RESEARCH PAPER

Isolation of Protein from Watermelon Seeds and Fabrication of Bioplastic Films

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ABSTRACT

Background

The problem of environmental pollution due to non-biodegradable plastics has increased the demand for sustainable solutions. Plant proteins show great potential as a renewable resource for biodegradable polymers.

Objective

The main aim of the study is to isolate protein from the seeds of watermelon (Citrullus lanatus) and use it for preparing biodegradable bioplastic films.

Materials and Methods

The procedure for protein extraction was done by alkaline solubilization followed by isoelectric precipitation. A qualitative test, UV spectroscopy and FTIR analysis were used to verify the isolated protein. The production of bioplastic films employed the solvent casting method where gelatin was used as a co-film former whilst glycerol acted as a plasticizer. Using a Universal Testing Machine, the mechanical properties were measured, while the soil burial testing was performed to determine the biodegradability.

Results

Following UV examination, an absorbance peak within the range of 260 to 280 nm indicated the presence of aromatic amino acids in the solution. FTIR spectra revealed the presence of amide I and II bands, suggesting the presence of peptide bonds. The tensile strength of the created bioplastic film was 1.66 MPa. The film was completely degraded in soil burial tests.

Conclusion

To conclude, the utilization of protein from watermelon seeds can lead to the production of biodegradable bioplastics which can be used instead of plastics.

Keywords: Watermelon seed protein, Bioplastic, Biodegradable film, Protein extraction, Sustainable polymer.

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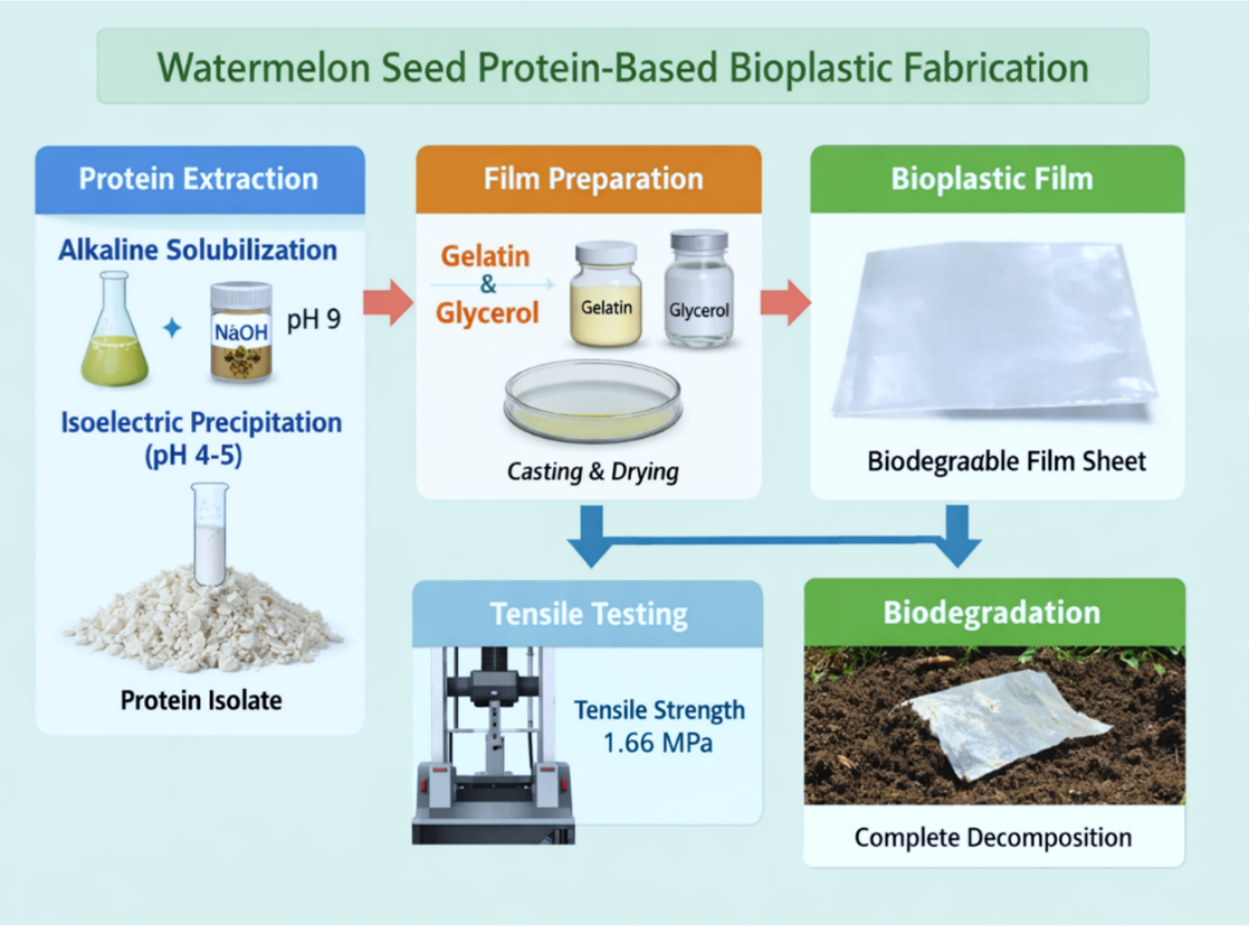

