PRODUCTION OF KERATIN BASED CHITOSAN INFUSED BIOPLASTIC FILM FROM WASTE MATERIALS

Submitted in partial fulfilment of the requirements for the award of Bachelor of Technology Degree in Biotechnology

by

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DEPARTMENT OF BIOTECHNOLOGY ENGINEERING
SCHOOL OF BIO-CHEMICAL ENGINEERING

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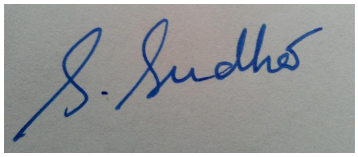
BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Sandra Samson (38230029), B. Vishnupriya (38230042)** who carried out the project entitled **"Production of Keratin based Chitosan infused Bioplastic Film from Waste Materials"** under my supervision from **17-01-2022** to **10-04-2022**



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DR. S. SUDHA

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DR V RAMESH KUMAR

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ATTENDANCE & PUNCTUALITY	: 18	OUT OF 20
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REPORTING SKILLS	: 42	OUT OF 50
IMPLEMENTATION EFFORTS	: NIL	OUT OF 10
ATTENDANCE & PUNCTUALITY	: 18	OUT OF 20
ATTITUDE & BEHAVIOUR	: 18	OUT OF 20
VIVA	: 42	OUT OF 50
TOTAL MARKS (IN %)	: 86.6 %	
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DECLARATION

I **Sandra Samson, B.Vishnupriya** hereby declare that the Project Report entitled **Production Of Keratin Based Chitosan Infused Bioplastic Film From Waste Materials** done by us under the guidance of **Dr S.Sudha** is submitted in partial fulfilment of the requirements for the award of **Bachelor of Technology degree in Biotechnology.**

DATE: 16-03-22

PLACE: CHENNAI

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to the **Board of Management of Sathyabama** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr V. Ramesh Kumar, PhD, Dean, School of Bio and Chemical Engineering** and **Dr V. Ramesh Kumar, PhD, Head of the Department, Dept. of Biotechnology** for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide,
Dr S. Sudha for her valuable guidance, suggestions and constant encouragement that paved the way for the successful completion of my project work.

I would also like to thank the Biolim Institute of Research for guiding us and letting us pursue the initial phase of our project there.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology who were helpful in many ways for the completion of the project.

ABSTRACT

Bioplastic films with increased degradation rate and non-toxic were produced using waste materials like feathers and fish scales. Keratin was extracted by pretreatment followed by chemical degreasing and centrifugation. Chitosan was produced using fish scales, which were washed and cleaned and underwent three processes, namely Demineralization, Deproteinization and Deacetylation to produce Chitosan from Chitin. Keratin and Chitosan solutions were prepared and combined together along with a few other binding agents like Gelatin, Glycerol and Carboxymethylcellulose. The film prepared using these compounds was tested under different concentrations and underwent different Analyses like SEM, TGA, XRF and FTIR to understand its structural and mechanical properties under different circumstances and environments. As per the results, the film degrades faster than 80% of the films in use at the moment, is completely non-toxic and has Anti-microbial properties and extended shelf life, hence proving to be a viable asset to the Bioplastic industry.

KEYWORDS: Keratin, Chitosan, Bioplastic film, Plastic, Packaging, Anti-microbial, Non-toxic, Degradation

INDEX

LIST OF ABBREVIATIONS

CMC	-	Carboxymethyl Cellulose
D.W	-	Distilled water
FTIR	-	Fourier transform infrared
g	-	Gram
HCL	-	Hydrochloric acid
Hr	-	Hour
Min	-	Minute
mg	-	Milligram
ml	-	Millilitre
mm	-	Millimetre
N	-	Normality
mM	-	Molecular Mass
NaOH	-	Sodium hydroxide
nm	-	Nanometre
pH	-	Potential of hydrogen
Rpm	-	Revolutions per minute
SEM micro	-	Scanning electron

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1. INTRODUCTION

1.1 PLASTIC

Plastics are the most versatile and ubiquitous materials ever invented and have become a necessity in almost all parts of life, right from our toothbrushes, packaging food to transport and machinery. Even though these plastics are very efficient to use and produce, In recent times, due to the issues plastic gives rise to, there is a need to replace these artificial plastics with bioplastics which are less harmful to the environment. Organic Waste materials, when they undergo degradation, produce a gas called methane which is a major greenhouse gas leading to Global warming. Thus, it is important that this organic waste gets reused and recycled at least once before it is discarded as waste. Bioplastic materials with research can gradually work as a substitute for various materials based on plastic.

Fig 1.1: Plastic Film

1.2 KERATIN

Keratin is the major structural fibrous protein providing outer coverings such as hair, wool, feathers, nail, and horns of mammals, reptiles and birds. [1] Feathers are composed of 90% Keratin which is an insoluble, fibrous and structural protein. It is among the most abundant forms of hard protein present in nature [2]

1.2.1 CHARACTERISTICS

Keratin is rich in cysteine, arginine, threonine and hydrophobic amino acids, with high nutrient potential [3]. Feather Keratin has also been studied to develop various valuable bio-based materials [4] It contains β-sheet crystallites and is highly cross-linked by cysteine 7 mol% [5]. One of the interesting uses of feather Keratin is in eco-composites andBioplatic's [4]

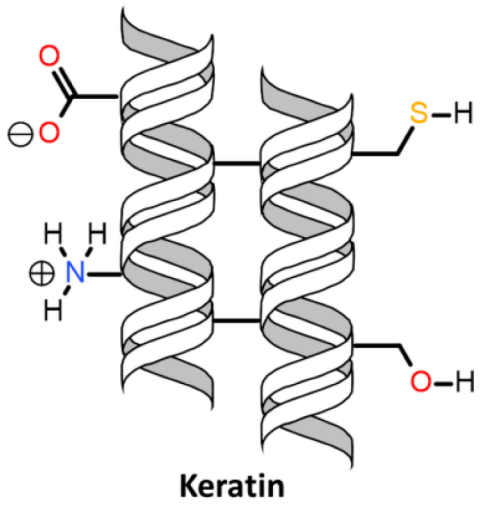


Fig 1.2 : Keratin structure

1.3 CHITOSAN

Chitosan is one in all the promising renewable polymeric materials for his or her broad application within the pharmaceutical and biomedical industry for enzyme immobilization [7]. Chitosan is employed within the chemical wastewater treatment and food industry for food formulation as a binding, gelling, thickening and stabilizing agent [6]. Mainly extracted within the kind of chitin from crustaceans' waste, squid, and oysters, Chitosan are widely studied for its applicability within the wastewater treatment field as an honest chelating agent and supports other nano-sized materials. Several Bioplastic Films which have been produced over the years claim to be harmless and producing these bioplastic films from Keratin and infusing Chitosan are some things that have not been studied to a good extent thus giving us a chance to pursue the unique properties of Chitosan.

1.3.1.CHARACTERISTICS

Chitin is created a linear chain of acetylglucosamine groups while chitosan is recovered by removing enough acetyl groups (CH3-CO) from chitin therefore the chitin molecule and therefore the resultant product is found to be soluble in most dilute acids. The particular variation between chitin and chitosan is the acetyl content of the polymer.[6]. Chitosan is well-known for its ability to create layer (film) and anti-bacterial properties [12].

Fig 1.3: Chitosan Structure

1.4 POLYHEXANIDE

Polyhexamethylene biguanide hydrochloride/polyhexanide/polyaminopropyl biguanide (PHMB) is employed as a disinfectant and antiseptic. [9] PHMB is that the most generally used antiseptic, thanks to its excellent antimicrobial activity, chemical stability, low toxicity, and reasonable cost. Furthermore, it's shown that polyhexanide in low concentrations stimulates cell proliferation and promotes wound healing.[11]

1.4.1 CHARACTERISTICS

Polyhexamethylene biguanide hydrochloride/polyhexanide/polyaminopropyl biguanide could be a biguanide, the molecules of which form a series (polymer). Biguanides are strong bases, and PHMB substances are therefore highly charged at physiological pH. this can be thought to be of considerable significance for his or her mechanism of action, which isn't fully understood[10].

