

VALORIZATION OF POULTRY FEATHER WASTE INTO SUSTAINABLE BIOPLASTICS: COMPARATIVE ANALYSIS OF KERATIN-BASED BIOPLASTICS WITH CORN AND POTATO STARCH

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ABSTRACT

The feather waste released by poultry industry is serious environmental concern globally. Conventional approaches to dispose poultry waste cause extreme damage to environment. There by, bioconversion of feather waste in to useful products is sustainable strategy that allows the proper disposal of waste along with fabrication of eco-friendly biomaterials for commercial applications. In current study, keratin was extracted from chicken feather waste through alkali treatment. The estimated keratin yield was recorded to be 42.21% using 10 g feathers (w/w) with 0.11mg/ml total protein content. After that, extracted keratin was utilized for the development of bio plastic films in combination with corn and potato starches individually. The comparative study on the properties of developed keratin based bio-plastic films blended with corn and potato starch was done. The moisture content of keratin-corn starch bioplastic film was 5.90% while keratin-potato starch bioplastic film possessed 8.99% moisture content. The transparency of bioplastic films was reduced as the concentration of keratin was increased. The keratin-corn starch bioplastic has low solubility (23.89%) as compared to keratin-potato starch bioplastic (29.88%). On the other hand, the different blends of bioplastic films approximately reduced 40% of original dry weight after 12 days of soil burial. SEM analysis indicated an improvement in the structure of bioplastic film on the basis of smoothness, rigidity and porosity with increased concentration of keratin. According to the results of this work, keratin-corn starch bioplastic film has more desirable attributes for commercial applications as compared to keratin-potato starch based bioplastic.

Keywords: Bioplastic, Corn starch, feathers, keratin, potato starch, poultry

INTRODUCTION

Presently, the most concerned challenges of world are unusual climatic changes and depletion of natural materials. The adverse environmental changes are associated with the burning of fossil fuels that ultimately leads to the emission of harmful greenhouse gases (GHG) including carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), and chlorofluorocarbons (CFCs) (Arif *et al.*, 2023). Overuse of synthetic plastics promotes severe environmental contamination (Abbasi, 2020). Although, conventional plastics are cheap; they are having low molecular mass with improved thermal features but they are resistant to biodegradation (Muneer *et al.*, 2021). The non-biodegradable nature of synthetic plastic is a serious threat to ecosystem and human health (Rhodes, 2018). Approximately, 34 million tons plastics are manufactured annually worldwide and only 7% is reused while rest of 93% plastic waste is disposed off in to sea and landfills. In order to overcome these difficulties, the demand of biodegradable plastic has interestingly increased. A lot of efforts have been made to replace fossil fuel based synthetic plastic with bioplastic (Hatti-Kaul *et al.*, 2020). Bioplastic is the form of polymer that is synthesized from natural and renewable resources for instance agro-industrial and food waste that are rich in polysaccharides, protein, lipids etc. Bacteria and algae are also capable to produce bioplastics. Bioplastics can easily be decomposed upon microbial action (Demirbas, 2007). Despite of being eco-friendly and safe, the process of bioplastic production encounters some obstacles especially costly production or weak barrier and physical attributes (Rahman, 2019). To cope up with these limitations, cheap raw materials like animal waste (poultry feathers, exoskeleton of arthropods etc.) can be consumed to synthesize bioplastic. Additionally, blends of two or more biopolymers can be utilized to improve the mechanical and barrier characteristics of bioplastic (Shubha and Srivastava, 2019).

Globally, the poultry industry plays a vital role in supplying protein rich diet like meat and eggs to human along with the generation of large amount of waste. This waste biomass mainly includes feathers, bones, blood, fats and non-edible parts of meat (Sharma *et al.*, 2018). In poultry industry, a large fraction of waste constitutes with

feathers. Every year, around 5 million tons of feathers waste is released worldwide (Tesfaye *et al.*, 2017; Poole *et al.*, 2008), from which a small portion is used for synthesis of low grade animal feed meanwhile a huge quantity of feathers waste is managed as landfills (Reddy and Yang 2007; Bertsch and Coello 2005). The dumping of feathers in land eventually leads to the accumulation of toxic heavy metals in higher proportions and emergence of harmful pathogens that have deleterious impacts on ecosystem and groundwater. The traditional approaches of feathers waste disposal are expensive and unhealthy that gives rise to global environmental issue. So there is a need to seek an eco-friendly technology that can valorize feather waste in to value added products. One promising approach is to utilize feathers for the production of bioplastics. Feathers contain a polymeric protein that can be extracted and used in the development of biodegradable plastics. Thus in this manner, sustainable consumption of feathers biomass for the production of bioplastics results in environmental protection and combats the overuse of synthetic plastic. Such bioplastics can be utilized for material packaging purpose.

