	30	50:50	2.34	28.97

S_{24}	80	30	70:30	2.29	27.71
S_{25}	80	30	90:10	2.28	27.55
S_{26}	80	40	10:90	2.14	20.50
S_{27}	80	40	30:70	2.05	20.55
S_{28}	80	40	50:50	2.01	20.16
S_{29}	80	40	70:30	2.00	18.66
S_{30}	80	40	90:10	2.00	17.85
S_{31}	90	20	10:90	1.89	24.80
S_{32}	90	20	30:70	1.90	25.55
S_{33}	90	20	50:50	1.94	25.70
S_{34}	90	20	70:30	1.90	22.98
S_{35}	90	20	90:10	1.85	22.08
S_{36}	90	30	10:90	1.75	23.96
S_{37}	90	30	30:70	1.80	23.96
S_{38}	90	30	50:50	1.76	21.88
S_{39}	90	30	70:30	1.79	21.11
S_{40}	90	30	90:10	1.69	20.66
S_{41}	90	40	10:90	1.78	21.11
S_{42}	90	40	30:70	1.72	19.63
S_{43}	90	40	50:50	1.78	19.06
S_{44}	90	40	70:30	1.75	18.45
S_{45}	90	40	90:10	1.75	17.60

4.3.1. Effect of Keratin to Starch Mixing Ratio on Mechanical Properties

The effect of the keratin-to-starch mixing ratio on tensile strength and elongation at break was investigated by varying the ratio at 10:90, 30:70, 50:50, 70:30, and 90:10, while maintaining a processing temperature of 80 °C and a processing time of 30 minutes, under which the highest tensile strength and elongation were obtained. As shown in Table 4, tensile strength increases from 2.34 to 2.37 MPa as the keratin content increases to 30%, and then slightly decreases to 2.28 MPa when the keratin content reaches 90%. The increase in mechanical strength at 30% keratin content is attributed to hydrogen bond formation between

the NH_4^+ groups on the keratin backbone and the OH^- groups in the starch matrix. A similar trend was reported on keratin-based green biofilms from waste chicken feathers [32]. Similarly, the effect of the starch-to-keratin mixing ratio on the elongation at break of the biofilm is shown in Table 4. The elongation at break slightly increases from 32.25% to 32.99% as the keratin content rises from 10% to 30%. However, further increases in keratin content beyond 30% result in a decrease in elongation at break, with values dropping sharply from 32.99% to 27.55% as the keratin content increases from 30% to 90%. A similar trend was reported on waste chicken feathers as a sustainable resource for producing bio-plastic films with keratin and polyvinyl alcohol [46].

The mechanical strength of keratin-based biofilms is largely influenced by the nature of the film-forming ingredients [17]. The highest tensile strength obtained in this study (2.37 MPa) is higher than that of goat hoof keratin/gelatin/sodium alginate-based biofilms, which recorded 2.1 MPa [17]. This higher tensile strength may be attributed to the keratin extracted from sheep hair forming a more extensive network, enhanced interactions between sheep hair keratin and cornstarch, or differences in biofilm thickness.

4.3.3. Effect of Processing Time on Mechanical Properties

The influence of processing time on the tensile strength and elongation at break of sheep hair keratin–cornstarch biofilms was examined by varying the contact time to 20, 30, and 40 minutes, while maintaining a constant processing temperature of 80 °C and a keratin-to-starch ratio of 30:70. As presented in Table 4.3, the tensile strength exhibited a pronounced increase from 1.90 MPa to 2.37 MPa when the processing time was extended from 20 to 30 minutes. This enhancement indicates improved molecular interaction and stronger interfacial bonding between keratin and starch components during moderate processing durations. However, further prolongation of the processing time to 40 minutes led to a reduction in tensile strength to 2.05 MPa, possibly due to partial thermal degradation or over-plasticization of the matrix, which weakens the polymeric network. A comparable pattern was observed for elongation at break, which increased from 30.61 % to 32.99 % between 20 and 30 minutes, suggesting enhanced flexibility and ductility of the biofilm. Nevertheless, extending the time to 40 minutes caused a sharp decline to 18.66 %, reflecting embrittlement likely induced by excessive chain scission or loss of moisture content. This behavior aligns with the findings of Walid Abotbina et al., who also reported that prolonged thermal exposure

beyond the optimum processing duration led to deterioration in mechanical properties of biopolymer-based films [6].

4.3.6. Effect of processing temperature on Tensile Strength and Elongation of Bioplastic

Processing temperature is a crucial parameter that significantly affects the mechanical properties of sheep hair keratin–cornstarch biofilms, particularly the tensile strength and elongation at break. To evaluate its influence, the processing temperature was varied at 70 °C, 80 °C, and 90 °C, while maintaining constant conditions of 30 minutes processing time and a 30:70 keratin-to-starch ratio. As presented in Table 4.3, the tensile strength exhibited a noticeable rise followed by a sharp decline with increasing temperature. Specifically, tensile strength increased from 1.74 MPa at 70 °C to a maximum value of 2.37 MPa at 80 °C, indicating that moderate heating promotes enhanced interaction and compatibility between keratin and starch molecules. However, further elevation of the temperature to 90 °C resulted in a decrease in tensile strength to 1.80 MPa, suggesting that excessive thermal exposure weakens the structural integrity of the biofilm. A similar trend was observed in the elongation at break. Elongation at break increased from 28.91 % at 70 °C to 32.99 % at 80 °C, implying improved ductility and molecular mobility within the biopolymer matrix. Beyond this optimal temperature, elongation at break declined markedly to 23.96 % at 90 °C, which can be attributed to thermal degradation, moisture loss, and potential crosslink disruption within the keratin–starch network.

These findings indicate that both tensile strength and elongation at break improve as the processing temperature and time rise from low to moderate levels but deteriorate significantly at excessive thermal conditions. At lower temperatures and shorter durations, incomplete hydrolysis or inadequate chain mobility may restrict effective molecular interactions, leading to weaker film formation. In contrast, prolonged heating or exposure to higher temperatures can cause denaturation of keratin proteins and partial gelatinization or degradation of starch, thereby compromising the mechanical integrity and flexibility of the resulting biofilm.