Figure 1: Graphical Abstract:
The extraction of protein from watermelon seeds is achieved through a process involving alkaline solubilization and isoelectric precipitation. This extracted protein is then utilized to produce biodegradable bioplastic films, incorporating gelatin and glycerol. The resulting films were assessed for their mechanical properties, revealing a tensile strength of 1.66 MPa. Additionally, their biodegradability was verified through soil burial tests, which confirmed their complete environmental decomposition.

1. INTRODUCTION

The global production of synthetic plastics has increased dramatically over the last few decades, leading to severe environmental pollution due to their non-biodegradable nature and persistence in ecosystems.[1] It is estimated that more than 350 million tons of plastic waste are generated annually worldwide, and a significant portion accumulates in landfills, oceans, and natural environments.[2] These plastics degrade very slowly and can persist for hundreds of years, resulting in ecological imbalance, microplastic contamination, and potential risks to human health.[3] Consequently, there is a growing demand for sustainable and environmentally friendly alternatives to conventional petroleum-based plastics.

Biodegradable polymers derived from renewable resources have emerged as promising alternatives to synthetic plastics because they can degrade naturally through microbial and enzymatic processes.[4] Natural polymers such as starch, cellulose, and proteins have been widely explored for biodegradable material development due to their biocompatibility, renewability, and eco-friendly nature.[5] Among these materials, plant-based proteins have attracted considerable attention for the fabrication of biodegradable films and packaging materials because of their ability to form intermolecular networks through hydrogen bonding and electrostatic interactions.[6]

Recent research has demonstrated that protein-based biodegradable films can exhibit desirable mechanical

and barrier properties when combined with suitable plasticizers and stabilizers. For example, studies conducted between 2020 and 2023 reported successful development of biodegradable films using soy protein isolate, wheat gluten, and pea protein for food packaging applications.[7,8] These materials show good film-forming ability and moderate mechanical strength; however, their large-scale industrial application is sometimes limited due to high cost, competition with food resources, and variability in physicochemical properties.[9] Therefore, researchers are increasingly exploring alternative protein sources derived from agricultural by-products and food processing waste.

Watermelon (*Citrullus lanatus*) is one of the most widely cultivated fruits worldwide, producing significant quantities of seed waste during processing and consumption. Although these seeds are often discarded, they contain substantial amounts of proteins, lipids, and essential amino acids. Previous studies have reported that watermelon seeds contain approximately 30–40% protein, indicating their potential as a valuable source of natural biopolymers.[11[10] The utilization of such agro-industrial waste materials not only provides an economical raw material for biodegradable polymer production but also supports sustainable waste management and circular economy principles.[11]

In recent years, several researchers have investigated the use of plant seed proteins for biodegradable polymer fabrication. For instance, protein extracted from sunflower seeds, pumpkin seeds, and rice bran has been successfully used to produce biodegradable films with promising mechanical properties and biodegradability.[12] These findings highlight the potential of seed-derived proteins as sustainable polymer matrices for environmentally friendly plastic substitutes. Protein extraction from plant materials is commonly achieved using alkaline solubilization followed by isoelectric precipitation, a method that allows efficient separation of protein fractions from plant biomass.[13] The extracted protein isolates can subsequently be processed into biodegradable films using plasticizers such as glycerol or sorbitol, which improve film flexibility and reduce brittleness.[14] Such biodegradable films can be applied in various fields, including food packaging, agriculture, biomedical materials, and environmentally friendly disposable products.[15]