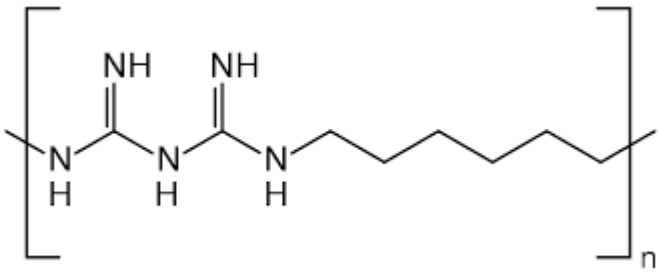


Fig 1.4: Polyhexanide Structure

Fig 1.5: Polyhexanide vial

1.5. GLYCEROL

The bioplastic film is formed more efficient by adding glycerol because the plasticizer. It increased the enduringness of the film. zero toxicity. Higher glycerol concentration makes bioplastic more flexible (higher elongation at break), weaker (low tensile strength), and easier to degrade under wet and dry soil.[12] Glycerol as a plasticizer has the function to enhance the flexibility of bioplastic in absorbing water and as a crystal-forming agent. Other characteristics of glycerol are associated with toxicity and Low Mass transfer as natural plasticizers [13]. Glycerol may be a non-toxic, edible, and biodegradable substance that's good for the environment.

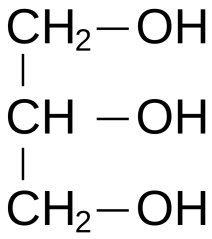


Fig 1.6: Glycerol structure

1.6.GELATIN

Gelatin is obtained by controlled hydrolysis of collagen, a fibrous insoluble protein, which is widely found in nature as the major constituent of skin, bones and connective tissue. This biopolymer has been extensively used in the food and pharmaceutical industry for its gelling and texturing properties.[15]The water solubility of gelatin permits a solvent-free approach but consequently induces water sensibility for materials application. For food packaging applications, gelatin cross-linking becomes essential to maintain film stability under humid conditions [14].

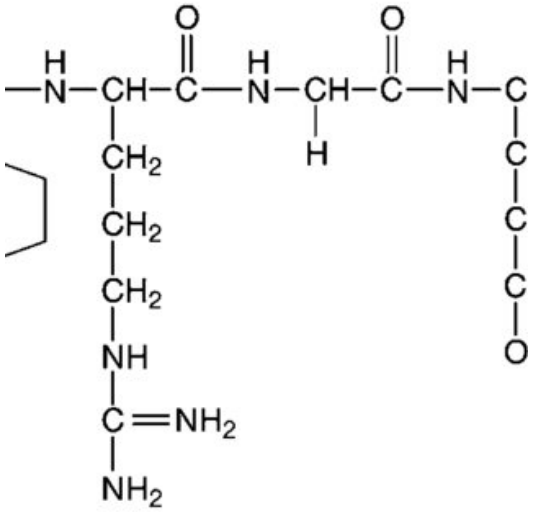


Fig 1.7: Gelatin Structure

1.7. CARBOXYMETHYL CELLULOSE

CMC which may be a thickening agent, when added gives the film a far more enduringness, elasticity and biodegradability. CMC may be a high relative molecular mass anionic polymer. It may be accustomed stabilize clay particles thanks to electrostatic interactions between polymeric anionic chains and electrical charges at the perimeters of clay particles. Further, CMC has been wont to improve the mechanical properties of the resulting composite films[16].

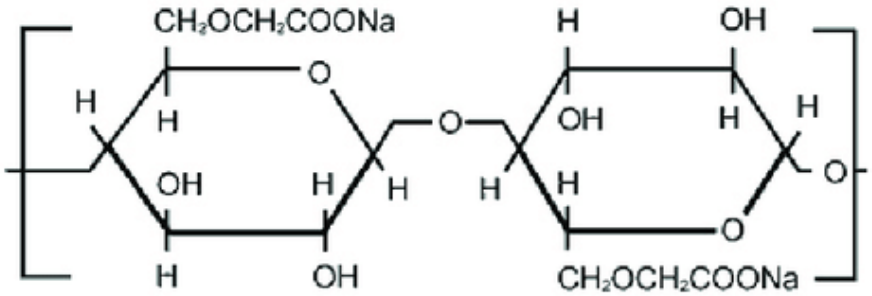


Fig 1.8: Carboxymethyl cellulose structure

2. LITERATURE SURVEY

YEAR	AUTHOR NAME	INFERENCE
2018	Sharma S, Gupta A, Kumar A, Kee CG, Kamyab H, Saufi SM	Pre-treatment of feathers and Keratin extraction and Analysis
2014	Kumari S, Rath PK	Isolation of chitosan by Demineralisation, Decolorization and Deacetylation
2021	Oluba OM, Obi CF, Akpor OB, Ojeaburu SI, Ogunrotimi FD, Adediran AA, Oki M	Preparation and Analysis of Bioplastic Film.
2021	Oluwasina, O. O., & Awonyemi, I. O. (2021).	Fish Packaging Analysis
2021	Anugrahwidya, R., Armynah, B., & Tahir, D. (2021)	Trial and error in obtaining the perfect concentration for Keratin and chitosan solutions
2017	Sharma, Swati; Gupta, Arun; Bin Tuan Chik, Syed Mohd Saufi; Gek Kee, Chua Yeo; Poddar, Pradeep Kumar (2017)	Keratin extraction
2019	Alashwal, Basma Y.; Saad Bala, Mohamed; Gupta, Arun; Sharma, Swati; Mishra, Puranjan (2019).	To improve properties of Keratin

TABLE 2.1

3. AIM AND SCOPE OF EXPERIMENT

3.1 AIM

- To produce a bioplastic film that is easily degradable, cheap, environmentally friendly and has zero toxicity while also having good mechanical properties.
- To determine the various physical and chemical properties of the biofilm.
- Its antimicrobial properties were also tested.
- To perform degradation analysis and packaging analysis to understand the packaging and degradation potential of the biofilm produced.

3.2 SCOPE

- The bioplastic produced this way will be minimal in toxicity, cost and highly efficient.
- Thus can be used in a day to day life for fish packaging, food packaging, substitutes for other harmful packaging, cosmetic packaging, surgical gloves and equipment.
- The film offers a wide scope that is yet to be explored as Keratin and chitosan are a great combination that showed great properties as we were trying to produce the film itself.

4. EXPERIMENTAL METHODS AND MATERIALS USED

4.1 MATERIALS

1. Waste Chicken feathers were collected from the local chicken shop in Kolathur, Chennai.
2. Waste Fish scales were collected from the local fish market in Thiru. Vi.Ka Nagar and Marina beach, Chennai.
3. Petroleum Ether
4. Sodium Sulfide(Na_2S)
5. Hydrochloric Acid (HCl)
6. Potassium Permanganate($KMnO_4$)
7. Citric Acid
8. Polyhexanide
9. Gelatin
10. Glycerol
11. Carboxymethyl Cellulose(CMC)
12. Tween- 18
13. Sodium Hydroxide (NaOH) and
14. Glacial Acetic Acid
15. D.W from the local Petrol Banks was used to clean and soak the feathers and the scales.
16. Whattman Filter paper
17. Protective Gloves

4.2 METHODS

4.2.1 EXTRACTION OF KERATIN

4.2.1.1 Pre-Treatment Of Feathers

Chicken feathers were collected from the local chicken shop and cleaned multiple times by running them under both tap water and D.W to remove the blood, sand and other impurities like any chicken food from the feathers. This process was repeated until the colour of the feathers turned white showing us it was rid of impurities. The clean feathers after washing were left in a ventilated oven at 40 °C overnight for drying.

Fig 4.2.1 : Cleaning of Feathers

Fig 4.2.2: Cleaning of Feathers

4.2.1.2 Degreasing Of Feathers

25g of clean feathers were weighed and chopped into small pieces (10–25 cm) and were completely immersed in petroleum ether for 24 hours in a rotatory shaker. The degreased feathers were again washed with D.W multiple times till the feathers stopped smelling like petroleum Ether. Cleaned defatted feathers were dried in a hot air oven for 24 h and chopped further into very small pieces of 2-5cm in size and stored at 4^{o}C for further use [4].