4.4. Physiochemical Characterization of Keratin/Starch Composite Biofilm

4.4.1. Film Thickness

Film thickness is a critical physicochemical parameter that directly affects the mechanical, barrier, and optical properties of biofilms [47]. In the present study, the keratin–starch biofilm

SS_{22} was selected for detailed characterization of film thickness. Measurements were performed in three independent trials using 40 g of film-forming solution per casting, with strict control of the casting and drying conditions to ensure reproducibility. Following complete drying to remove residual moisture, the biofilm exhibited an average thickness of 0.22 ± 0.01 mm, reflecting excellent consistency and uniformity among replicates. This narrow variation in thickness demonstrates that the applied processing parameters resulted in a homogeneous film-forming dispersion and a stable polymeric network during film formation. The uniformity of thickness aligns well with the observed enhancement in tensile strength and elongation at break, suggesting that the molecular interactions between keratin and starch produced a densely packed and evenly distributed matrix structure. Such a compact configuration suggests stress distribution across the biofilm, thereby improving its overall mechanical stability.

Comparable findings have been reported in other starch–protein composite systems, such as gelatin–starch and soy protein–starch blends, where uniform film thickness was found to correlate positively with mechanical integrity, microstructural uniformity, and reduced defect density [48]. The thickness results obtained in this study further confirm that the synthesized keratin–starch formulation produced a structurally coherent, reproducible, and high-quality biofilm, demonstrating strong potential for biodegradable packaging and related sustainable applications.

4.4.2 Moisture Content (MC)

The moisture content (MC) of the sheep hair keratin–cornstarch films was determined to be 24.45 %. For the analysis, three film specimens (2 × 2 cm^2) were dried in a hot-air oven at 105 ± 1 °C until a constant weight was achieved. The relatively uniform values across the replicates suggest consistent water retention within the biopolymer matrix, confirming the reproducibility of the film preparation process. This moderate moisture level is significant, as it contributes to the flexibility and mechanical stability of the bioplastic films. Excessive moisture can weaken hydrogen bonding, reducing tensile strength, while too little moisture may cause brittleness and reduced elongation. The observed MC, therefore, indicates a favorable balance between plasticization and structural integrity. These results are consistent with earlier reports. Walid A. et al. observed comparable moisture contents for keratin-based biofilms, noting that MC values typically range between 20–30 % depending on plasticizer content and drying conditions. Similar trends have been reported in starch protein composites, where glycerol and other hydrophilic additives promote moderate moisture

retention, enhancing elasticity without severely compromising strength [49]. Thus, the present results confirm that the keratin starch films possess an optimal MC profile, suitable for use in flexible biodegradable packaging applications.

4.4.3 Biofilm Solubility

Water solubility (WS) is a critical property of biopolymer films, especially for food packaging applications, where a certain level of water resistance is required to maintain product integrity [41]. The water solubility of SS_{22} was evaluated in three independent trials, yielding value of with an overall average of 36.26 % ± 0.1%. This level of solubility reflects the hydrophilic character of the composite and its interaction with aqueous environments. The results are consistent with earlier studies on starch-based films. Walid Abotbina et al. reported similar solubility ranges for starch glycerol bioplastics, while Tesfaye et al. noted that the hydrophilic nature of starch is the dominant factor influencing solubility, with higher starch content leading to increased water uptake and film disintegration [7, 34]. By contrast, pure keratin-based films typically show lower solubility due to the presence of hydrophobic amino acids and stable disulfide crosslinks [49]. The relatively high solubility observed here can, therefore, be attributed to the starch-rich composition of the film, which enhances water absorption and dissolution. From an application perspective, the solubility level of 36 % suggests that while the keratin–starch bioplastic offers promising biodegradability, its water resistance remains limited. For packaging applications requiring moisture barriers, modification strategies such as crosslinking with acetic acid, incorporation of hydrophobic additives (e.g., waxes or essential oils), or blending with Nano cellulose could be employed to reduce solubility without compromising biodegradability. Thus, the present findings highlight both the advantages and limitations of starch-dominant keratin bioplastics in practical applications.

4.4.4. Moisture Absorption

Moisture intake (MI) is an important property for starch-based films, as water acts as a natural plasticizer. Films with higher water content generally exhibit greater flexibility [4], although increased moisture absorption can reduce mechanical strength. In this study, the weight gain of all specimens was monitored until a constant weight was reached. The results indicate that all films, including the control, reached saturation within 2 hours of immersion, after which further water absorption was negligible. The SS_{22} sample exhibited an average water absorption of approximately 196.83%, which is comparable to the 194.3% reported by

[7]. The relatively high moisture intake is attributed to the higher starch content in the biofilm, highlighting the significant role of starch in water uptake and film flexibility.

4.4.5. Biodegradability of Film

The biodegradability of the keratin starch bioplastic films was evaluated under controlled composting conditions. The film S_{22}, were found to be fully degraded within 9 days. Pure keratin films prepared under similar conditions also degraded completely within the same timeframe, whereas only 16% of commercial starch-based plastic bags degraded within 12 days [2]. The rapid degradation observed in the keratin–starch biofilms can be attributed to their high swelling index, which enhances water uptake, facilitates microbial penetration, and accelerates enzymatic surface attack, ultimately leading to significant weight loss. The presence of glycerol may also play a role by altering the hydrogen bonding network and introducing partial cross-linking interactions with keratin macromolecules, thereby increasing the accessibility of microbial enzymes. Meanwhile, commercial starch-based bags remained largely non-biodegraded even after composting, reflecting slower degradation kinetics. Taken together, these results demonstrate that the SS_{22} keratin–starch–glycerol formulation not only achieves rapid biodegradability but also maintains sufficient mechanical integrity prior to degradation. This highlights its potential as a sustainable alternative to conventional starch-based films in short-term packaging applications.