Despite the promising potential of plant-based protein bioplastics, the utilization of watermelon seed protein for biodegradable film fabrication remains relatively underexplored. Considering the high protein content and widespread availability of watermelon seed waste, further research is required to evaluate its potential as a sustainable raw material for biodegradable polymer production[16]

Therefore, the present study aims to isolate protein from watermelon seeds and utilize the extracted protein for the fabrication of biodegradable bioplastic films. The prepared films are further characterized for physicochemical properties, mechanical strength, and biodegradability.[17] This research highlights the potential application of watermelon seed protein as an eco-friendly and renewable material for biodegradable plastic production, contributing to sustainable material development and waste valorization strategies[18,19].

2. MATERIALS AND METHODS

2.1. Materials

Watermelon seeds were collected from local markets in Pune, India. Sodium hydroxide (0.1 M), hydrochloric acid (0.1 N), chloroform, gelatin, glycerol, and distilled water were used. All chemical were purchased from **Loba Chemie Pvt. Ltd., Mumbai, India.**

Preparation of Seed Powder

Fresh watermelon (*Citrullus lanatus*) seeds were collected from local fruit markets in Pune, India. The seeds were thoroughly washed with distilled water to remove adhering pulp and impurities. After washing, the seeds were sun-dried for several hours and subsequently dried in a hot air oven at 45°C to eliminate residual moisture. Drying of seeds is essential to prevent microbial growth and to facilitate efficient grinding of the material. The dried seeds were then pulverized using a laboratory grinder to obtain a fine powder. The powdered material was stored in airtight containers until further use. Similar seed preparation techniques have been reported for the extraction of plant proteins from oil seeds and agro-waste materials.[20]

2.2. Method

2.2.1. Defatting Procedure

Prior to protein extraction, the seed powder was subjected to a defatting process in order to remove lipids that may interfere with protein isolation. The powdered sample was mixed with chloroform in a 1:5 (w/v) ratio and stirred continuously for approximately 3 hours. Chloroform acts as an effective organic solvent for dissolving non-polar lipid components present in seed matrices. After the extraction period, the mixture was

filtered through filter paper to separate the defatted residue from the solvent phase. The obtained solid residue was then air-dried at room temperature to allow complete evaporation of residual chloroform. Removal of lipids improves protein solubility and increases the efficiency of subsequent protein extraction processes.da[21]

2.2.2. Alkaline Solubilization

Protein extraction from the defatted seed powder was carried out using the alkaline solubilization technique, which is widely employed for isolating plant seed proteins. The defatted powder was dispersed in 0.1 M sodium hydroxide solution in a 1:20 (w/v) ratio. The mixture was maintained at 50°C under continuous stirring for 2 hours to promote the solubilization of proteins into the alkaline medium. Under alkaline conditions, protein molecules become negatively charged and dissolve readily in the aqueous phase. After extraction, the mixture was filtered to remove insoluble plant residues.[22]

2.2.3. Isoelectric Precipitation

The protein-containing filtrate was then subjected to isoelectric precipitation, a method commonly used to recover proteins from solution. The pH of the alkaline extract was gradually adjusted by adding 0.1 N hydrochloric acid dropwise while continuously monitoring the pH. Protein precipitation occurred when the pH reached approximately 4–5, which corresponds to the isoelectric point of many plant storage proteins. At this pH, the net electrical charge of the protein molecules becomes neutral, resulting in aggregation and precipitation. The precipitated protein was subsequently collected by centrifugation, washed with distilled water, and dried to obtain the isolated protein powder.[23]

2.2.4. Preparation of Bioplastic Film

Preparation of Bioplastic Film

Bioplastic films were prepared using the solvent casting method, which is commonly employed for the fabrication of protein-based biodegradable films. Initially, the extracted protein isolate was dissolved in distilled water to form a homogeneous solution. Gelatin was then added as a co-film forming agent to improve the mechanical strength and structural stability of the resulting film. Subsequently, glycerol was incorporated into the solution as a plasticizer, which helps increase flexibility and reduce brittleness of the film by enhancing molecular mobility within the polymer matrix.