Chicken feathers are mainly comprised of 90% keratin protein- an insoluble, tough and fibrous protein that provides structural strength (Reddy and Yang 2007; Onifade *et al.*, 1998). Keratin contains cysteine, threonine, arginine and hydrophobic amino acids with high nutritional value (Tiwary and Gupta, 2010). Keratins are categorized in to α and β keratins (Skieresz-Szewczyk *et al.*, 2017). α -keratin are present in all vertebrates, while β -keratin are exclusively present in reptiles and birds (Fraser and Parry, 2017). In birds, β -keratin exists in feathers, beaks and claws. Keratin protein is suitable candidate for the fabrication of bioplastics because of its biodegradability, biocompatibility and stability (Gupta *et al.*, 2012). For the enhancement strength and physical properties of biopolymer, feather-extracted keratin can be used in conjugation with other synthetic or natural polymers in blended forms (Sharma *et al.*, 2018). The main focus of current study is the extraction of keratin from chicken feathers using alkali treatment; fabrication of bioplastic film using extracted keratin along with corn and potato starch (blends) and characterization of developed bioplastic films. This study also compares the properties of keratin-corn starch and keratin-potato starch bioplastic films.

MATERIAL AND METHODS

Materials

The chicken feathers were locally procured from poultry shop (Gole Market, Karachi, Pakistan). The chemicals used in this study included Sodium hydroxide (Dae Jung), hydrochloric acid (Sigma Aldrich), glycerol (BDH Chemicals Ltd.), corn and potato starch (Sigma Aldrich). These all chemicals were of analytical grade.

Pretreatment of Raw Feathers

The collected chicken feathers were cleansed thoroughly with detergent, followed by vigorous rinsing with water to remove any traces of the blood and grease. Then the feathers were treated with sodium hypochlorite solution (0.5%) for disinfection. For neutralization, feathers were exposed to sodium thiosulphate. It was further followed by washing with distilled water. The feathers waste was allowed to dry at 70 °C in dry oven till constant weight. The dried feather biomass was turned in to small pieces by cutting them mechanically (Oluba *et al.*, 2021).

Extraction of Keratin

Ten g of pre-treated chicken feathers were soaked in 0.5N NaOH (100 ml) solution. Then the flask was kept for incubation at 70°C for 6 hours under constant stirring. After incubation, the resulting mixture was filtered using filter paper. The filtrate was further centrifuged at 8000 rpm for 15 minutes at 1°C in order to remove waste biomass. Then alkaline pH of supernatant was adjusted using 1N hydrochloric acid to achieve neutralization. After that, the sediments of keratin protein were collected by centrifugation at 8000 rpm for 10 minutes at 1°C. Finally, the precipitates were washed, dried and stored for further experimentation.

Percent extraction yield of Keratin

The percent yield of extracted keratin was measured by using initial weight of pre-treated feathers and total dry weight of obtained protein till constant weight as shown in equation (1):

$$\% \text{ Extraction Yield} = \frac{M'}{M} \times 100 \quad (1)$$

Where M' stands for the weight of extracted keratin sample while M represents the initial weight of processed feathers. The difference in weight was estimated. Bradford Method (1976) was employed in order to assess the

unknown concentration of extracted keratin using Bovine Serum Albumin (BSA) as standard. All the experiments were carried out in three replicates.

Synthesis of keratin-starch bioplastic film

For the preparation of keratin-starch based bioplastic films, two types of starch were used i.e. corn starch and potato starch. The synthesis of bioplastic films was carried out using a protocol described by Tesfaye *et al.*, (2018) with little alterations. A 5% keratin solution was prepared in 0.1N sodium hydroxide then the mixture was kept on heating at 70 °C for 15 minutes with continuous shaking. A starch solution with 5% concentration was made and heated at 70 °C for gelatinization under constant shaking. The different combinations of starch and keratin solutions were made as follows; 40:0 (control), 40:2, 40:6, and 40:10 (v/v starch:keratin). 2 ml glycerol was added as plasticizer to each combination of starch and keratin. The mixture was allowed to heat at 70°C for 15 minutes with constant agitation. The viscous solution was casted in aluminum foil boat which was kept in dry oven at 60°C for 24 hours. The resulting bio-composite film was carefully peeled off from the aluminum foil boat and stored at cool place.

Characterization of Keratin-starch Bioplastic Film

Moisture content

The moisture content of the prepared bioplastic films was estimated by keeping film in an oven at 100°C until the weight became constant. The original weight prior to drying was considered as W1. The moisture content was calculated by using equation (2):

$$\text{Moisture content \%} = \frac{W_1 - W_2}{W_1} \times 100 \quad (2)$$

Where W1 is the original weight of biofilm prior to drying and W2 is the final weight after drying.