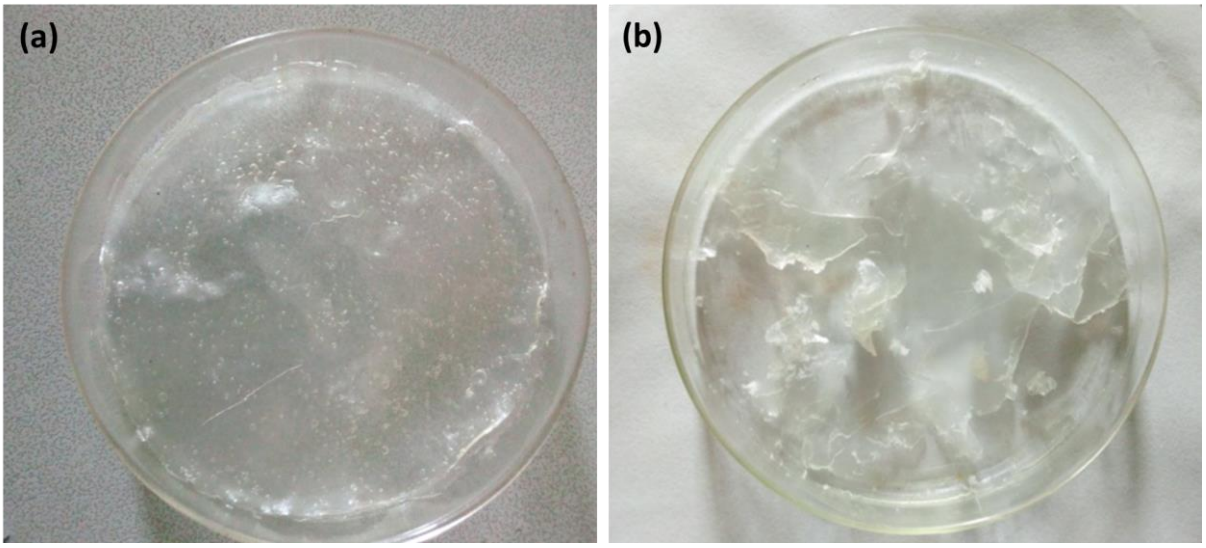


Figure7. S_{22} bioplastic film: (a) before biodegradation; (b) after three days of biodegradation.

4.4.6. Optical Characterization by UV-Vis Spectroscopy

The UV–Vis spectrum of the keratin–starch bioplastic film exhibits a strong absorbance band in the 190–230 nm region, characteristic transitions of the peptide backbone (amide bonds) in

proteins. This deep-UV band is widely reported for keratin and other structural proteins [43]. A shoulder extending toward 260–280 nm reflects the presence of aromatic amino acids such as tyrosine, phenylalanine, and tryptophan, also consistent with spectra of inherent keratin films [44]. Starch alone shows only a weak absorption near 200 nm [45], confirming that the additional features arise primarily from keratin. Above 300 nm, absorbance declines steadily to about 1 AU by 600 nm, indicating excellent transparency in the visible range. Compared with other study values, the present spectrum aligns with keratin isolated by mild alkaline extraction. Keratin films prepared under moderate alkali exhibit a dominant 210–220 nm peak and a minor 270–280 nm shoulder, whereas harsher treatments shift the maximum toward shorter wavelengths and reduce aromatic-band intensity [49]. Similar dual-band profiles have been reported for keratin–polysaccharide blends, such as keratin–chitosan and keratin–cellulose films, where the starch-like component contributes minimal absorbance above 220 nm but enhances film clarity [50]. The retention of both peptide and aromatic signals here suggests that the low-temperature alkaline method preserved the keratin's primary and secondary structures, avoiding extensive cleavage of disulfide bonds that would otherwise diminish UV absorbance. Strong UV absorption below 280 nm provides natural UV-shielding, while the low absorbance above 400 nm ensures high visible-light transparency, a desirable combination for food or cosmetic packaging. Studies on keratin–starch and keratin–gelatin composites shows that films with preserved high-molecular-weight keratin exhibit superior tensile strength and barrier properties compared with those extracted under harsher conditions [51]. The present film therefore combines the optical clarity typical of starch-based plastics with the UV-protective and mechanical benefits of intact keratin, demonstrating the effectiveness of the gentle alkaline extraction and the suitability of the composite for sustainable packaging and other bioplastic applications.