The prepared film-forming solution was poured onto clean glass plates and spread evenly to obtain uniform thickness. The solution was allowed to dry at ambient conditions until complete evaporation of the solvent occurred, leading to the formation of thin bioplastic films. The dried films were carefully peeled from the casting surface and stored under controlled conditions prior to further characterization. The solvent casting technique has been widely used for producing biodegradable films from protein and polysaccharide matrices.[24]

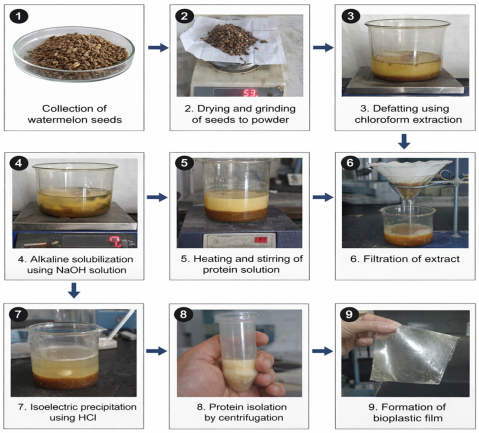

Figure 2: Experimental Workflow for Isolation of Watermelon Seed Protein and Preparation of Bioplastic Film

Stepwise experimental procedure illustrating watermelon seed preparation, defatting, alkaline protein extraction, isoelectric precipitation, centrifugation, and final fabrication of biodegradable bioplastic film.

3. Characterization

Qualitative Protein Tests

The presence of protein in the extracted watermelon seed sample was verified through several conventional qualitative biochemical assays. These preliminary analytical tests are commonly used in protein chemistry to detect functional groups and characteristic reactions associated with amino acids and peptide linkages. The extracted sample was subjected to Biuret test, Xanthoproteic test, Lead acetate test, Heat coagulation test, and Ammonium sulphate precipitation test to confirm its proteinaceous nature.

In the Biuret test, the addition of alkaline copper sulfate solution resulted in the development of a violet coloration, which indicates the presence of peptide bonds formed between amino acids. This reaction occurs due to the complex formation between copper ions and the

nitrogen atoms of peptide linkages in proteins. The Xanthoproteic test produced a yellow coloration after treatment with concentrated nitric acid, suggesting the presence of aromatic amino acids such as tyrosine, tryptophan, and phenylalanine. These amino acids undergo nitration reactions under acidic conditions, leading to the formation of colored compounds.

The Lead acetate test resulted in a dark precipitate, which indicates the presence of sulfur-containing amino acids such as cysteine or cystine. This reaction occurs when sulfur atoms in the amino acid side chains interact with lead ions to form insoluble lead sulfide. Additionally, heat coagulation of the sample led to the formation of a white precipitate due to thermal denaturation and aggregation of protein molecules, which is a characteristic property of many globular proteins.

Furthermore, ammonium sulphate precipitation resulted in visible protein precipitation due to the salting-out effect, a phenomenon in which high salt concentrations reduce protein solubility and promote aggregation. The successful precipitation of protein under these conditions indicates the presence of globulin-type proteins in the watermelon seed extract. Collectively, the outcomes of these qualitative biochemical tests confirm the successful isolation of protein from watermelon seeds and support its suitability for further characterization and bioplastic film development.[25–30]

Test	Procedure	Observation	Inference
Biuret Test	The sample was treated with sodium hydroxide followed by the addition of 1–2 drops of copper sulfate solution.	Violet coloration observed.	Confirms the presence of peptide bonds indicating protein.
Xanthoproteic Test	The sample was treated with concentrated nitric acid and gently heated.	Yellow precipitate observed.	Indicates the presence of aromatic amino acids such as tyrosine and tryptophan.
Lead Acetate Test	The sample was treated with sodium hydroxide followed by lead acetate solution.	Dark blue to black precipitate formed.	Suggests the presence of sulfur-containing amino acids.
Heat Coagulation Test	The sample solution was heated in a test tube.	Formation of white coagulated precipitate.	Indicates the presence of albumin-type proteins.
Ammonium Sulphate Test	Saturated ammonium sulphate solution was added to the sample.	White precipitate formed.	Confirms the presence of globulin proteins due to salting-out effect.

Table 1. Qualitative biochemical tests used for identification of watermelon seed protein extract.