Fig 4.2.3: Degreasing of Feathers

Fig 4.2.4: Drying of Feathers

4.2.1.3 Digestion Of Feathers

The degreased feathers were further immersed in 500 mM of sodium sulfide separately and then digested using a mechanical stirrer for 1–6hr. [17] The dissolved Feathers were filtered twice using Whatman filter paper of 110 mm diameter and centrifuged using Eppendorf Centrifuge 5810 R at 5000 Rpm for 30 min to separate the supernatant from un-dissolved feathers. This process of centrifugation was repeated multiple times in order to extract Keratin with no impurities. The Keratin particles were collected and dried overnight until they reached a powder form. The Keratin particles were blended using a Prestige Mixer Grinder to get fine Keratin powder. The Keratin powder was stored at 4^{o}C for further use.[18]

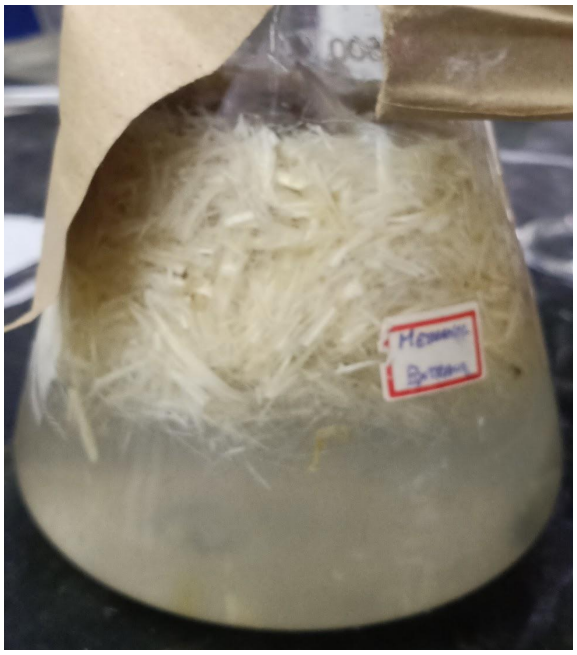


Fig 4.2.5 : Digestion of Feathers

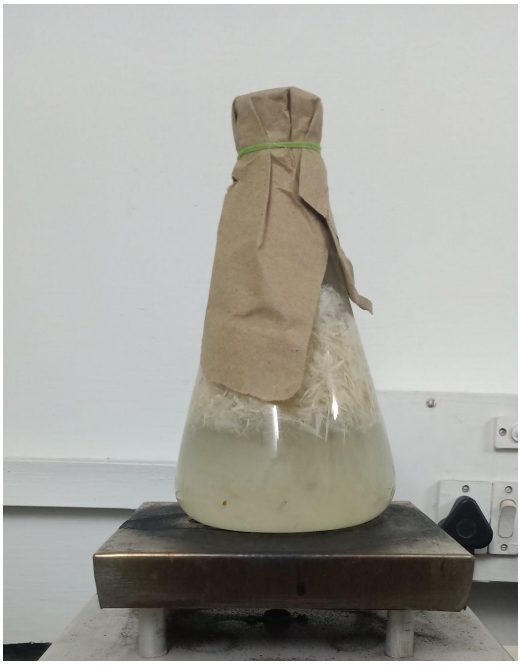


Fig 4.2.6: Digestion of feathers in stirrer

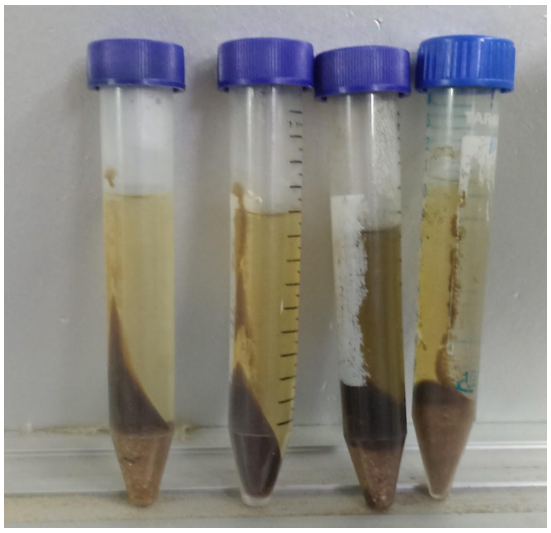


Fig 4.2.7: Centrifuging the filtrate and collecting the sediments

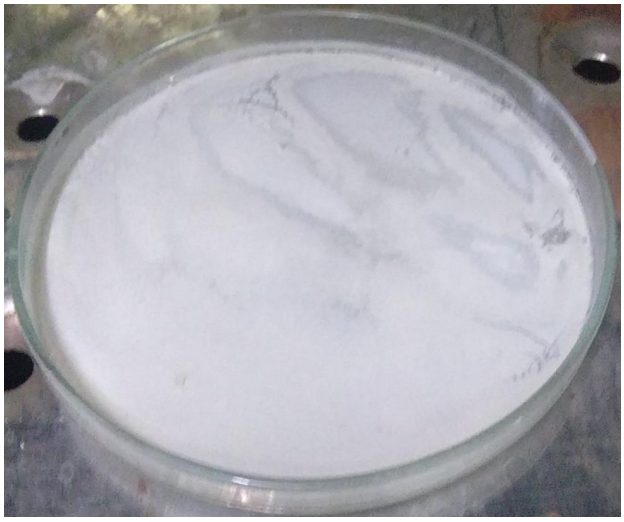


Fig 4.2.8: Keratin Powder

4.2.2 EXTRACTION OF CHITOSAN

4.2.2.1 Pre-Treatment of Fish Scales

The collected fish scales and fish skins from the local markets were cleaned multiple times until the sand particles and other impurities like fish blood and skin were completely removed. Left the washed fish scales for drying in the hot air oven overnight at 40°C for 52 hours.

Fig 4.2.9 : Washing of Fish Scales

Fig 4.2.10: Cleaning of Fish Scales

Fig 4.2.11: Drying of Fish Scales

4.2.2.2 Demineralisation of Fish Scales

The dried fish scales were demineralised with a HCl solution (1.0 M) at 30°C, using a ratio of solid to an acidic solution of 1:5 (w/v). This resulted in the fish scales becoming quite squashy. Further, the 60g fish scales were added to 30ml of 38% HCl for 24hours. It was then washed and set to be dried in the oven at 30°C overnight. The dried scales were kept in NaOH(1:1 m/v) solution for 36 hours for demineralisation.

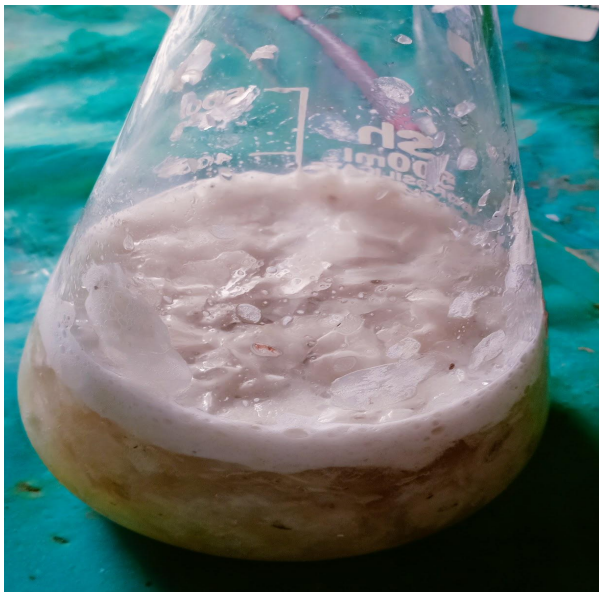


Fig 4.2.12 : Demineralisation (HCL)

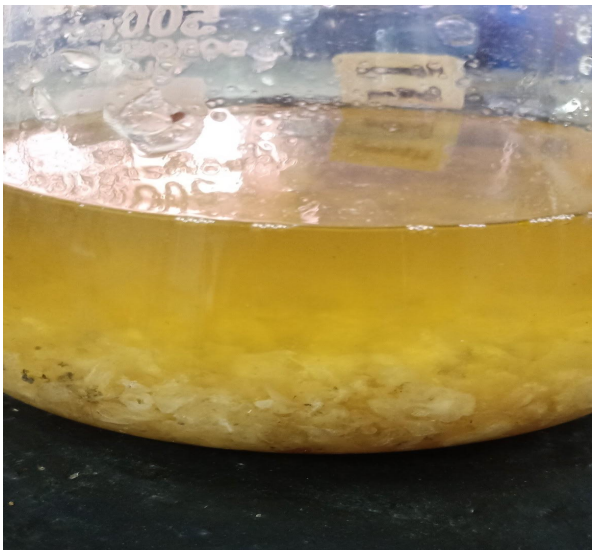


Fig 4.2.13: Demineralisation(NaOH)

4.2.2.3 Decolourization of Fish Scales

The next step in this process was decolourization in which the demineralised fish scales were dissolved in Potassium permanganate solution for 1 hour, followed by keeping them in CItric acid for the process of decolourization.