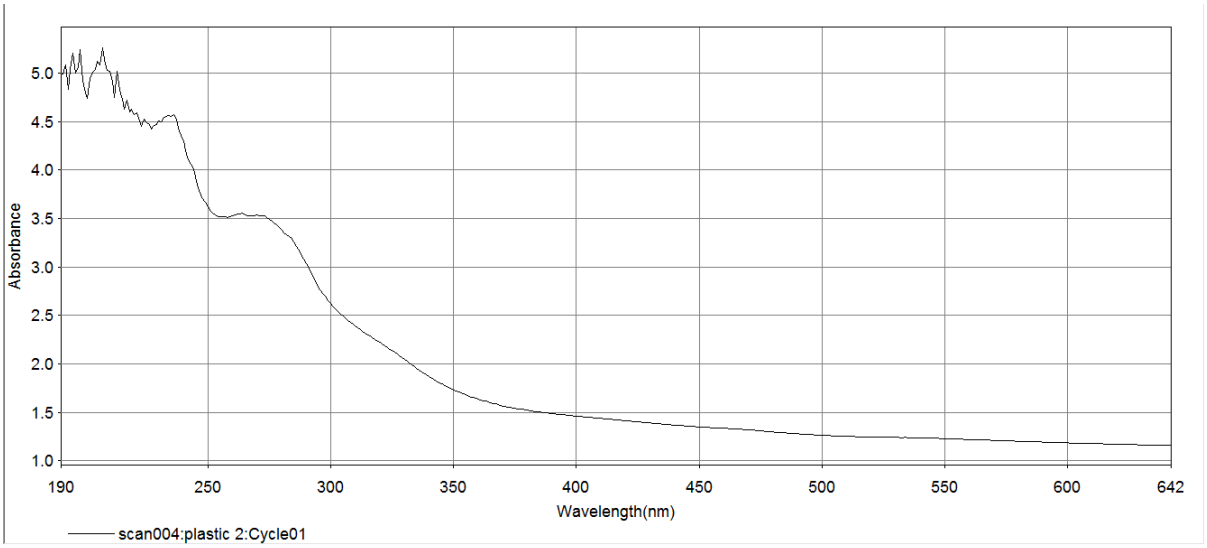


Figure 8: UV-Vis Spectroscopy of the S_{22} biofilm

4.4.7. XRD-of the Biofilm

The X-ray diffraction (XRD) pattern of the keratin–starch composite biofilm (Figure 9) indicates that the material is predominantly amorphous, as evidenced by the absence of sharp diffraction peaks. The diffractogram of the keratin–starch bioplastic film shows a broad halo centered around $2\theta \approx 20°$, with minor shoulders between 15–25° and a gradual decline in intensity toward higher angles (up to 80°). This pattern is characteristic of amorphous or semi-crystalline biopolymer matrices. Native starch generally presents distinct A- or B-type crystalline peaks near 15°, 17°, 18°, and 23° depending on its botanical origin [49]. The absence of sharp reflections here indicates that blending with keratin and the film-forming/alkaline treatment disrupted starch's native crystallinity. The broad maximum near 20° also corresponds to the α-helical keratin signature, commonly reported for wool and hair keratin powders and films [50]. Comparable keratin films typically show a dominant amorphous hump at 19–22° and weak β-sheet contributions near 9° and 21° [51]. The absence of a pronounced β-sheet peak suggests that the mild alkaline extraction preserved mostly random-coil/α-helical domains rather than inducing extensive β-sheet aggregation, which aligns with the UV–Vis evidence of intact protein structure. Similar amorphous halos have been observed in other protein–polysaccharide composites such as keratin–chitosan [52] and keratin–gelatin films [53], where hydrogen bonding and electrostatic interactions between the two biopolymers interfere with polysaccharide crystallite formation. The relatively low and broad intensity beyond 30° further confirms that the composite film is largely non-crystalline, a feature that can enhance flexibility and transparency. The XRD

result demonstrates that the keratin–starch bioplastic is an amorphous, well-integrated blend, with disrupted starch crystallinity and preserved keratin secondary structure properties favorable for producing clear, flexible, and mechanically resilient biodegradable packaging materials.

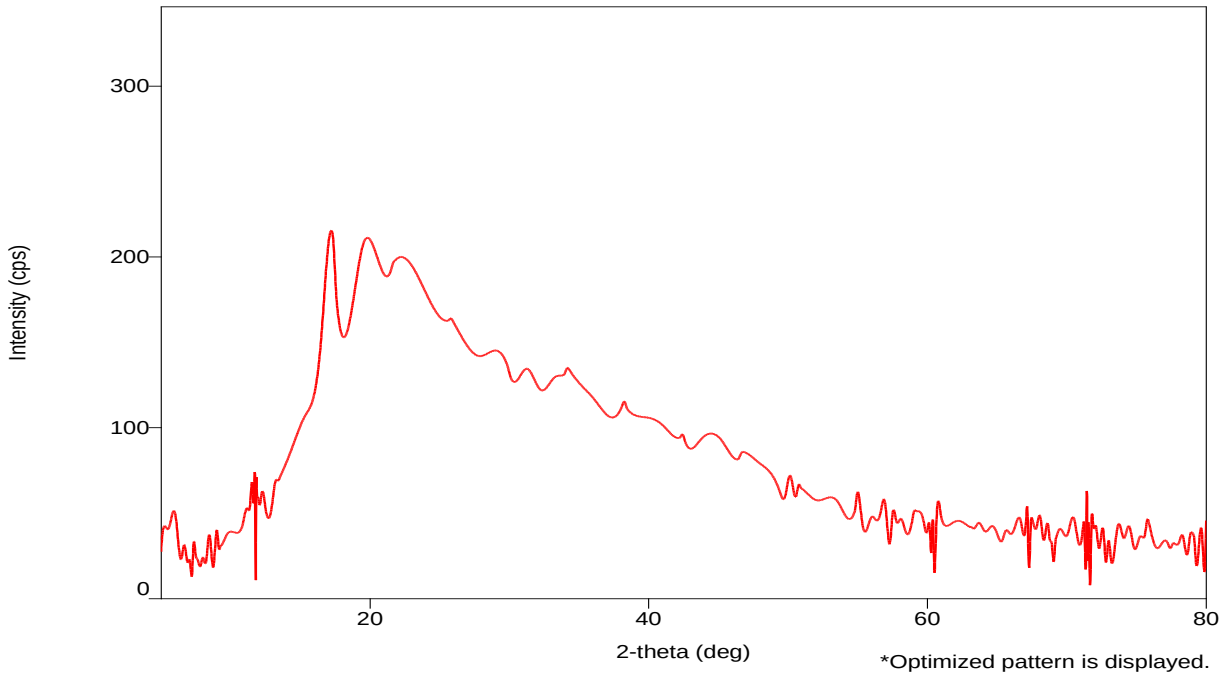


Figure 9: XRD plot for keratin starch S_{22} biofilm

4.7.8. Fourier Transform Infrared (FTIR) Spectroscopic Analysis of Waste Sheep Hair Keratin-Corn Starch Biodegradable Film.

The FTIR spectrum of the sheep hair keratin–cornstarch biodegradable film is shown in Figure 10. The broad absorption band observed around 3250 cm^{-1} corresponds to O–H and N–H stretching vibrations, indicating the presence of hydroxyl groups from starch and amide groups from keratin. Peaks at 2928 and 2887 cm^{-1} are attributed to C–H stretching vibrations of methylene groups, reflecting the organic backbone of both components. The strong peak at 1643 cm^{-1} can be assigned to the C=O stretching of the amide I band, characteristic of keratin's protein structure, while the band at 1413 cm^{-1} corresponds to the C–N stretching and N–H bending of the amide II band. Additional peaks at 1334 and 1151 cm^{-1} are associated with C–H bending and C–O stretching of the starch matrix. The absorption at 1105 and 1078 cm^{-1} further confirms the presence of C–O–C linkages typical of polysaccharides.

Peaks at 923 and 854 cm^{-1} can be attributed to out-of-plane bending vibrations of –CH groups in the polymer backbone.

The FTIR spectra indicate successful molecular interactions and compatibility between keratin and starch, primarily involving hydrogen bonding between the N–H groups of keratin and the O–H groups of starch, as evidenced by the shifts and broadening of the O–H/N–H and C=O absorption bands. This molecular interaction supports the formation of a homogeneous amorphous network, consistent with the XRD results and mechanical property findings, contributing to the flexibility and biodegradability of the films. The spectrum confirms that the keratin–starch composite retains characteristic functional groups of both components while forming a stable, physically cross-linked biofilm matrix suitable for biodegradable plastic applications.