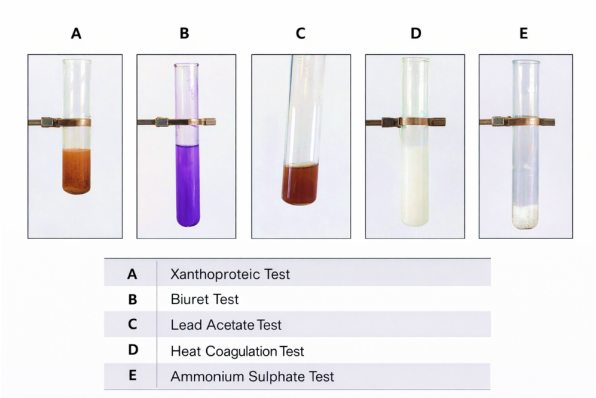

Figure 3:Confirmatory test:

2.4.2 UV Spectroscopy

Ultraviolet (UV) spectroscopic analysis was conducted to verify the presence of protein in the extracted watermelon seed sample. The analysis was performed using a UV-1900 series spectrophotometer. For sample preparation, 4 mg of the isolated protein was dissolved in 250 mL of solvent, and the absorption spectrum was recorded using both distilled water and phosphate buffer (pH 7.4) as solvents.

The protein solution exhibited characteristic maximum absorbance peaks (λmax) at 264.5 nm in distilled water and 272.5 nm in phosphate buffer. Proteins typically demonstrate strong absorption in the 260–280 nm region, primarily due to the presence of aromatic amino acids such as tryptophan, tyrosine, and phenylalanine, which contain conjugated ring structures capable of absorbing ultraviolet radiation.[23,26]

The detection of absorbance within this range indicates the presence of these aromatic residues and confirms the proteinaceous nature of the extracted material. Additionally, the slight variation in λmax observed between the two solvents may be attributed to changes in solvent polarity and minor conformational adjustments in the protein structure, which can influence the electronic environment of aromatic amino acid residues.[29]

Experimental parameters

- Instrument: UV-1900 Series spectrophotometer
- Sample concentration: 4 mg in 250 mL solvent
- Observed λmax: (Placeholder1)
    - 264.5 nm (distilled water)
    - 272.5 nm (phosphate buffer)

These results confirm the presence of aromatic amino acids and support the successful isolation of protein from watermelon seeds.

2.4.3 FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was employed to investigate the functional groups and structural characteristics of the extracted protein. FTIR analysis is widely used to identify molecular vibrations associated with protein functional groups and to confirm the presence of peptide linkages in proteinaceous materials.[30]

The FTIR spectrum displayed a broad absorption band near 3300 cm^{-1}, which corresponds to N–H stretching vibrations (Amide A band). This peak is characteristic of peptide linkages and hydrogen bonding interactions present within protein structures. A strong absorption peak observed at approximately 1650 cm^{-1} represents the Amide I band, mainly attributed to C=O stretching vibrations of peptide bonds, and is closely related to the secondary structure of proteins such as α-helices and β-sheets. Another significant peak detected around 1550 cm^{-1} corresponds to the Amide II band, arising from N–H bending coupled with C–N stretching vibrations.

In addition to these characteristic protein bands, absorption peaks within the 700–800 cm^{-1} region were also observed. These bands are associated with aromatic ring vibrations, indicating the presence of aromatic amino acids such as tyrosine and phenylalanine.

Overall, the FTIR spectral profile confirms the presence of peptide bonds and typical functional groups associated with proteins. The results further demonstrate that the extracted material maintains its protein structural integrity, which is essential for the formation of stable bioplastic films.[30]

Sample concentration: 4 mg in 250 mL

Observed λmax: 264.5 nm (water) and 272.5 nm (phosphate buffer)

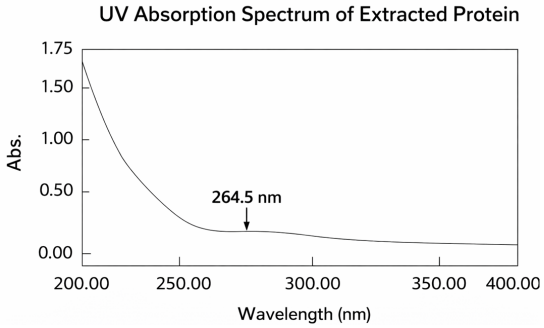

Figure 4: UV–visible absorption spectrum of watermelon seed protein extract recorded between **200–400 nm**. The absorbance maximum at **264.5 nm** is attributed to electronic transitions of aromatic amino acids, confirming

the presence of protein in the isolated extract.