Solubility analysis

The solubility of biofilm was analyzed according to the method of Pavin *et al.* (2010). A circular bioplastic film of about 2cm diameter was put into petri plates, dried in an oven at 105°C for 24 hours and weighed as W1. After that, the bioplastic film was completely immersed in 50ml of distilled water in beaker for 24 hours at room temperature. After that, the insolubilized portion of the film was removed from water, dried at 105°C and weighed as W2. The solubility of biofilm was calculated by employing equation (3):

$$\text{Solubility \%} = \frac{W_1 - W_2}{W_1} \times 100 \quad (3)$$

Biodegradability test

The procedure suggested by Pavin *et al.* (2010) was followed for biodegradability testing. For a period of 16 days, a weighed bioplastic film was vertically buried in the soil at 3-5 cm depth to facilitate the aerobic degradation conditions at 25°C-35°C. After every 4 days, the bioplastic film was taken out from soil, washed, dried till constant weight and weighed. The percent weight loss of film was calculated by using equation (4):

$$\% \text{ Weight} = \frac{W_1 - W_2}{W_1} \times 100 \quad (4)$$

Where W1 represents the initial weight of biofilm and W2 indicates the weight of bioplastic film after biodegradation

Evaluation of surface density

The surface density of the prepared bioplastic film was calculated by using equation (5);

$$\text{Surface Density} = \frac{\text{Average Weight}}{\text{Area}} \quad (5)$$

Morphological Studies of Bioplastic Film

The morphological studies were carried out using Scanning Electron Microscopy (JSM 6380A Jeol, Japan) to evaluate the structural properties of the extracted keratin and fabricated bioplastic films.

Statistical analysis

All the experiments were carried out in three replicates and data was represented as mean ± SD.

RESULTS AND DISCUSSION

Keratin extraction from chicken feathers

Keratin protein possesses insolubility in water, weak acids or weak alkalis and non polar solvents. Thus various processes like hydrolytic activation, oxidation or reduction of cysteine amino acid in peptide chain can facilitate the solubilization of keratin (Endo *et al.*, 2008). The hydrolytic cleavage of peptide bonds associated with cysteine residues weakens the strength of peptide chain of keratin however resulting in the formation of new hydrogen bonds (Schrooyen *et al.*, 2000). In this study, a cheap and convenient protocol has established in order to catalyze disulphide linkages and hydrogen bonds with in compact structure of keratin using strong alkaline solution (0.5N NaOH) as extractant with slight alterations as mentioned by Oluba *et al.* (2021). The concentration of alkali has significant impact on the dry mass and final percent yield of obtained keratin from feathers. It was observed that approximately 42.21% crude protein was extracted using 0.5 N NaOH from 10g of feathers at 70°C reaction temperature with 0.11mg/ml total protein content. Similarly, Mengistu *et al.* (2024) described that 0.65N NaOH gave 89.71% yield of extracted keratin at 70°C from poultry feathers. Nagai and Nishikawa (1970) demonstrated 0.1N sodium hydroxide optimally extracted keratin at 90°C. Several reports highlight the utilization of alkalis other than NaOH for the extraction of keratin from feathers. Gindaba *et al.* (2019) reported the 0.5M sodium sulphide extracted 63.25% pure keratin from 25g of chicken feathers at 30°C.

Development of keratin based bio-plastic film in combination with corn and potato starch

The developed bioplastic films prepared from different volumes of keratin blended with corn and potato starch (40:0, 40:2, 40:6 and 40:10) along with glycerol as plasticizer is shown in Fig. 1. This study reported the successful development of homogenous, thin and flexible of bioplastic film from varied ratios of starches and keratin. Following properties of prepared bioplastic films were analyzed.

Determination of moisture content and transparency of bioplastic films

It was noticed that as the concentration of keratin was increased the moisture content of bioplastic film was significantly declined. 5.9% moisture content was present in a bioplastic film comprised of keratin and corn starch with the ratio of 40:10. While keratin-potato starch (40:10) possessed 8.99% moisture content. On the other hand, the control biofilms that were fabricated only with starches (40:0) have higher moisture content (Table 1). On contrary the keratin bioplastic film belnded with corn starch has less moisture content (improved feature) than bioplastic film formed from keratin and potato starch. The moisture absorption potential of bioplastic films is very crucial with respect to their processing, shelf life, transportation and packaging. During the course of processing, the moisture content of product might influence an inter and intra-molecular interactions with in and/or among molecules exist in product, consequently impacting its final weight and durability. Bioplastic films with higher moisture content are heavier and extremely susceptible to rapid disintegration (Kausar *et al.*, 2018; Munawar and Subiyanto, 2014). In current research, the addition of chicken feather extracted- keratin in developed bioplastic films significantly declined the moisture content. These results highlight the improved shelf life of product due to reduced microbial action.

The transparency of prepared bioplastics was observed on visual basis. The opaqueness was increased proportionally with increased concentration of keratin. The color of bioplastic film was turned from white to yellow as the concentration of keratin was gradually raised that led to increase in solid particles in filmogenic solution thereby the degree of transparency of prepared films was decreased. Bioplastic films with increased opaqueness exhibit great UV resistant attribute, a desirable property for materials of food packaging. Similar features were reported when keratin and avocado pear kernel were used to fabricate bioplastic film (Tesfaye *et al.*, 2018)

Table 1. Moisture content (%) of bioplastic films

Bioplastic samples	Starch/ Keratin (v/v)			
	40:0	40:2	40:6	40:10
Keratin-Corn starch film	50.44 ± 0.26	27.47 ± 0.28	10.83 ± 0.09	5.90 ± 0.07
Keratin-Potato starch film	53.11 ± 0.30	35.38 ± 0.206	22.97 ± 0.09	8.99 ± 0.003