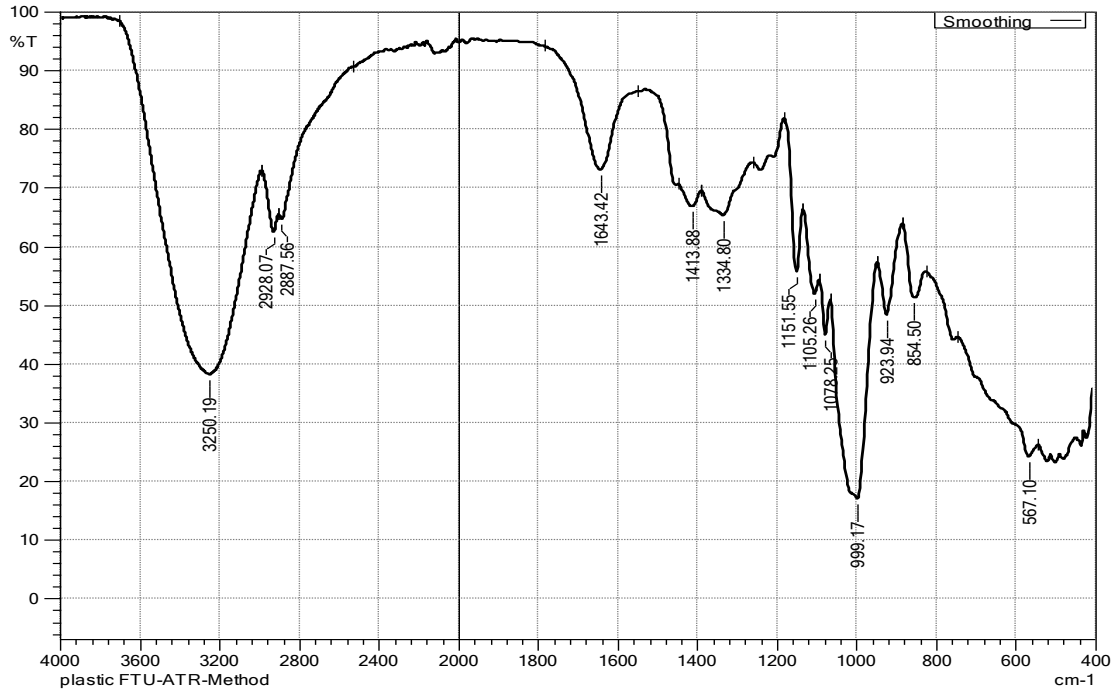


Figure 10: FTIR of waste sheep hair Keratin and corn starch biodegradable film

5. Conclusion and Recommendation

5.1 Conclusion

This study successfully demonstrated the extraction of keratin from waste sheep hair and its incorporation with corn starch to develop a fully biodegradable keratin–corn starch bioplastic film. Structural characterizations using FTIR, XRD, and UV–Vis spectroscopy confirmed that the low-temperature alkaline extraction process effectively preserved the primary peptide backbone of keratin while promoting uniform blending with starch, resulting in an amorphous, optically transparent composite. Mechanical testing, supported by instrumental analyses, revealed that processing temperature, time, and keratin–starch ratio exert significant effects on the film's mechanical properties. The combination of 80 °C processing temperature, 30 minutes duration, and a 30:70 keratin-to-starch ratio yielded the highest tensile strength of 2.37 MPa and elongation at break of 32.99 %, values comparable to those reported for other protein–polysaccharide-based films in the literature. In addition, the biofilm exhibited rapid biodegradability, with complete degradation observed within five days, underscoring its potential as an eco-friendly alternative to conventional petroleum-derived plastics. Beyond its functional performance, this approach presents a value-added utilization pathway for tannery by-products by transforming low-value waste sheep hair into a high-value, sustainable material. The findings in this study demonstrate that keratin–corn starch bioplastics can serve as a viable substitute for packaging and single-use plastics, while concurrently addressing environmental and waste management challenges associated with the leather and wool industries.

5.2. Recommendation

Based on the findings of this study, it is recommended that up to 30 % of the starch can be replaced with keratin derived from waste sheep hair when formulating corn-starch bioplastic films. This substitution level maintained desirable film integrity and provided improved biodegradability while utilizing an abundant industrial by-product. For future research, it is advisable to explore alternative plasticizers and crosslinking agents for example, sorbitol, citric acid, or naturally derived polymers in order to further enhance mechanical strength, flexibility, and moisture resistance. Investigating different processing conditions such as pH, drying temperature, and mixing techniques and optimizing could also yield films with improved performance and broader application potential. Finally, long-term stability and

scalability studies including aging tests, barrier property evaluation, and pilot-scale production are recommended to validate commercial feasibility and to ensure that the keratin-starch films meet the requirements for food packaging and other biodegradable plastic applications.

Reference

1. K. R. Ramya, Ramar T., Balaraman Madhan Comparative analysis of the chemical treatments used in keratin extraction from red sheep's hair and the cell viability evaluations of this keratin for tissue engineering applications. Process Biochemistry. 2019.11.015

2. Borja Fernandez-d ArlasTough and functional cross-linked bioplastics from sheep wool keratin. International Agrophysics. 2019;9;14810.

3. Walid Abotbina, S.M.Sapuan, et al.Development and characterization of cornstarch-based bioplastics packaging film using a combination of different plasticizers. 2021, 13, 3487.

4. Punam Sen, Arun.C.M, et el. A Pilot-scale study on the Extraction and optimization of keratin from humanhair-An Adapted strategy for the control of enviromental Menace 2020;29(10):2625-43.

5. Palmu PT, Crosso F. Development and characterization of edible films based on gluten from semi-hard and soft Brazilian wheat flours. Food Sci Technol. 2003;23:264-9.

6. Kanagaraj J, Velappan KC, Chandrababu NK, Sadulla S(2006), Solid waste generation in the leather industries and its utilization for cleaner environment—an review. J Sci. Ind. Res 65:541–548

7. Grasso G., Santoprete G. and Detpezzo L. (1990), "L'industriadellaConciaedelCuoio", G. Giappichelli Editor- Torino

8. Chaosri P, Thonpho A, Srihanam P. Properties and drug release profile of keratin/starch blend films. J Sci Technol MSU. 2014;33(6):554-560.

9. Cui L, Gong J, Fan X, et al. Transglutaminase-modified wool keratin film and its potential application in tissue engineering. Engineering in Life Sciences. 2013;13(2):149-55.

10. Leather Industry Development Institute (LIDI) and United Nation Development Program(UNID) (2010), Processing of National workshop on enhancing the Ethiopia Leather Industry and its Market Competitiveness ,Adama, Ethiopia.

11. Sothornvit R, Krochta JM. Water vapor permeability and solubility of films from hydrolyzed whey protein. Journal of Food Science. 2000;65(4):700-3.

12. Oladayo OO, Joseph OS, Oluwasegun W. Physicochemical properties of cassava starch and starch-keratin prepared biofilm. Songklanakarin J Sci and Technol. 2016;38(4).