Fig 4.2.14 : Decolorization of Fish Scales

4.2.2.4 Deacetylation and Chitosan Production

These processes resulted in chitin as the product which was further treated with 50% w/v NaOH for the process of deacetylation resulting in chitosan as the end product. Chitosan was centrifuged and the supernatant was centrifuged again for 25 minutes each. The resulting particles were dried in the hot air oven overnight. The dried chitosan was blended with and stored for future purposes. [6]

Fig 4.2.15 : Chitin in 50% NaOH

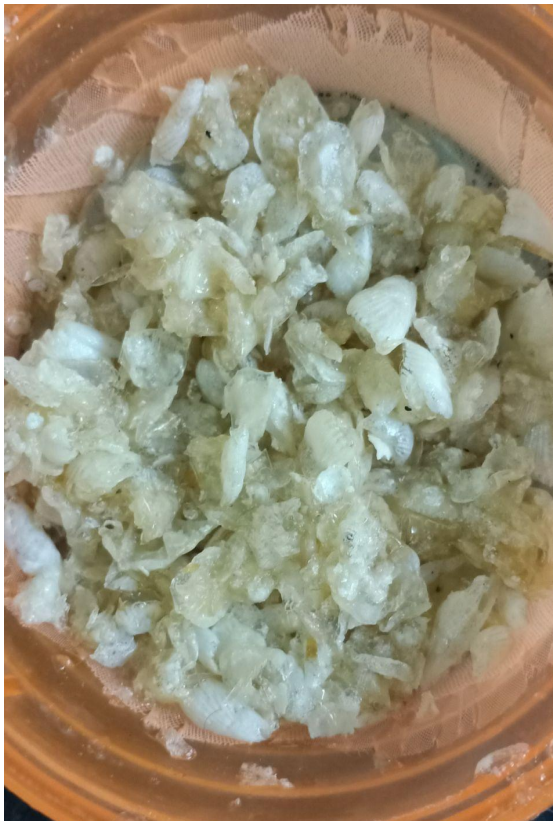


Fig 4.2.16 : Chitosan

4.2.3 BIOPLASTIC FILM PREPARATION

Keratin solution was prepared by dissolving 10% off Keratin powder in 2N NaOH. Chitosan solution was made by dissolving Chitosan Powder in 75% of acetic acid [19]. After complete dissolution of the keratin and Chitosan particles in the solutions, The keratin and Chitosan solutions were combined together.

2% Gelatin and 2% Glycerol were added to the Keratin-Chitosan solution as the Plasticizer. Further, 2% CMC was measured and added to this composite solution for the film formation [20]. The solution was kept in the mechanical stirrer for 10 mins with a constant temperature of 70 to 100^{o}C until the solution was completely dissolved. The solution was cooled a little and then poured onto a plastic sheet which was greased by a greasing agent. The film was set to dry in the hot air oven at 40^{o}C for 48-52hrs. The film was peeled off from the plastic and analyzed for various properties.[19]

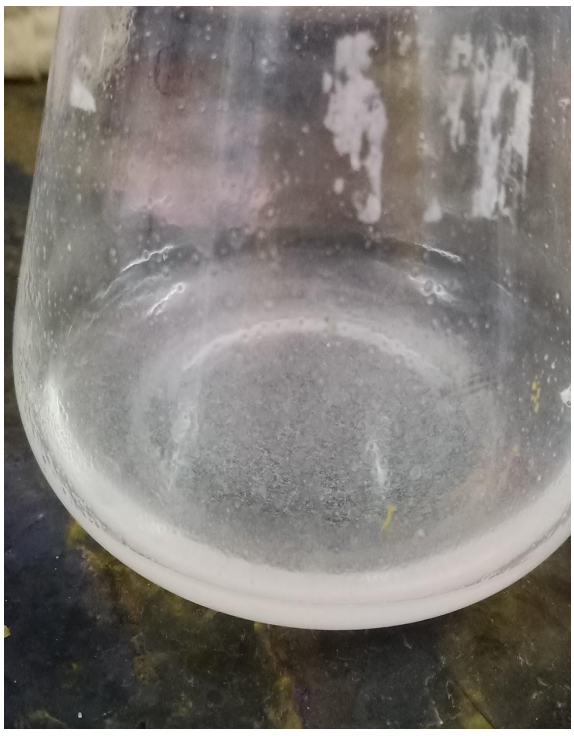


Fig 4.2.17: Keratin solution

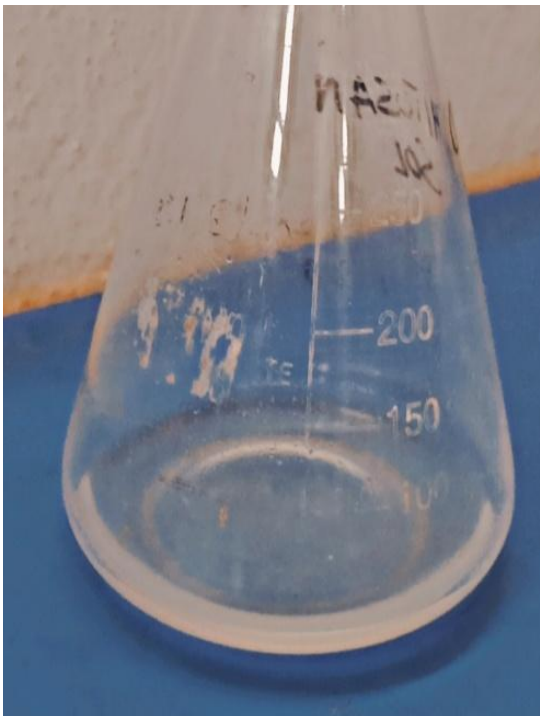


Fig 4.2.18: Chitosan solution

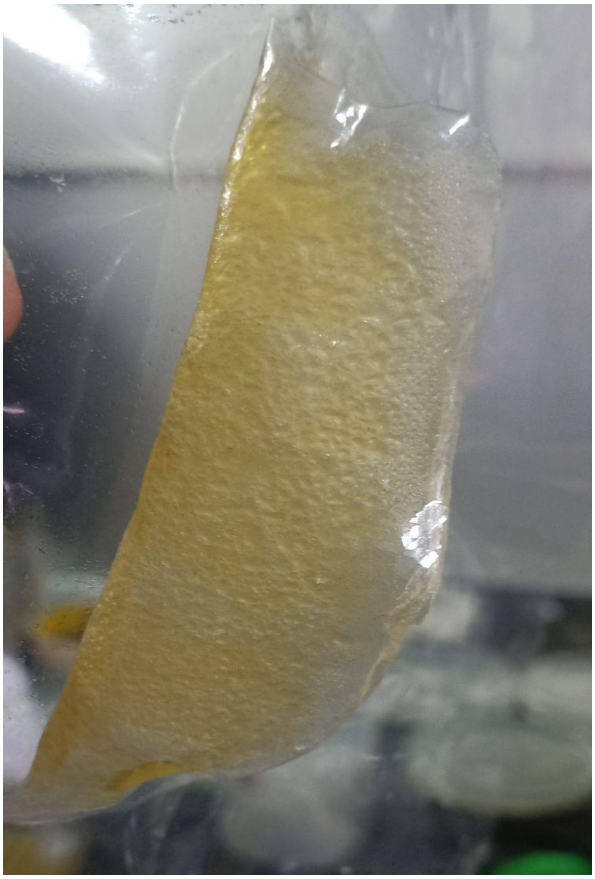


Fig 4.2.19 : Kn-Cn Bioplastic Film

Fig 4.2.20 : Kn-Cn Bioplastic Film

4.2.4 DRUG ENHANCED ANTIMICROBIAL ACTIVITY OF BIOPLASTIC FILM

To the original Bioplastic film solution which was prepared a unique polymeric drug with Anti-microbial and Disinfectant Properties was added to check its influence on the Bioplastic film.