Data is represented as mean ± standard error (SE) for three replicates. Note: 40:0, 40:2, 40:6 and 40:10 = starch/keratin (v/v)

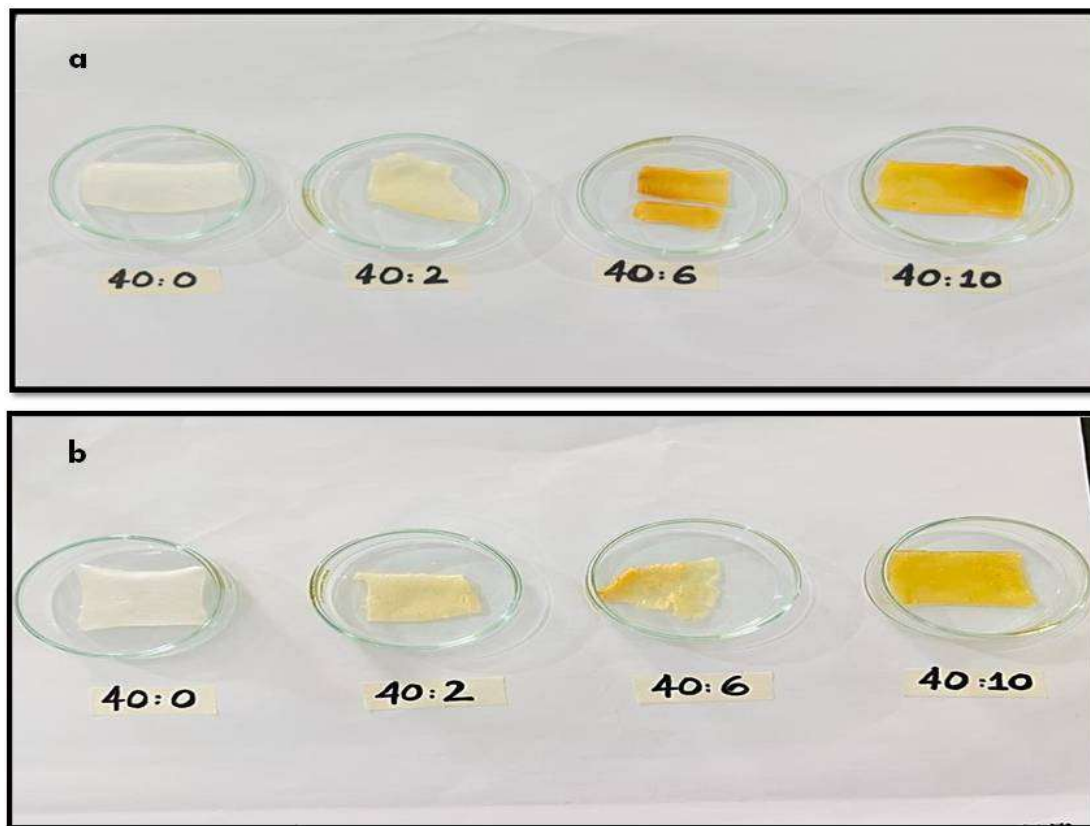


Fig.1. Bioplastic films with different ratios of keratin and starch. **a** Keratin-Corn starch bioplastic, **b** Keratin-Potato starch bioplastic. Note: 40:0, 40:2, 40:6 and 40:10 = starch/keratin (v/v)

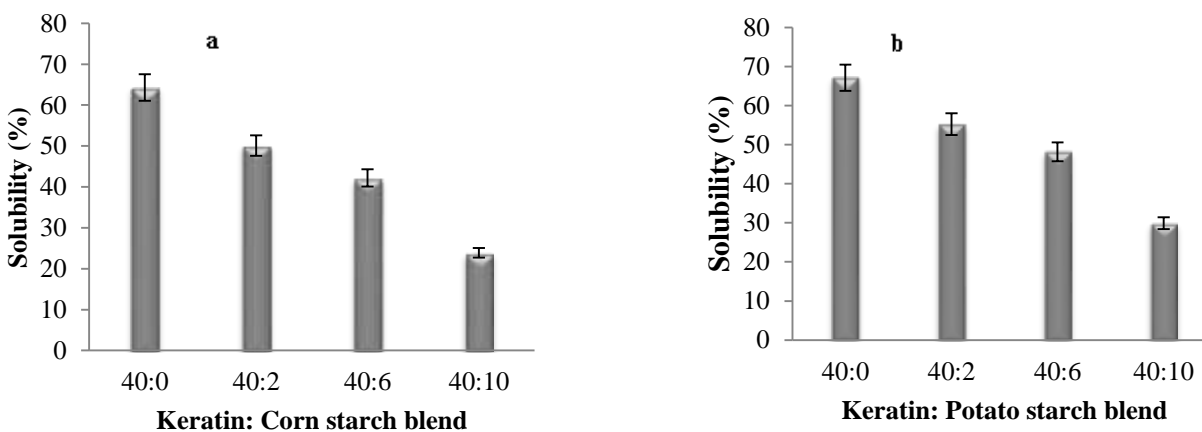


Fig.2. Assessment of solubility (%) of bioplastic films. **a** Solubility profile of keratin and corn starch based bioplastic, **b** Solubility profile of keratin- potato starch based bioplastic