2.4.3 FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was performed to confirm the functional groups and structural characteristics of the extracted protein. The FTIR spectrum showed a broad peak around ~3300 cm^{-1}, which corresponds to N–H stretching vibrations (Amide A band) and indicates the presence of peptide linkages. A prominent peak observed near ~1650 cm^{-1} represents the Amide I band, primarily associated with C=O stretching vibrations of peptide bonds, which is a key indicator of protein secondary structure. Another significant peak at ~1550 cm^{-1} corresponds to the Amide II band, arising from N–H bending and C–N stretching vibrations. These two bands (Amide I and Amide II) are characteristic signatures of proteins and confirm the presence of intact peptide chains.

Additionally, absorption bands in the region of 700–800 cm^{-1} are attributed to aromatic ring vibrations, suggesting the presence of aromatic amino acids such as tyrosine and phenylalanine. The overall FTIR spectral pattern confirms that the extracted material retains its proteinaceous structure and functional groups necessary for film formation.

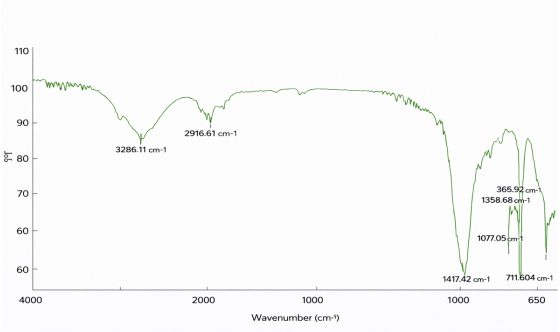

Figure 5: FTIR spectrum of the sample showing characteristic absorption peaks at **3286.11 cm^{-1}, 2916.61 cm^{-1}, 1417.42 cm^{-1}, 1077.05 cm^{-1}, 865.92 cm^{-1}, 711.60 cm^{-1}, and 1358.68 cm^{-1}**, corresponding to different functional groups present in the material.

2.5 Tensile Strength

Tensile Strength and Thickness Analysis

The mechanical performance of the prepared bioplastic film was evaluated using a TA.XT Plus Texture Analyzer, operating as a universal testing machine to determine the tensile properties of the film material. Film samples were cut into standardized strips, and their thickness and width were measured precisely prior to testing to ensure accurate calculation of stress values. Each strip was mounted securely between the instrument grips to maintain proper alignment and avoid uneven distribution of applied force during the experiment.

During the test, a tensile force was applied at a constant rate until rupture occurred. The maximum load at break (Fmax) was recorded from the resulting force–extension curve generated by the instrument. Tensile strength was calculated by dividing the breaking load by the cross-sectional area of the film (thickness × width), according to the following equation:

Tensile Strength = Load at break / (Thickness × Width)

The recorded load at break was 500 N, while the thickness and width of the film were 0.01 m and 0.03 m, respectively. Based on these measurements, the tensile strength of the fabricated bioplastic film was calculated to be 1.66 MPa. Tensile strength represents the maximum stress a material can withstand before failure and is an essential parameter for determining the mechanical suitability of biodegradable polymer films for packaging and related applications.[30]

Although the obtained tensile strength is lower than that of conventional petroleum-based plastics, the value indicates moderate mechanical stability, making the material suitable for biodegradable packaging and other low-load applications. The presence of gelatin in the polymer matrix likely contributed to improved mechanical strength due to the formation of intermolecular protein networks, while glycerol functioned as a plasticizer, enhancing film flexibility and reducing brittleness by increasing polymer chain mobility.[31]

2.6 Biodegradation Study

Soil Burial Test

The biodegradability of the developed bioplastic film was investigated using the soil burial test, a commonly used method for evaluating the environmental degradation behavior of biodegradable polymers. This technique relies on the natural microbial activity present in soil, where microorganisms such as bacteria and fungi secrete enzymes capable of degrading organic polymer structures. Through enzymatic hydrolysis and microbial metabolism, complex polymer chains are gradually broken down into simpler compounds including carbon dioxide, water, and microbial biomass.[32]

Protein-based biopolymers are particularly susceptible to microbial degradation because their peptide bonds can be hydrolyzed by proteolytic enzymes produced by soil microorganisms. As a result, soil burial testing is considered an effective and realistic approach for

assessing the biodegradability of protein-derived polymer materials under natural environmental conditions.[33]

In this study, the initial weight of the bioplastic sample was recorded as 0.10 g. The sample was buried at a depth of approximately 4–5 cm in moist garden soil, ensuring adequate exposure to microbial activity. The soil was maintained under damp conditions without excessive water accumulation, which supports microbial growth while preventing anaerobic conditions. The experimental setup was kept in a well-ventilated area at room temperature to simulate natural environmental conditions that facilitate polymer degradation.