2ml of the drug called Polyhexanide was added to the liquid composition followed by the general composition of Gelatin, Glycerol and CMC as discussed above. The drug was added to further enhance the Anti-microbial properties of the film which could also be explored further as a wound dressing based on Bioplastic film as Polyhexanide is a drug that is very commonly used in Surgical areas to inhibit post-surgical infections.

Fig 4.2.21 : Dye infused Film

Fig 4.2.22 : Polyhexanide infused Film

Fig 4.2.23 : Film with tween-18

4.2.5 CHARACTERIZATION

4.2.5.1 SEM

The surfaces of synthesized bioplastic films were studied under a Hitachi's Tabletop Electron Microscope TM3030 Plus at an accelerating voltage of 15 kV. SEM is used to identify the molecular structure of the sample to check if the mixtures are well-mixed during the polymerization process. [22]

4.2.5.2 FTIR

The FTIR (Fourier Transform InfraRed) is a characterization technique based on the vibrations of atoms or molecules by passing infrared radiation through a material or sample in which the energy corresponds to the wave frequency [35]. Fragments of bioplastic were dried and cleaned to remove all the deposits formed on their surface. The variation of peaks intensity and wavenumbers provides qualitative information about the chemical change of the polymeric structure, the specific degradation process of Keratin and Chitosan and the effect of the Plasticizers used on the film and its structure. [30]

4.2.5.3 XRF/ FOOD SAFETY TEST

The elemental composition of the Keratin-chitosan film was done with XRF. XRF (X-Ray Fluorescence) is ideally suited for very fast qualitative elemental analysis. Typically all elements from sodium through to uranium can be detected simultaneously, with good quality spectra obtained in seconds/minutes. Measurements of a bioplastic material composition are necessary to determine the content of heavy metals, which results in food security for the reference product.

4.2.5.4 TGA

Thermal test bioplastics by TGA (Thermogravimetric analysis) aims to determine the nature and thermally stable bioplastic components of skin cassava has produced. This data is a reference in determining the optimum conditions of bioplastic packaging when exposed to high temperatures or resistance of the packaging in wrapping the food. [36].TGA was carried out using a thermogravimetric analyser. The bioplastic film samples were cut into pieces (2.5 to 5 mg) and transferred to the sample holder.

4.2.5.5 Chemical Analysis

The Bioplastic film was cut into four pieces and each piece was immersed in four different chemicals namely NaOH, NaCl, Ethanol and Hcl to observe their Chemical reactivity in alkaline and acidic natures.

Fig 4.2.24 : CHEMICAL ANALYSIS

5. RESULTS AND DISCUSSION

5.1 RESULTS

Keratin and Chitosan solutions were combined to produce a Bioplastic Film using Glycerol, Gelatin and CMC. Keratin was Produced from Waste chicken feathers after following the ASTM methods. This was further scraped and Blended to produce Keratin in a powder format. Chitosan was further successfully produced after following the materials and methods used above. Instead of Oxalic acid, Citric acid and HCl were used as Decolourising agents. Finally, Keratin and Chitosan solutions were produced in different concentrations until a Feasible Concentration was identified under the Trial and Error method. The Keratin and Chitosan solutions produced under optimum concentrations were made into a Bioplastic Film using Gelatin and Carboxymethyl as plasticizers and thickening agents to enhance the mechanical properties of the film. The film with chitosan held natural antimicrobial properties when tested against various parameters. But this property was enhanced for the packaging analysis by adding a drug called polyhexanide. This film had a good elasticity and stability and at even high temperatures it wasn't dissolved. They were degradable but reusing the same again and again was not possible.

Among all films, about 24.43% reduction in contact angle proved that the lower side of PVP/CMC/BC/GG film was suited as packaging exterior as a result of its better hydrophobicity to prevent water permeability than the PVP/CMC film (61.40°).

For bioplastics by adding chitosan composition 5% w, bioplastic is used to wrap food sausage that does not need to be peeled again when cooked (direct consumption). The sausages are wrapped in transparent plastic that needs to be peeled before cooking. Besides being less effective, plastic also causes problems in the environment. While the use of bioplastics can provide three benefits at once, namely: more effective (no need to peel), not a waste, mineral content is good for the body.

Chitosan %	Keratin %	Visual Appearance Result
10	10	The film had not formed any bonds and hence was still in the form of a semi-solid composition. No film like structure was observed.
20	20	The liquid composition was much thicker than the previous concentration but film formation was still not complete and hence peeling off the film was not possible.
30	20	To check which product among keratin and Chitosan was helping in film formation we increased the chitosan percentage to observe that film formed was extreme but increasing the percentage did lead to film formation.
30	40	Increasing the keratin concentration seemed to increase the consistency of the film and the film was much thicker and stronger and was peelable to a certain extent.
50	50	Increasing both chitosan and keratin to an equal level produced a stable film that formed strong bonds and after sun drying for a few days gave us the perfect film which was thick and strong with visible plasticity when pulled.

TABLE 5.1 Kn-Cn % used in the experiment

5.1.1 Visual Appearance of the Bioplastic films

The films were experimented with by adding and changing various factors around the film and also in the composition of the film.

The film with dye: To the original composition of the film, a natural food colour saffron was added for commercial marketing purposes and it was observed that it did not affect the stability of the film in any way while also retaining shape and structure. The film was pink and vibrant looking.

The film with Polyhexanide: 1ml of Polyhexanide drug was added to the original composition and was compared to the original film, while the drug enhanced the antimicrobial properties, it was observed that adding the drug made the film take a longer time to dry, but also did increase the rate at which the film degraded. The film looked a little more wet and oily.

The Film with tween 18: 1ml of tween 18 was added to one of the original compositions of bioplastic solution, this film was more ductile and had a greasy texture compared to the other films. Tween 18 also gave the film a little more of an added yellow colour compared to the other films.

The film with Polyhexanide and Tween 18: Though the Tween 18 still have the Bioplastic solution a greasy texture the drug settled down and the film was not oily or wet after a few more days in the hot air oven. The film was a shade more in the yellow colour spectrum but not as much as the film with just tween 18. Polyhexanide and Tween 18 blended well with the composition of the bioplastic and overall as well.