Analysis of solubility of bioplastic films

Solubility assessment of bioplastic films is an important step as it determines chemical features and suitability for specified commercial applications. Solubility of bioplastic elucidates the degree of dissolution of bioplastic in different solvents and level of susceptibility to disintegration. Small pieces of prepared bioplastic films were

immersed in water for 2 hours and it was noticed that as the concentration of keratin was increased there was a significant reduction in solubility of biofilms (Fig. 2). While control bioplastic films (40:0) developed only from starch has greater solubility. Same outcomes were reported by Oluba *et al.* (2021). The 40:10 ratio of corn starch and keratin bioplastic film possessed 23.89% solubility meanwhile potato starch and keratin biocomposite revealed 29.88% solubility. In comparison, keratin bioplastic film blended with corn starch has low solubility than keratin bioplastic film blended with potato starch.

For food packaging applications, the partial solubility of bioplastic films in water is required in order to maintain the robustness of packed product; thereby the evaluation of solubility of specific products prior to utilization is useful. The current study elaborates the low solubility of bioplastic films due to inclusion of keratin which might be due to the existence of non polar amino acid residues with in keratin molecule (Barone and Gregoire, 2006). Lower solubilities of bioplastic films contribute their stability and make them attractive candidates for industrial applications.

Assessment of biodegradability

The objective of screening of biodegradable potential of bioplastic is to evaluate how rapid and conveniently bioplastic is degraded in their natural environment. The biodegradability screening of bioplastics gives perception about the deterioration mechanisms, sustainability and suitability of product for certain applications. All bioplastic films samples were analyzed for their biodegradability. The results of biodegradability showed that with the passage of time, there was a decline in the total mass of bioplastic films (Fig. 3). After 12 days of burial in soil, almost 40% bioplastic films were decomposed. Keratin-corn starch blended bioplastic films led to more reduction in total original weight than keratin-potato starch blended biofilms. This highlights that the developed bioplastic is decomposable and eco-friendly. It is suggested that soil microflora including bacteria and fungi release keratinases enzyme extracellularly that cleave the complex structure of keratin present in bioplastic (Watie, 2017).

Estimation of surface density

The surface density of bioplastic film is crucial physical property as it elaborates extent to which atoms or particles of bioplastic film are tightly packed. Moreover, the relationship between bioplastic film and surface density reveals the physical and barrier features along with sustainability of environment and cost. However, it's very important to study this relationship in order to optimize the fabrication process and application of biodegradable plastic in different commercial sectors. It was noticed that as the keratin proportion was increased with constant volume of starch, there was also increase in surface density of bioplastic films (Fig. 4). It is suggested that keratin protein has higher density than starch that is why increasing the concentration of keratin leads to higher surface density of fabricated bioplastic films. The corn starch-keratin bioplastic has more density (1.47 g/cm^3) than potato starch-keratin bioplastic film (1.36 g/cm^3) at 40:10 starch and keratin ratio. So the synthesized bioplastic films in this study have high density as compared to conventional PE plastic having density ranges from 0.917 to 0.930 g/cm^3 . So it is proposed that keratin- starch bioplastic films have more densely packed particles. Similar results were also reported by Abdullah *et al.* (2021).

Scanning Electron Microscopy

The surface morphology of extracted keratin and bioplastic films was investigated using SEM analysis. According to SEM examination, it is revealed that extracted keratin possess highly porous morphology with multitude properties such as specimen seems globular, spongy with a very thick layer. The extracted keratin consists of microporous fragments which are randomly arranged in aggregated microstructures. The surface of keratin particles appeared to be smooth with aspheric configuration. Fig. 5 shows tightly packed small microspheres with diameter $50 \mu\text{m}$, $10 \mu\text{m}$ and $5 \mu\text{m}$.

Various previous researches also reported the similar morphologies of dried keratin extracted from wool and feather (Zhang *et al.*, 2013; Yin *et al.*, 2013; Rad *et al.*, 2012). Scanning electron microscopy was also done to evaluate the morphology of starch-keratin prepared bioplastic films. The SEM micrographs of bioplastic films displayed multiple variations according to their respective formulation. From SEM images it is assumed that the smoothness of film surface was increased as the concentration of keratin was increased (Fig. 6 and 7). Increased concentration of keratin reduced the roughness. Good and compatible surface structures were observed without pores and cavities. This indicates stable interactions among the components of bioplastic films i.e. starch, keratin and glycerol. Glycerol (2mL) was used as plasticizer which forms homogeneous mixture with keratin and starches used in this study i.e. potato and corn starch. The whole combination allowed the formation of firmed film with biodegradability potential. These results highlight that smooth appearance of keratin bioplastic films blended with corn and potato starch is highly dependent upon the ingredients used in bioplastic films formulation. Bioplastic

characteristics like surface topology and texture is greatly affected by the ratios of starch and keratin solutions. The degree of smoothness was enhanced as the starches showed roughness. Similar results were also reported by Oluba *et al* (2021).