After the predetermined burial period, the sample was carefully removed from the soil. It was gently washed with distilled water to remove soil particles, dried thoroughly, and weighed again to determine the remaining mass.

The percentage weight loss was calculated using the following equation:

Weight loss (%) = $(W_0 - Wt) / W_0 \times 100$

where W_0 represents the initial weight of the sample and Wt represents the final weight after burial.

The final recorded weight of the film was 0 g, indicating complete degradation of the material. Consequently, the calculated weight loss was 100%, demonstrating that the fabricated bioplastic film is fully biodegradable under natural soil conditions. The rapid degradation observed in this study can be attributed to the protein-based polymer matrix, which is highly susceptible to microbial enzymatic breakdown in soil environments.[34]

3. Results and Discussion

Protein Extraction from Watermelon Seeds

Protein extraction from watermelon seeds was successfully achieved through alkaline solubilization followed by isoelectric precipitation. During the extraction process, the protein components present in the seed powder were solubilized under alkaline conditions and subsequently precipitated when the pH of the solution was adjusted to approximately 4–5, which corresponds to the isoelectric point of many plant storage proteins. At this pH, the net electrical charge of the protein molecules becomes neutral, leading to aggregation and precipitation from the solution. The formation of a visible protein precipitate indicated effective separation and recovery of protein from the seed material. Similar extraction techniques have been widely applied for isolating proteins from plant seeds and oilseed residues due to their efficiency and reliability.[35]

Qualitative Protein Identification

The presence of protein in the isolated sample was confirmed through a series of qualitative biochemical tests. In the Biuret test, the development of a violet coloration indicated the presence of peptide bonds formed between amino acids, which is a characteristic reaction of proteins. The Xanthoproteic test produced a yellow precipitate upon treatment with concentrated nitric acid, demonstrating the presence of aromatic amino acids such as tyrosine and tryptophan.

The Lead acetate test produced a dark-colored precipitate, suggesting the presence of sulfur-containing amino acids such as cysteine or cystine. Additionally, heat coagulation of the sample resulted in the formation of a white precipitate due to thermal denaturation of protein molecules, while the ammonium sulphate precipitation test produced a visible precipitate due to the salting-out effect, confirming the presence of globulin-type proteins.

Collectively, the outcomes of these qualitative tests verified that the extracted material contained protein fractions suitable for further characterization and bioplastic film fabrication. Similar biochemical tests are commonly employed for preliminary confirmation of proteins in plant-derived extracts.[36]

UV Spectroscopic Analysis

Ultraviolet spectroscopic analysis of the extracted protein solution revealed maximum absorbance peaks at 264.5 nm in distilled water and 272.5 nm in phosphate buffer. Proteins generally exhibit strong absorption within the 260–280 nm wavelength range due to the presence of aromatic amino acids, particularly tryptophan, tyrosine, and phenylalanine, which contain conjugated ring systems capable of absorbing ultraviolet radiation.

The observed absorption peaks therefore confirm the presence of aromatic amino acid residues within the isolated protein sample. The slight variation in the observed wavelength between the two solvent systems may be attributed to differences in solvent polarity and minor conformational changes in the protein structure, which can influence the electronic environment surrounding aromatic residues. Similar UV absorption characteristics have been reported for several plant-derived protein isolates used in biodegradable film production.[37]

FTIR Spectroscopic Analysis

Fourier Transform Infrared (FTIR) spectroscopy was used to further confirm the presence of functional groups associated with proteins. The FTIR spectrum displayed a broad absorption band around 3300 cm^{-1}, corresponding to N–H stretching vibrations associated with peptide linkages (Amide A band). A prominent peak observed near 1650 cm^{-1} corresponds to the Amide I band, which is primarily related to C=O stretching vibrations of peptide bonds and provides important information regarding the secondary structure of proteins.

Another characteristic peak was observed around 1550 cm^{-1}, representing the Amide II band, which arises from N–H bending and C–N stretching vibrations within the peptide backbone. These two bands are widely recognized as diagnostic peaks for protein structures.