5.1.2 PERFORMANCE ANALYSIS

5.1.2.1 SEM

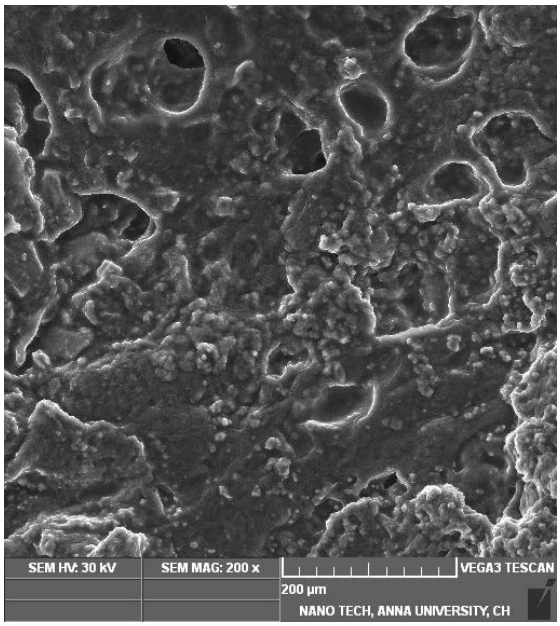


Fig : 5.1 (a)

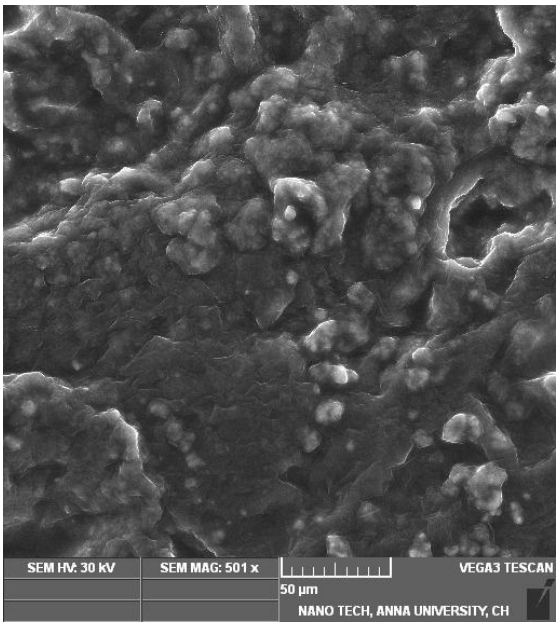


Fig : 5.1(b)

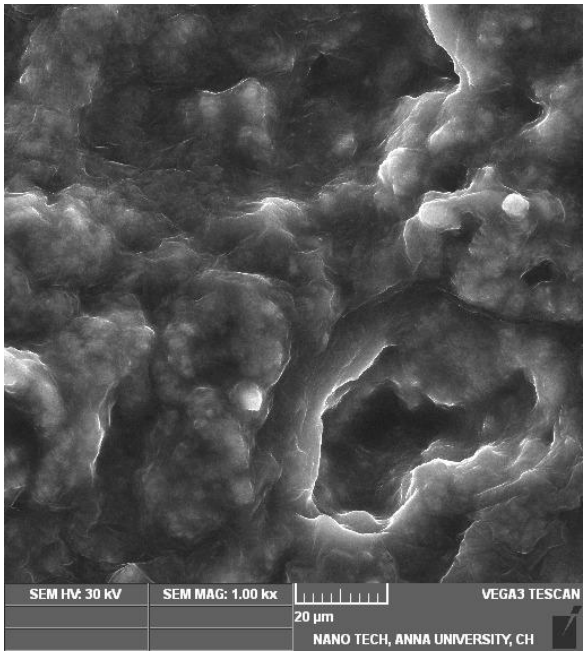


Fig : 5.1(c)

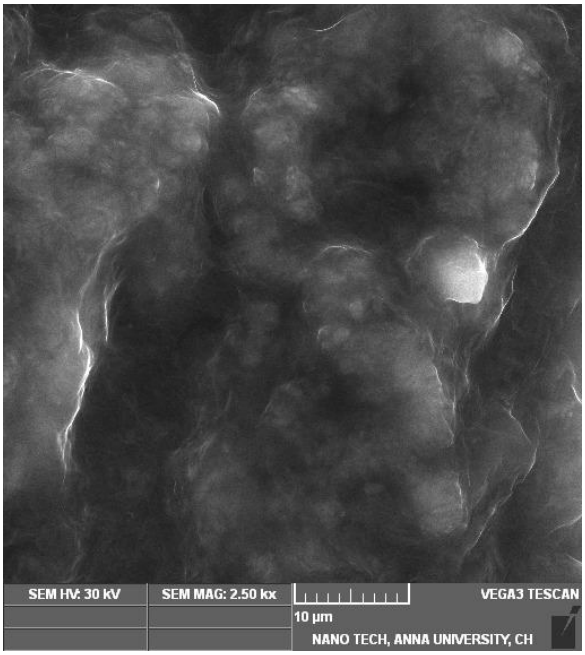


Fig : 5.1(d)

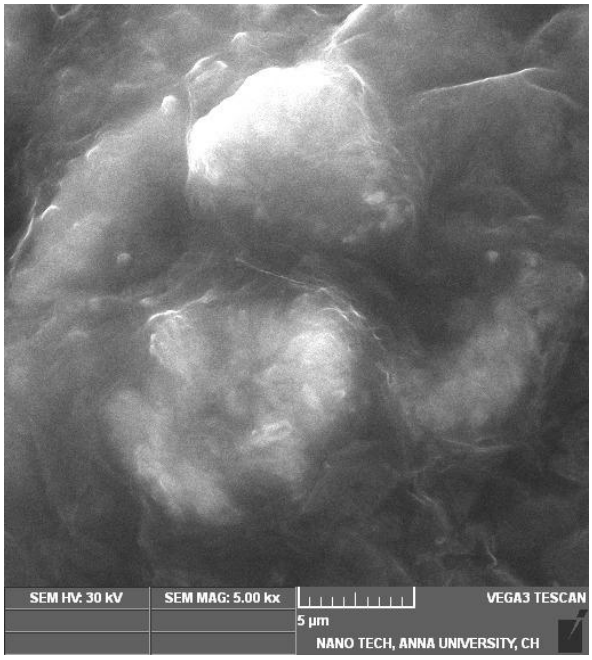


Fig : 5.1(e)

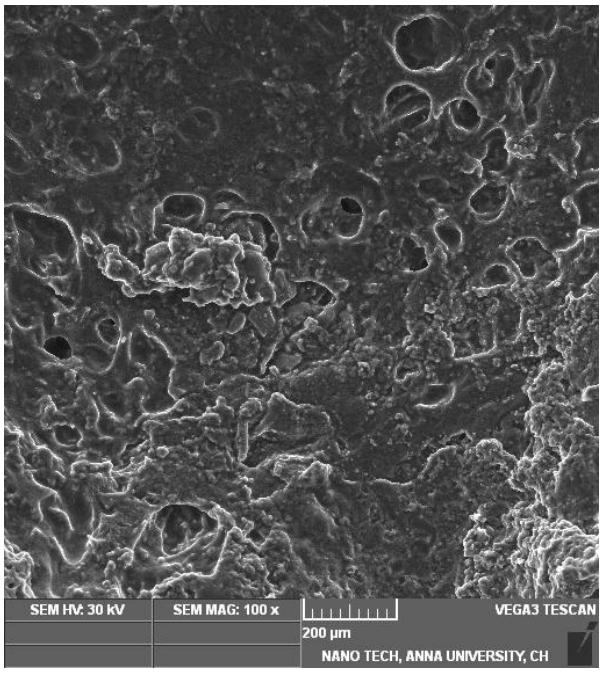


Fig : 5.1(f)

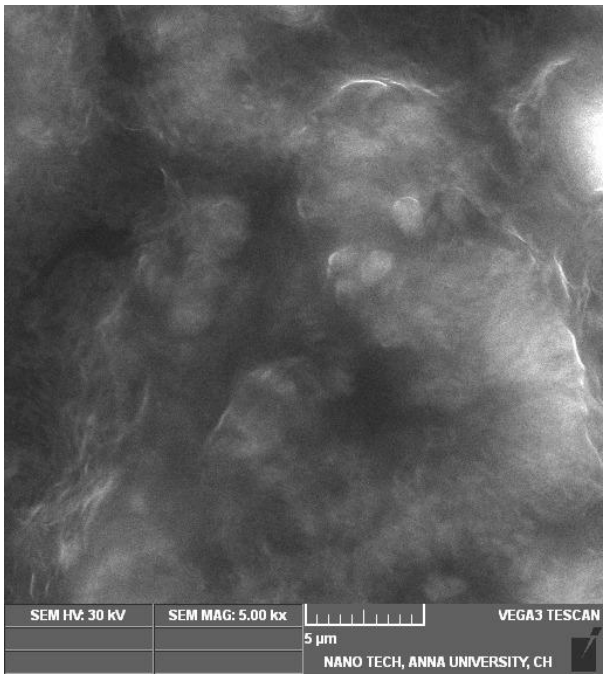


Fig : 5.1(g)

The above pictures were recorded as the results of the SEM analysis of 2%wt glycerol in the bioplastic film at various magnifications like 100x, 500x,1 kx,2.5kx, 5kx. The images indicate the presence of a heterogeneous microstructure.The main evidence of the material is the presence of some circular spots whose dimensions range in the order of a few hundred nm probably composed of Chitosan.