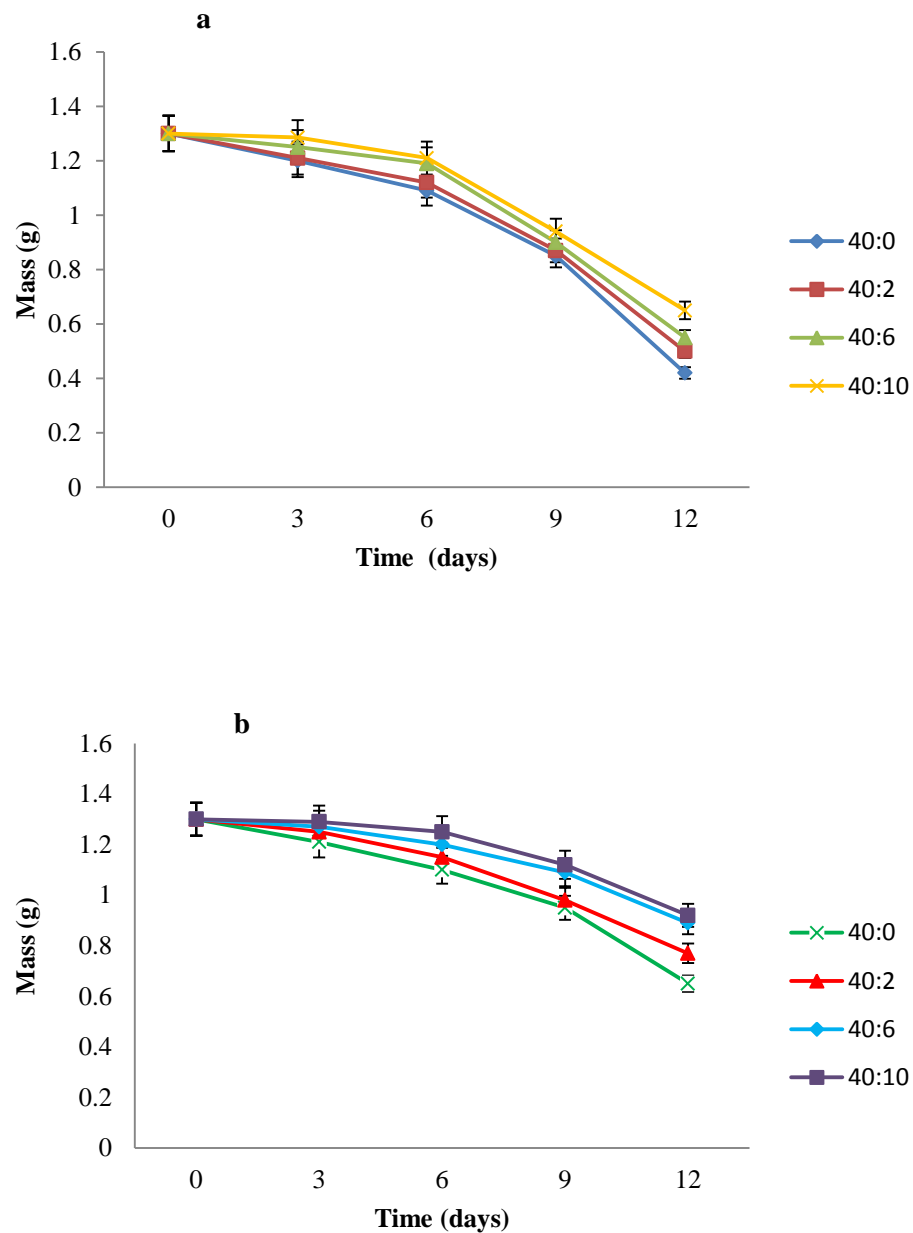


Fig.3. Evaluation of biodegradability of bioplastic films. **a** Biodegradability of keratin-corn starch based bioplastic, **b** Biodegradability of keratin and potato starch based bioplastic

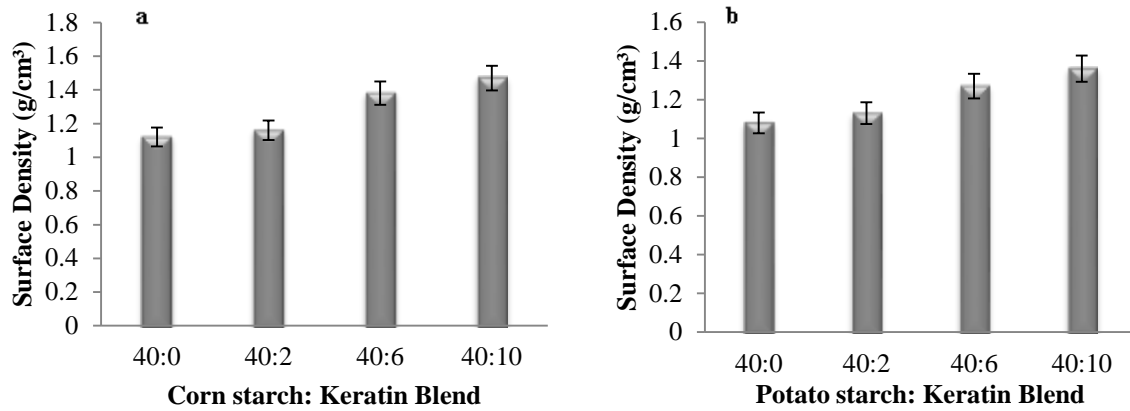


Fig.4. Evaluation of surface density of biplastic films. **a** keratin and corn starch based biplastic, **b** keratin- potato starch based biplastic.