Additionally, peaks observed in the 700–800 cm^{-1} region were attributed to aromatic ring vibrations, indicating the presence of aromatic amino acids within the protein matrix. The overall FTIR spectral pattern confirms that the extracted material retained its proteinaceous structure and functional groups, which are essential for forming intermolecular networks during film formation. Similar FTIR spectral characteristics have been reported for plant protein-based biodegradable films.[38]

Mechanical Properties of the Bioplastic Film

The fabricated bioplastic film exhibited measurable mechanical strength when tested using a Universal Testing Machine. The tensile strength of the film was calculated to be 1.66 MPa based on the recorded load at break and the measured film dimensions. Tensile strength is an important parameter for evaluating the structural stability and durability of biodegradable films intended for packaging applications.

Although the tensile strength obtained in this study is lower than that of conventional petroleum-based plastics, the observed value indicates moderate mechanical stability, which is comparable to other protein-based biodegradable films reported in previous studies. The addition of gelatin likely contributed to the formation of a stronger polymer network by enhancing intermolecular interactions, while glycerol acted as a plasticizer, improving flexibility and reducing brittleness by increasing polymer chain mobility. The use of plasticizers such as glycerol has been widely reported to improve the mechanical performance of protein-based biodegradable films.[39]

Biodegradation Study

The biodegradability of the prepared bioplastic film was assessed using the soil burial method. The results demonstrated complete degradation of the film during the experimental period. The initial weight of the sample (0.10 g) decreased to 0 g, corresponding to 100% weight loss. This observation confirms the biodegradable nature of the developed material under natural soil conditions.

The rapid degradation of the film can be attributed to the protein-based polymer matrix, which is highly susceptible to enzymatic degradation by microorganisms present in soil. Microbial enzymes break down peptide bonds within the protein structure, leading to fragmentation of the polymer network and eventual mineralization into simpler compounds such as carbon dioxide, water, and microbial biomass. Such biodegradation behavior is characteristic of natural protein-based polymers used in environmentally friendly plastic substitutes.[40]

Overall Interpretation

The results obtained from qualitative tests, spectroscopic characterization, mechanical analysis, and biodegradation studies collectively demonstrate that watermelon seed protein can serve as an effective raw material for biodegradable bioplastic film production. The fabricated film exhibited adequate mechanical strength and complete biodegradability, indicating its potential suitability for sustainable packaging materials and other low-load biodegradable applications. Furthermore, the utilization of watermelon seed waste provides an environmentally beneficial approach for valorizing agricultural by-products while reducing dependence on synthetic plastics.

4. Conclusion

In this study, protein was successfully isolated from watermelon seeds using the alkaline solubilization and isoelectric precipitation method, demonstrating that watermelon seed waste can serve as a valuable source of plant-derived protein. The presence of protein in the extracted material was confirmed through qualitative biochemical tests and spectroscopic analyses, including UV and FTIR studies, which indicated characteristic features of protein structures.

Biodegradable bioplastic films were successfully prepared using the solvent casting technique, incorporating gelatin as a co-film forming agent and glycerol as a plasticizer to enhance film flexibility. The fabricated films exhibited moderate mechanical strength, with a tensile strength of 1.66 MPa, indicating their potential suitability for biodegradable packaging and other low-load applications.

Furthermore, the soil burial biodegradation test demonstrated complete degradation of the film, confirming its environmentally friendly nature. Overall, the findings suggest that watermelon seed protein represents a promising renewable resource for the development of biodegradable plastics, while also providing an effective approach for the utilization of agricultural waste and promoting sustainable material development.

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Authorship Contribution

Pallavi Shrirang Dhekale designed the study, performed the experimental work including protein extraction, bioplastic film preparation, and characterization studies, and prepared the initial draft of the manuscript. Sarah Ranganekar and Sonali Pawar assisted in laboratory experiments, data collection, and interpretation of the analytical results. Poonam Taru and Reshma Todkari contributed to data analysis, preparation of figures and tables, and revision of the manuscript. Dr. Ashwini Ohol supervised the research work, guided the experimental design, critically reviewed the manuscript, and approved the final version for submission. All authors read and approved the final manuscript.

Conflict of Interest

The author declares no conflict of interest.

Funding Source

The authors declare that **no external funding was received** for conducting this research work. The study was carried out using the available laboratory facilities of the respective institution

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