13. Sando L, Kim M, Colgrave ML, et al. Photochemical crosslinking of soluble wool keratins produces a mechanically stable biomaterial that supports cell adhesion and proliferation. Journal of Biomedical Materials Research Part A. 2010;95(3):901-11.

14. Zhong QP, Xia WS. Physicochemical properties of edible and preservative films from chitosan/cassava starch/gelatin blend plasticised with glycerol. Food Technology and Biotechnology. 2008;46(3):262-9.
15. Fang Y, Tung MA, Britt IJ, et al. Tensile and barrier properties of edible films made from whey proteins. J Food Sci. 2002;67(1):188-93.
16. Gennadios A, Weller CL. Edible films and coatings from soymilk and soy protein. Cereal Foods World, USA. 1991.
17. Lim LT, Mine Y, Tung MA. Transglutaminase cross-linked egg white protein films: tensile properties and oxygen permeability. J Agri Food Chem. 1998;46(10):4022-9.
18. Saul JM, Ellenburg MD, de Guzman RC. Keratin hydrogels support the sustained release of bioactive ciprofloxacin. J Biomed Mat Res Part A. 2011;98(4):544-53.
19. Van Dyke ME, Timmons SF, Blanchard CR. et al. Absorbent keratin wound dressing. U.S. Patent 6,270,793. 2001.
20. Yamauchi K, Khoda A. Novel proteinous microcapsules from wool keratins. Colloids and Surfaces B: Biointerfaces. 1997;9(1-2):117-9.
21. De Carvalho RA, Grosso CRF. Characterization of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. Food Hydrocolloids. 2004;18(5):717-26.
22. Handa A, Gennadios A, Hanna MA. et al. Physical and molecular properties of egg-white lipid films. J Food Sci. 1999;64(5):860-4.
23. Yang L, Paulson AT. Effects of lipids on mechanical and moisture barrier properties of edible gelatine film. Food Research International. 2000;33(7):571-8.
24. Hill P, Brantley H, Van Dyke M. Some properties of keratin biomaterials: Kerateines. Biomat. 2010;31(4):585-93.
25. Jiang Y, Tang CH, Wen QB, et al. Effect of processing parameters on the properties of transglutaminase-treated soy protein isolate films. Innovative Food Sci and Emerging Technol. 2007;8(2):218-25.
26. Oh JH, Wang B, Field PD, et al. Characteristics of edible films made from dairy proteins and zein hydrolysate cross-linked with transglutaminase. Int J Food Sci and Technol. 2004;39(3):287-94.
27. Rejak A, Moscicki L, Wójtowicz A, et al. Influence of keratin addition on selected mechanical properties of TPS film. Teka Komisji Motoryzacji i Energetyki Rolnictwa. 2012;12(1).
28. Tonin C, Aluigi A, Vineis C. et al. Thermal and structural characterization of poly (ethylene-oxide)/keratin blend films. J Thermal Analysis and Calorimetry. 2007;89(2):601-8.

29. Verma V, Verma P, Ray P, et al. Preparation of scaffolds from human hair proteins for tissue-engineering applications. Biomedical Mat. 2008;3(2):025007.
30. Tesfaye T, Sithole B, Ramjugernath D. Valorisation of chicken feathers: Recycling and recovery routes. Proceedings, Sardinia 2017, 16th International Waste Management and Landfill Symposium. S Margherita di Pula, IWWG: International Waste Working Group, CISA Publisher. Sardinia, Cagliari Italy. 2017a:511.
31. Tesfaye T, Sithole B, Ramjugernath D, et al. Valorization of chicken feathers: Characterization of physical properties and morphological structure. J Cleaner Prod. 2017b;149:349-65.
32. Tesfaye T, Sithole B, Ramjugernath D, et al. Valorization of chicken feathers: Characterization of chemical properties. Waste Management. 2017c;68:626-35.
33. Tesfaye T, Sithole B, Ramjugernath D, et al. Valorization of chicken feathers: Application in paper production. Journal of Cleaner Production. 2017d;164:1324-31.
34. Tesfaye T, Sithole B, Ramjugernath D. Valorisation of chicken feathers: A review on recycling and recovery route-current status and future prospects. Clean Technologies and Environmental Policy. 2017e;19(10):2363-78.
35. Tesfaye T, Sithole B, Ramjugernath D, et al. Valorization of chicken feather barbs: Utilization in yarn production and technical textile applications. Sustainable Chem and Pharmacy. 2018f;8:38-49.
36. Srisuwan Y, Thonpho A, Srihanam P. Effect of starch on property of silk fibroin/keratin blend films. Int J. 2016;11(28):2870-3.
37. Saul JM, Ellenburg MD, de Guzman RC. Keratin hydrogels support the sustained release of bioactive ciprofloxacin. J Biomed Mat Res Part A. 2011;98(4):544-53.
38. Tanabe T, Okitsu N, Yamauchi K. Fabrication and characterization of chemically crosslinked keratin films. Mat Sci and Eng. C. 2004;24(3):441-6.
39. Thonpho ANSAYA, Srihanam P. Preparation and characterization of keratin blended films using biopolymers for drug controlled release application. Oriental J Chem. 2016;32(4):1739-48.
40. Garcia MA, Martino MN, Zaritzky NE. Lipid addition to improve barrier properties of edible starch-based films and coatings. J Food Sci. 2000;65(6):941-4.
41.Ferreira AR, Alves VD, Coelhoso IM. Polysaccharide-based membranes in food packaging applications. Membranes. 2016;6(2):22.
42. Olarewaju M., Egosa O. Production and characterization of keratin-starch bio-composite film from chicken feather waste and turmeric starch. ELSEVIER. 2021;63(2):22-2.