The voids and the separation present in the images are directly proportional to the glycerol content present [25]. The dried keratin consists of spherical, tightly packed micro-particles and randomly arranged porous microstructures in 1(a), 1(b) 1(f).[27][29]. The images of 1(a) and 1(f) show that the presence of voids and distortions are due to the higher plasticizer content present and proves that they will form less voids and undispersed particles at lower plasticizer content.

5.1.2.2 FTIR

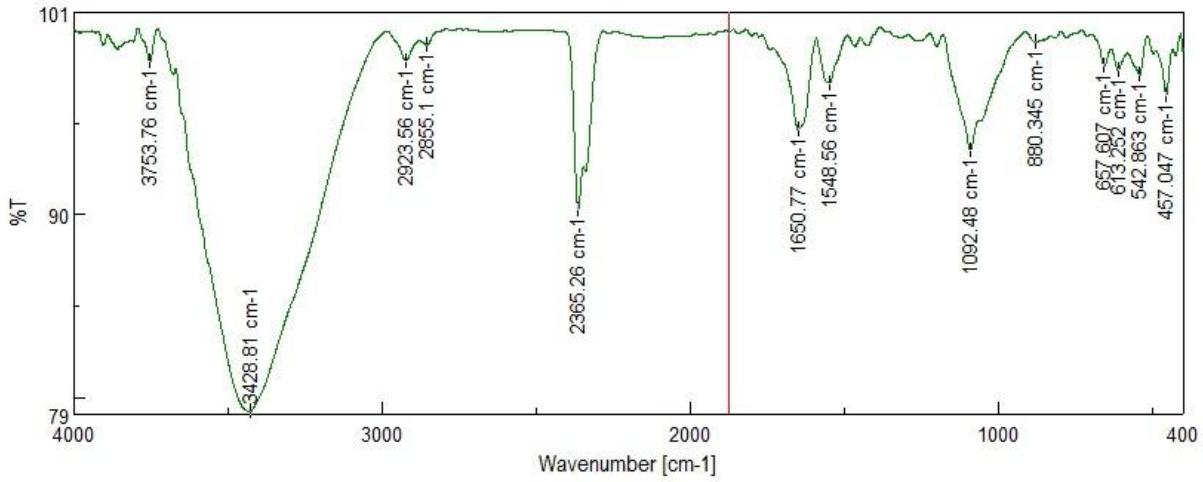


Fig 5.2 : FTIR Graph

The Figure reports the spectra in the wavenumber range between 4000 cm-1 to 400 cm-1, where the main diagnostic peaks are present. The major peaks are reported at 3428.81 cm-1, 2365.26 cm-1, and multiple other smaller peaks. In the presence of a

plasticizer, the graph shows a shifting peak towards a higher wavenumber. This is because of the decreased interaction of glycerol with β-sheets and more disordered structures. The amide II band is related to N-H bending and C-H stretching vibrations. Although it is much less conformationally sensitive than amide I, it is much more sensitive to the environment of the N-H group [31]. Therefore, the amide II band can be used to deduce changes to the environment of the N-H groups and respond to differences in hydrogen-bonding environments [32].

In general, stronger hydrogen-bonded N-H groups absorb at higher frequencies. Glycerol, containing more hydroxyl groups, can form a higher number of hydrogen bonds, thus increasing the amount of hydrogen-bonded peptide groups.[33] Therefore, it was observed at a higher wavenumber. The spectrum of cluster peaks below 2000cm-' down to around 400cm-' are the peaks from the stretching of spi-hybridized C-H bonds. The alcohol 0-H group peak is around or about 3300 cm. A sharp peak right around 1650 cm-' means that there is a carboxylic acids group in the mixture. Nevertheless, the criteria of the peaks meet the criteria of the existence of an ester group, which are carbonyl peak, alkyl peaks and ester peaks.[33]

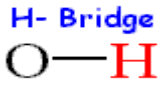
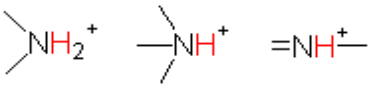
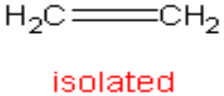
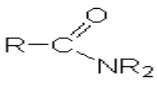
Peak wavenumber	Bonds	Intensity
3753.76 cm-1		Variable intensity
3428.81 cm-1	Hydrogen bond	Variable intensity , often sharp, frequency-independent from concentration
2923.56 cm-1		Strong to medium intensity
2855.1 cm-1		Strong intensity
2365.26 cm-1		Medium intensity
1650.77 cm-1		Weak to medium intensity bonds
1548.56 cm-1		Strong intensity
1092.48 cm-1		Medium intensity
880.345 cm-1	Aromatic Bonds	Weak intensity
657.607 cm-1	Alkyl Bonds	Strong Intensity
613.252 cm-1	Alkyl Bonds	Strong intensity
542.863 cm-1	C-Br (alkyl)	Strong Intensity
457.047 cm-1	Alkanes or Alkenes	Weak intensity

TABLE 5.2 BONDS PRESENT FROM FTIR ANALYSIS

5.1.2.3 TGA

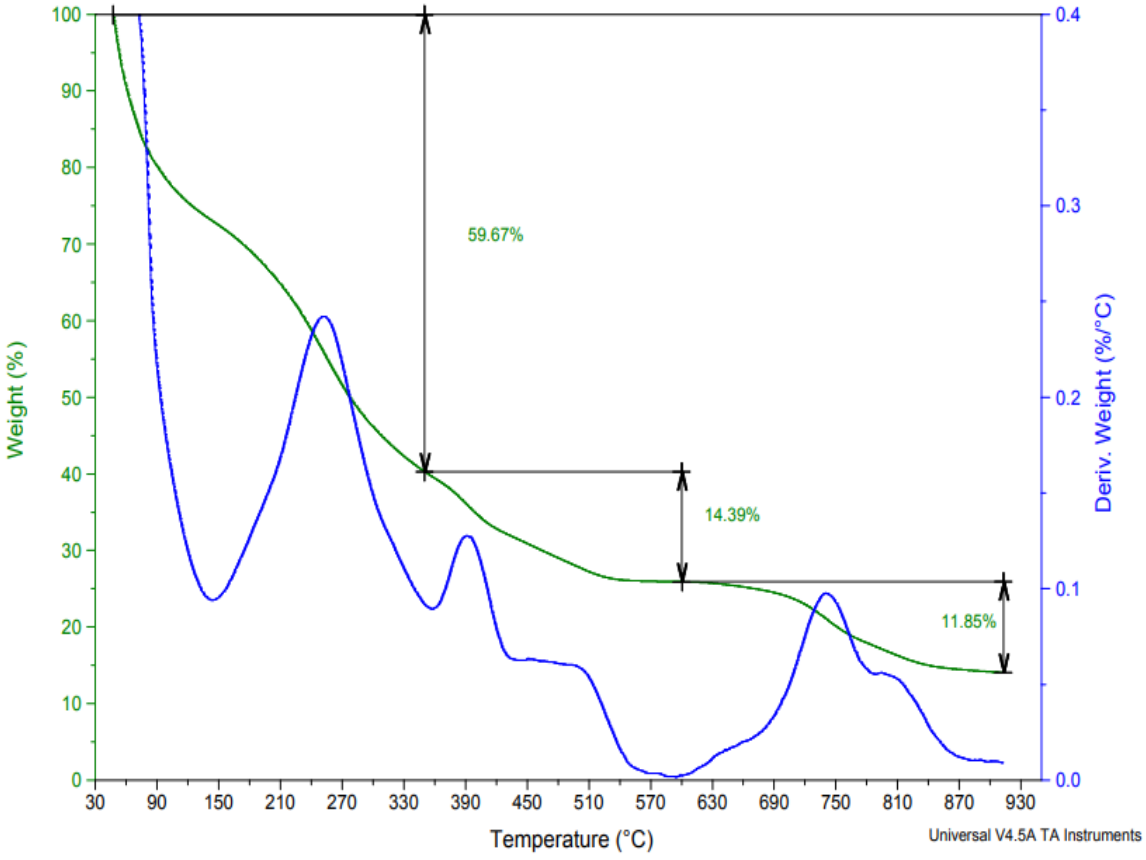


Fig 5.3 : TGA Graph

Thermogravimetric analysis (TGA) is an analytical technique used to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a sample is heated at a constant rate. This curve depicts multistage decomposition. This curve shows high volatility of 59.67%, low volatility of 14.39% and combustion of 11.85%. The weight of 59.67% happens due to the loss of plasticizers, 14.39% due to degradation and the rest is due to the loss of residual compounds like the polymer.