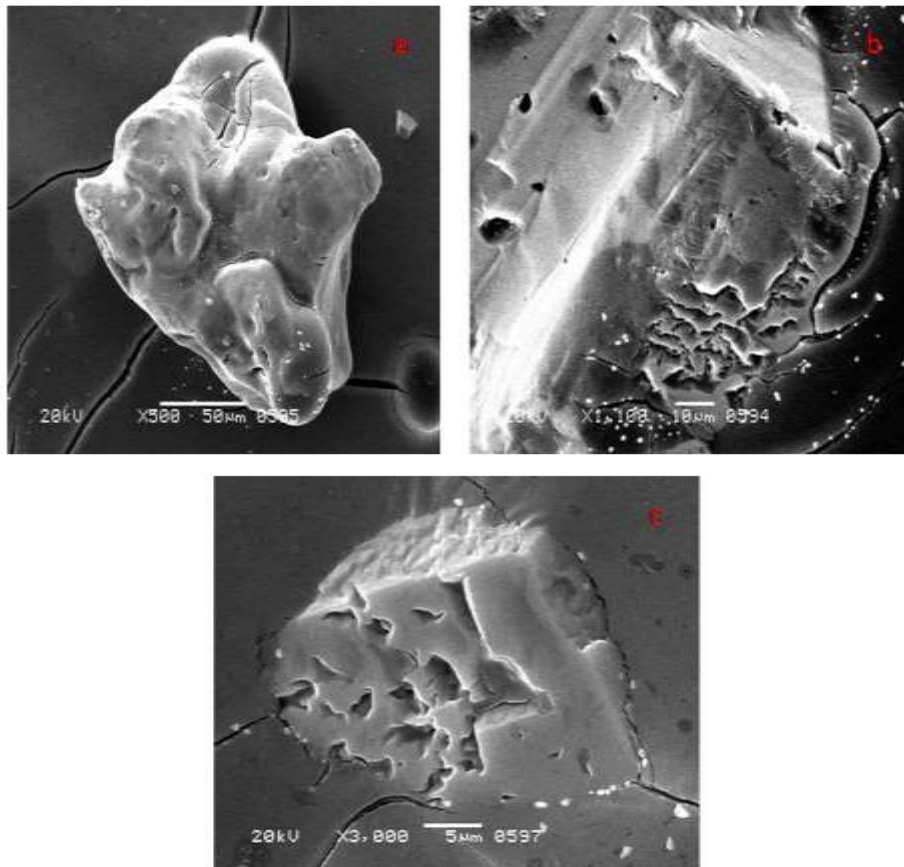


Fig.5. SEM micrographs of extracted keratin. **a** SEM image of keratin at 500x with diameter 50 µm, **b** SEM image of keratin at 1,100x with diameter 10 µm, **c** SEM image of keratin at 3000x with diameter 5 µm