44. Reese ET (1957) Biological degradation of cellulose derivatives. Ind Eng Chem 49(1):89–

45. Caddeo S, Boffito M, Sartori S (2017) Tissue engineering approaches in the design of healthy and pathological in vitro tissue models. Front Bioeng Biotechnol 5:40 - PubMed - PMC

46. Limongi T, Tirinato L, Pagliari F, Giugni A, Allione M, Perozziello G, Candeloro P, Di Fabrizio E (2017) Fabrication and applications of micro/nanostructured devices for tissue engineering. Nanomicro Lett 9(1):1 - PubMed

47. Kim J, Kang JW, Park JH, Choi Y, Choi KS, Park KD, Baek DH, Seong SK, Min H-K, Kim HS (2009) Biological characterization of long-term cultured human mesenchymal stem cells. Arch Pharm Res 32(1):117–126 - PubMed

48. Martin-Piedra MA, Garzon I, Oliveira AC, Alfonso-Rodriguez CA, Sanchez-Quevedo MC, Campos A, Alaminos M (2013) Average cell viability levels of human dental pulp stem cells: an accurate combinatorial index for quality control in tissue engineering. Cytotherapy 15(4):507–518 - PubMed

49. Tsai H-H, Yang K-C, Wu M-H, Chen J-C, Tseng C-L (2019) The effects of different dynamic culture systems on cell proliferation and osteogenic differentiation in human mesenchymal stem cells. Int J Mol Sci 20(16):4024 - PMC

50. Quent VM, Loessner D, Friis T, Reichert JC, Hutmacher DW (2010) Discrepancies between metabolic activity and DNA content as tool to assess cell proliferation in cancer research. J Cell Mol Med 14(4):1003–1013 - PubMed - PMC

51. Rutkovskiy A, Stensløkken K-O, Vaage IJ (2016) Osteoblast differentiation at a glance. Med Sci Monit Basic Res 22:95 - PubMed - PMC

52. Manoukian OS, Matta R, Letendre J, Collins P, Mazzocca AD, Kumbar SG (2017) Electrospun nanofiber scaffolds and their hydrogel composites for the engineering and regeneration of soft tissues. In: Biomedical Nanotechnology. Springer, New York, pp 261–278

53. Tatulian SA (2013) Structural characterization of membrane proteins and peptides by FTIR and ATR-FTIR spectroscopy, lipid-protein interactions. Springer, New York, pp 177–218

APPENDIX -A

Table 5: Experimental Setup of Full Factorial Designed Matrix for Bio plastic Film

Run order	**Temperature (^{O}C)**	**Time (minutes)**	**Mixing ratio (%)**	**TS**	**EA**
1	**90**	**40**	**50:50**		
2	**70**	**20**	**50:50**		
3	**70**	**20**	**10:90**		
4	**70**	**40**	**90:10**		
5	**80**	**20**	**90:10**		
6	**80**	**30**	**70:30**		
7	**70**	**40**	**30:70**		
8	**80**	**40**	**30:70**		
9	**70**	**40**	**50:50**		
10	**90**	**30**	**90:10**		
11	**90**	**20**	**10:90**		
12	**80**	**20**	**30:70**		
13	**80**	**20**	**50:50**		
14	**90**	**20**	**30:70**		
15	**90**	**30**	**10:90**		
16	**80**	**30**	**50:50**		
17	**70**	**30**	**30:70**		
18	**80**	**40**	**10:90**		
19	**90**	**40**	**30:70**		
20	**70**	**40**	**10:90**		
21	**70**	**30**	**50:50**		
22	**70**	**20**	**90:10**		
23	**90**	**30**	**70:30**		
24	**80**	**30**	**30:70**		
25	**80**	**40**	**90:10**		

26	**90**	**40**	**10:90**
27	**70**	**30**	**90:10**
28	**80**	**30**	**90:10**
29	**90**	**40**	**70:30**
30	**80**	**40**	**70:30**
31	**70**	**20**	**30:70**
32	**90**	**40**	**90:10**
33	**70**	**20**	**70:30**
34	**70**	**40**	**90:10**
35	**90**	**30**	**50:50**
36	**80**	**30**	**10:90**
37	**80**	**40**	**50:50**
38	**90**	**20**	**50:50**
39	**80**	**20**	**70:30**
40	**70**	**30**	**10:90**
41	**70**	**30**	**70:30**
42	**80**	**20**	**10:90**
43	**90**	**30**	**30:70**
44	**90**	**20**	**90:10**
45	**90**	**20**	**70:30**

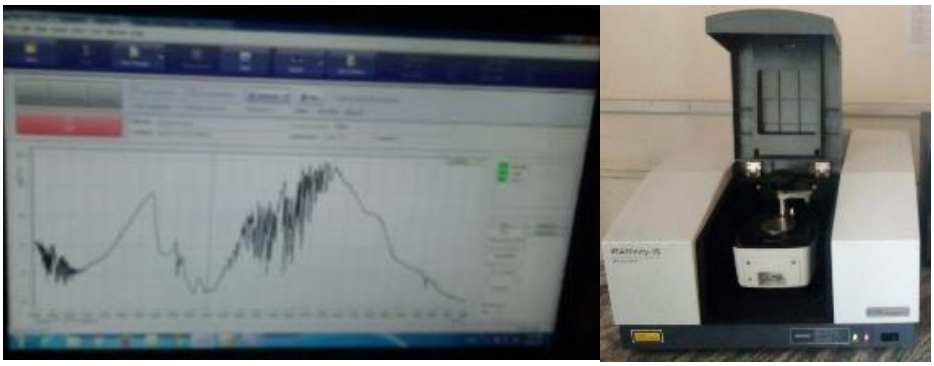
Table 6: Demonstrates the Interaction Effect of Operating Parameters on the Mechanical Property

No.	Temp.	Time	Percent	T_1	T_2	T_A	E_1	E_2	E_A
S_1	70	20	10:90	1.61	1.68	1.65	28.61	27.81	28.21
S_2	70	20	30:70	1.66	1.71	1.68	27.01	27.11	27.06
S_3	70	20	50:50	1.67	1.61	1.64	27.21	26.81	27.01
S_4	70	20	70:30	1.68	1.60	1.64	26.11	27.10	26.61
S_5	70	20	90:10	1.60	1.61	1.60	25.81	26.66	26.24
S_6	70	30	10:90	1.71	1.77	1.74	29.01	28.81	28.91
S_7	70	30	30:70	1.78	1.71	1.74	28.91	28.91	28.91
S_8	70	30	50:50	1.76	1.75	1.75	26.71	28.12	27.42
S_9	70	30	70:30	1.86	1.70	1.78	27.31	28.11	27.71
S_{10}	70	30	90:10	1.71	1.69	1.78	26.06	27.01	26.54
S_{11}	70	40	10:90	1.81	1.70	1.75	30.91	30.11	30.51
S_{12}	70	40	30:70	1.70	1.78	1.74	30.01	29.01	29.51
S_{13}	70	40	50:50	1.81	1.87	1.84	29.01	29.21	29.11
S_{14}	70	40	70:30	1.80	1.89	1.84	28.61	29.64	29.13
S_{15}	70	40	90:10	1.89	1.79	1.84	28.11	27.81	27.95
S_{16}	80	20	10:90	1.81	1.90	1.85	31.01	30.31	30.66
S_{17}	80	20	30:70	1.91	1.90	1.90	30.31	30.91	30.61
S_{18}	80	20	50:50	1.89	1.91	1.90	30.10	29.01	29.91
S_{19}	80	20	70:30	1.90	1.98	1.94	29.01	29.81	29.41
S_{20}	80	20	90:10	1.89	1.98	1.93	28.01	29.06	28.54
S_{21}	80	30	10:90	2.26	2.41	2.34	32.4	32.10	32.25
S_{22}	80	30	30:70	2.45	2.28	2.37	32.91	33.08	32.99