5.1.2.4 XRF/ FOOD SAFETY TEST

Quantitative Result

Analyte	Result	[3-sigma]	Proc.-Calc.	Line	Int.(cps/uA)
Ca	48.550 %	[0.502]	Quan-FP	CaKa	2.7107
S	37.777 %	[0.694]	Quan-FP	S Ka	0.6306
Mn	6.619 %	[0.183]	Quan-FP	MnKa	1.3780
Fe	3.008 %	[0.137]	Quan-FP	FeKa	0.8655
Cu	2.874 %	[0.096]	Quan-FP	CuKa	1.2538
Zn	0.611 %	[0.078]	Quan-FP	ZnKa	0.3127
Os	0.562 %	[0.219]	Quan-FP	OsLa	0.1121

TABLE 5.2 The readings of the metals present in the Bioplastic Film

Determination of the various levels of heavy metals helps us understand the food safety parameters. Testing of these heavy metals (Cd, Pb, Hg, Cr) contained in the bioplastic packaging is done by an Assay performed with measurement by XRF (X-Ray Fluorescence). Food safety testing is done by the polymer nanocomposite BPOM Head Regulation No. HK 00.05.55.6497. The above tabular column depicts that the sample has Calcium, Sodium, Manganese, Iron, Copper, Zinc and Osmium. From table 5.3 it appears that this bioplastic material does not contain heavy metals (Pb, Cd, Hg, and Cr). It showed that this bioplastic material is safe for consumption and hence can be used for food packaging due to its non-toxicity. Meanwhile, the content which is largest in this material is calcium in the form of CaO, which is a source of minerals needed by the body. This is because bioplastic is made from keratin derived from the feathers of Chickens.

From Table 5.3 it can be seen that the bioplastic produced does not contain metals that are harmful to health in any way, but rather they are minerals that the body needs and are minerals that can be attained by consuming foods that have been packaged or wrapped in the bioplastics we produced. When the Food is packed with the

Bioplastic which has a composition of 5% w chitosan,the wrapper need not be peeled and can be consumed directly.

5.1.2.5 Biodegradability Testing

The Bioplastic film was buried under natural clay soil which held moisture and was observed for a period of 1 week. The film on the first day exhibited no change except a little discolouration after 24 hours in the soil. On the second day, after being buried for 48 hours, The film became a little sticky but was still intact but was also fully brown in colour, showing full discolouration. On the third day, After 36 hours in soil, parts of the film were visually disintegrating and had broken down to just a few sticky pieces of film. At the end of 96 hours, only traces of the film were observed where a few chunky pieces, extremely sticky and almost semi-solid were observed. On the fifth and sixth days, the film getting degraded was visible clearly as the pieces went missing and started completely disintegrating to nothing. By the end of the week, the film was completely gone with no traces and it was almost like there was no film there. It was observed that the film produced had impeccable degradation properties.

Fig 5.4: Biodegradability Test

5.1.2.6 Chemical Analysis

5.1.2.7.1 NaOH - In NaOH, the film was completely dissolved. There was no trace of film and No film particles were observed.

5.1.2.7.2 NaCl - In NaCl, The film was completely dissolved as well. The Film particles were broken down into multiple small pieces and the particles are too small to observe

5.1.2.7.3 HCl - In HCl, The film had completely softened and was still in the form of a film and had not fallen apart or dissolved. It had a Jelly consistency.

5.1.2.7.4 Ethanol - In Ethanol, The film had softened but was not in the form of a film but had fallen apart into lumps of jelly-like film parts. These lumps seem to come apart more when shaken or stirred.

Fig 5.5.1 : HCL

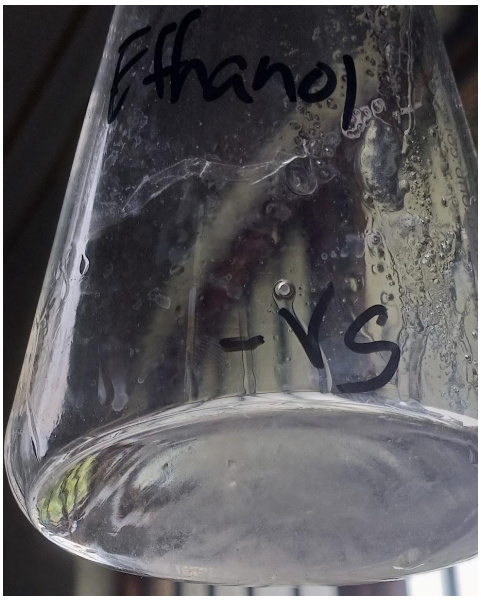


Fig 5.5.2 : ETHANOL

Fig 5.5.3 : NACL

5.2 DISCUSSION

The Keratin and chitosan produced was extracted from waste materials thus lowering the cost of production by half. The Keratin and chitosan powder that was obtained was not infected or has not disintegrated after being stored in various temperature and PH conditions even after so many months from the day it was produced. when they were dissolved with NaOH and Acetic acid to prepare Keratin and chitosan solutions they showed complete solubility. When these solutions were dried individually without any other external chemicals and in a hot air oven at 40°c it was observed that both Keratin and chitosan solution individually were capable of forming a film-like structure and making bonds when dried. The Keratin solution was a bit more dry compared to the chitosan solution which took a bit more time to dry up and make bonds, though it was observed that the chitosan solution formed a stronger structure showing good bond formation.

Later, When both the solutions were mixed and dried at 40°c, the consistency of the film was better and the bonds formed were firmer showing that Keratin and chitosan combined had enhanced properties.

Even though the Keratin and chitosan solutions by themselves were capable of forming film-like structures, they needed an anchor like base to obtain a Biofilm that we could peel off. So to this Keratin-Chitosan mixture, we added glycerol and Gelatin to plasticize and thicken the solution. We added gelatin as it was an organic thickening agent and as it was also another compound that was prepared from waste material like Pig Fat.

The more glycerol we added the more the film was distorted. So less amount of glycerol enhanced the properties of the film. When CMC was added the film was more oriented with stability and withheld higher temperatures. When film was produced just with keratin its packaging properties were not that enhanced but with chitosan as an

antimicrobial property intensified its packaging property. To make it more enhanced we added in a drug called Polyhexanide. Even a 0.5% concentration of this drug made its antimicrobial property increase. But when we added more of this drug the formation of the film did not withhold its surface property and declined to form a film with stability.

Later, the film was additionally made consistent and into a plastic-like consistency by adding Carboxymethyl which helped us get our Final end product which was further tested on how it reacts with alkali and Acid. The film obtained was placed in different environments with different temperatures and PH. The film was sun-dried and was also placed at room temperature for multiple days and was observed to be resilient and was shown to hold natural Antimicrobial properties.

6. SUMMARY AND CONCLUSION

These studies demonstrated and produced the successful fabrication of a Keratin and chitosan bioplastic film from chicken feather wastes and Fish Scales. The addition of Keratin and Chitosan as a mixture was observed to enhance the suitability of bio-composite for food packaging through improved quality attributes including reductions in moisture content, opacity or transparency, as well water solubility of the bioplastic films. The remarkable degradability of the bioplastic film is a testament to its environmental friendliness as it is non-toxic and is produced from organic waste materials. Overall, the Keratin and Chitosan bioplastic showed comparable qualities to the non-degradable polymers. Therefore, it could offer a viable, eco-friendly and sustainable solution to solid waste accumulation in the environment.

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