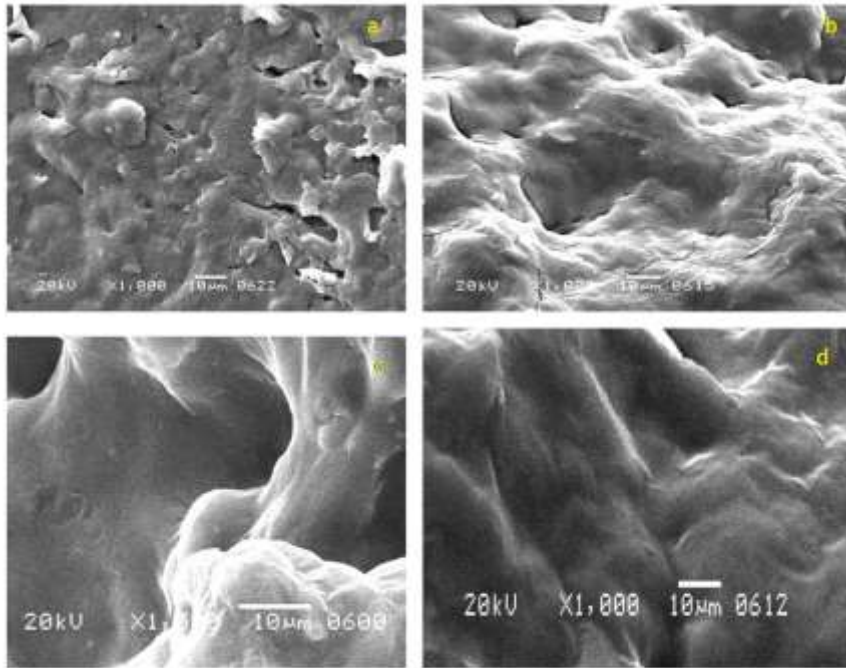


Fig. 6. SEM micrographs of keratin-corn starch bioplastic film. **a** SEM image corn starch bioplastic film (40:0) 1,000x with diameter 10 µm, **b** SEM image of keratin-corn starch bioplastic film (40:2) at 1,100x with diameter 10 µm, **c** SEM image of keratin-corn starch bioplastic film (40:6) at 1,300x with diameter 10 µm, **d** SEM image of keratin-corn starch bioplastic film (40:10) at 1,000x with diameter 10 µm. Note: 40:0, 40:2, 40:6, and 40:10 = starch/keratin (v/v).

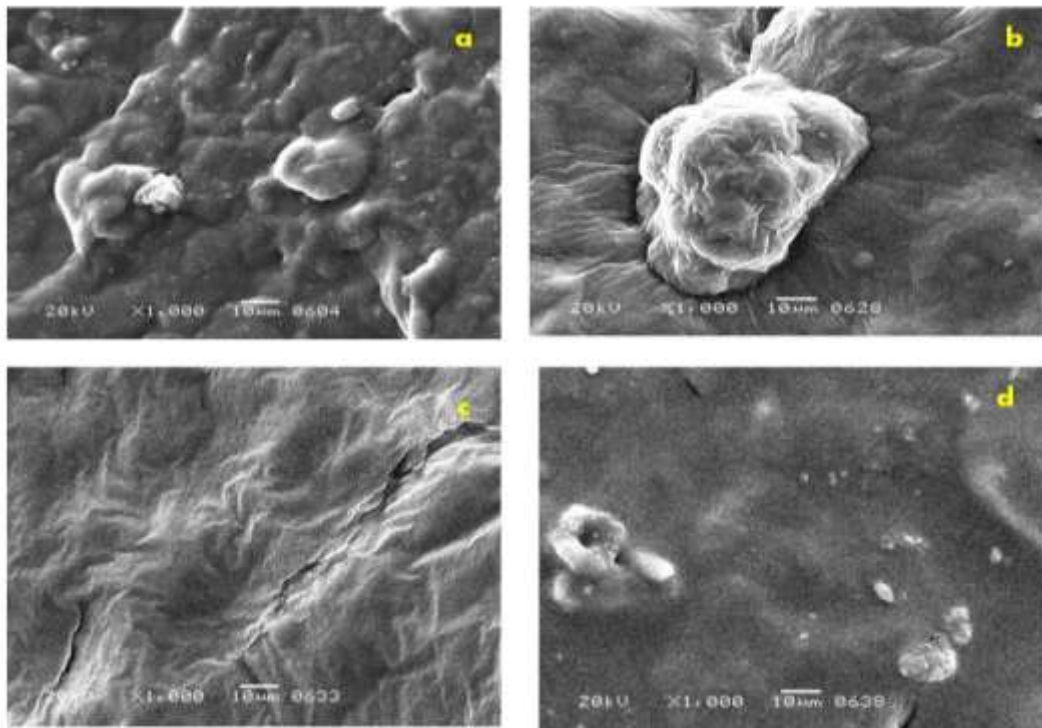


Fig. 7. SEM micrographs of keratin-potato starch bioplastic film. **a** SEM image potato starch bioplastic film (40:0) 1,000x with diameter 10 µm, **b** SEM image of keratin-potato starch bioplastic film (40:2) at 1,100x with diameter 10 µm, **c** SEM image of keratin-potato starch bioplastic film (40:6) at 1,100x with diameter 10 µm, **d** SEM image of keratin-potato starch bioplastic film (40:10) at 1,000x with diameter 10 µm. Note: 40:0, 40:2, 40:6, and 40:10 = starch/keratin (v/v).

CONCLUSION

In environmental and industrial biotechnology, valorization of environmental waste is most recent and promising approach. Management of poultry waste in eco-friendly manner is still challenging. Keratin is important biopolymeric protein, present in chicken feathers. However, keratin can be extracted from poultry feathers that can be utilized in the manufacturing of different products like anti-aging creams, shampoos, hair treatment products, wound healing creams and bioplastics. This research reveals a successful development of bioplastic films of chicken feather's keratin blended individually with corn and potato starch. In addition, the current study also compares the characteristic features of keratin-corn starch and keratin-potato starch bioplastic films. As compared to keratin-potato starch bioplastic film, the bioplastic film comprised of keratin and corn starch has much improved features like decreased moisture content, low solubility, biodegradability and increased surface density. Analysis of surface topologies of developed bioplastic films through SEM elaborated that increased keratin concentration provides smoother and less porous structure as compared to control (bioplastic film without keratin). In general, the fabricated bioplastics showed better properties for applications in food packaging. This strategy gives insights towards eco-friendly, viable and sustainable management of poultry waste.

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