S_{23}	80	30	50:50	2.38	2.29	2.34	28.14	29.80	28.97
S_{24}	80	30	70:30	2.31	2.28	2.29	28.11	28.41	27.71
S_{25}	80	30	90:10	2.29	2.28	2.28	28.01	27.09	27.55
S_{26}	80	40	10:90	2.19	2.08	2.14	20.01	21.00	20.50
S_{27}	80	40	30:70	2.09	2.00	2.05	21.10	20.00	20.55
S_{28}	80	40	50:50	2.01	2.01	2.01	20.01	20.31	20.16
S_{29}	80	40	70:30	2.00	2.01	2.00	18.31	19.01	18.66
S_{30}	80	40	90:10	2.01	2.00	2.00	18.10	17.60	17.85
S_{31}	90	20	10:90	1.90	1.89	1.89	24.60	25.01	24.80
S_{32}	90	20	30:70	1.99	1.81	1.90	25.10	26.01	25.55
S_{33}	90	20	50:50	1.98	1.99	1.94	24.6	26.81	25.70
S_{34}	90	20	70:30	1.91	1.89	1.90	23.16	22.81	22.98
S_{35}	90	20	90:10	1.81	1.90	1.85	22.16	22.01	22.08
S_{36}	90	30	10:90	1.71	1.80	1.75	23.91	24.01	23.96
S_{37}	90	30	30:70	1.81	1.79	1.80	23.91	24.01	23.96
S_{38}	90	30	50:50	1.71	1.81	1.76	22.91	20.86	21.88
S_{39}	90	30	70:30	1.77	1.81	1.79	20.31	21.91	21.11
S_{40}	90	30	90:10	1.67	1.71	1.69	21.31	20.01	20.66
S_{41}	90	40	10:90	1.78	1.79	1.78	21.31	20.91	21.11
S_{42}	90	40	30:70	1.76	1.69	1.72	20.18	19.09	19.63
S_{43}	90	40	50:50	1.76	1.80	1.78	19.81	18.31	19.06
S_{44}	90	40	70:30	1.80	1.70	1.75	18.90	18.01	18.45
S_{45}	90	40	90:10	1.81	1.69	1.75	18.08	17.18	17.60

APPENDIX - B

Figure B-1: Different images taken during laboratory work



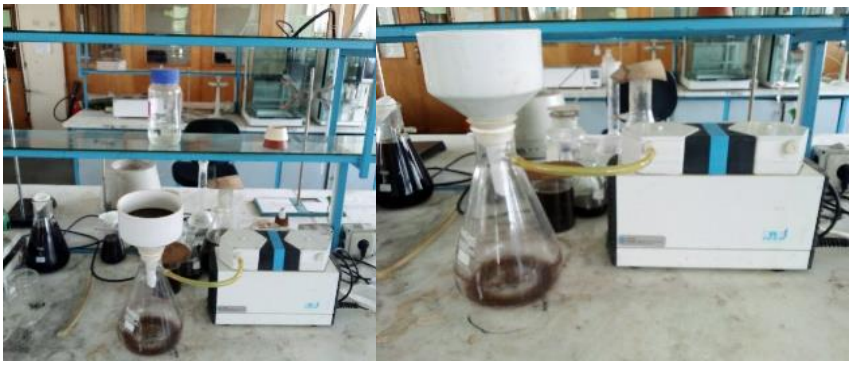


Figure B-2(a) Reaction between starch and acetic acid, and (b) Reaction between starch and glycerol.

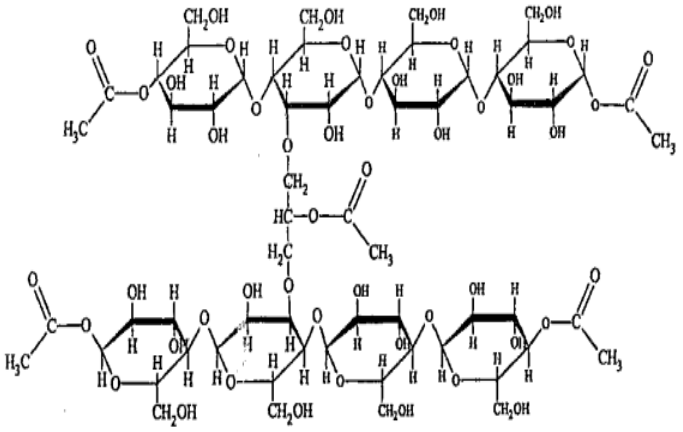


Figure B-2 (a) Reaction between starch and acetic acid

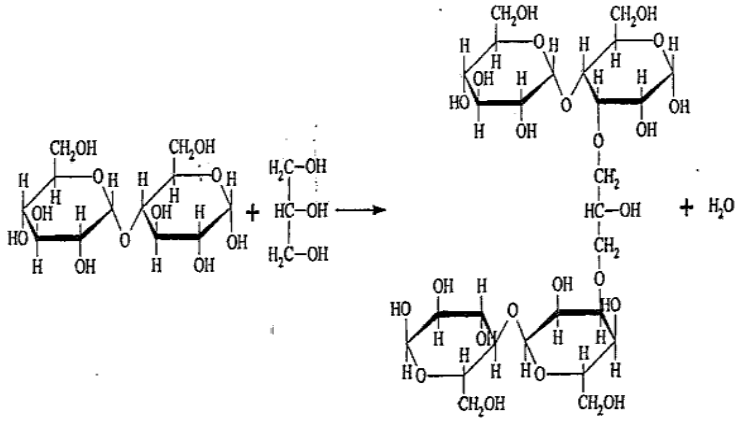


Figure B-2 (b) Reaction between starch and glycerol.