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Valorizing Agro Industrial Byproducts for Next Generation Sustainable Food Packaging: Wheat Germ Protein and Banana Peel Films

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ABSTRACT

The increasing environmental footprint of synthetic plastic garbage researchers are looking into biodegradable options from renewable resources. The goal of this study was to formulate and characterize banana peel powder (BPP) modified wheat germ protein (WGP) based edible and antimicrobial films for possible application as sustainable food packaging materials. Films were made from various concentrations of WGP (0%, 0.4% and 0.8%) with a constant concentration of BPP and the mechanical physicochemical antioxidant and antimicrobial properties were evaluated. The results showed that the use of WGP greatly improved film function. Mechanical analysis revealed that the tensile strength of the control sample was 5.0 ± 0.02 MPa whereas the tensile strength of the 0.8% WGP film was 4.0 ± 0.07 MPa which was a gradual decrease thus showing good flexibility. The water vapor permeability is increased by increasing WGP indicating increased barrier properties. The total phenolic contents (0.8 ± 0.2 mg GAE/g) and DPPH radical scavenging ($55 \pm 0.2\%$) were found to be quite high with increasing concentration of WGP indicating that it possesses bioactive potential in the film. Microbial assays showed effective inhibition of pathogenic microorganisms and color and surface morphology analyses (SEM and FTIR) indicated uniformity and compact structures of WGP enriched films. Based on the results of the study, the films made by incorporating wheat germ protein and banana peel powder were biodegradable, antioxidant, and antimicrobial, which makes them suitable for active packaging applications for food packaging. The results of this product contribute to the valorization of agro-industrial by products as viable and sustainable alternatives to synthetic plastics with an environmentally friendly focus.

Keywords— Film Forming Properties, Water Vapor Permeability (WVP), Mechanical Strength, Phenolic Compounds, Protein Polysaccharide Interaction, Bioactive Peptide

1. Introduction

Food packaging helps to maintain the quality and safety of food products, and prolong their shelf life from production to consumption. The traditional synthetic packaging materials which are mainly made of petroleum based plastics have been long dominating the industry owing to their cost effectiveness strength and versatility. The durability to degrade has created serious environmental pollution and waste management issues (Mangaraj et al., 2019). Further some plastics contain harmful chemical additives that can leach into food and cause health problems for the consumer (Shaikh et al., 2021). To overcome an increasing trend in the world for producing biodegradable renewable and edible packaging materials based on natural biopolymers (Siracusa et al., 2010).

The development of coatings and edible films is seen as a potential discretion to synthetic plastics as they are biodegradable nontoxic and can serve as gas moisture and solute permeable barriers (Suderman et al., 2019). They additionally have the capacity to be loaded with useful materials like antimicrobials antioxidants or taste

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improvements, boosting food safety and preference. Edible films are formed and functional depending on their formulation which mainly includes polysaccharide protein and lipid or composite of these components and their interaction with each other (Kurek and Ščetar, 2017; Roy et al., 2020).

In last few years the source of agro industrial by products for producing biodegradable films has gained a lot of research interest both for environmental protection and waste valorization (Arquelau et al., 2019). These banana peels a by product of fruit processing has a high cellulose, hemicellulose, lignin and pectin content which makes it an ideal material for film forming properties (Pelissari et al., 2021). It also has antioxidant and antimicrobial properties because of its rich polyphenols and flavonoids content (Hikal et al., 2022). In the same way wheat germ protein (WGP) a by product of milling wheat is an tremendous source of bioactive peptides phenol and essential amino acids. These compounds have excellent emulsifying water holding and film forming properties (Boukid et al., 2018; Zhang et al., 2023) and biological properties like antioxidant and antimicrobial activity (Garavand et al., 2022). Both these resources are underutilized and hence a unique opportunity to produce biodegradable and active packaging films with enhanced mechanical nutritional and protective properties.

The use of biopolymer matrices containing natural extracts or proteins to modify their functional properties has been pointed out in previous studies (Didar et al., 2023; Rossi et al., 2024). The best of the author's knowledge however little research has investigated the synergism effect of the wheat germ protein and banana peel components on the formulation of composite edible films. This valorization of these waste materials into eco functional films not only reduces the environmental pollution but also supports the circular bioeconomy and food packaging innovation.

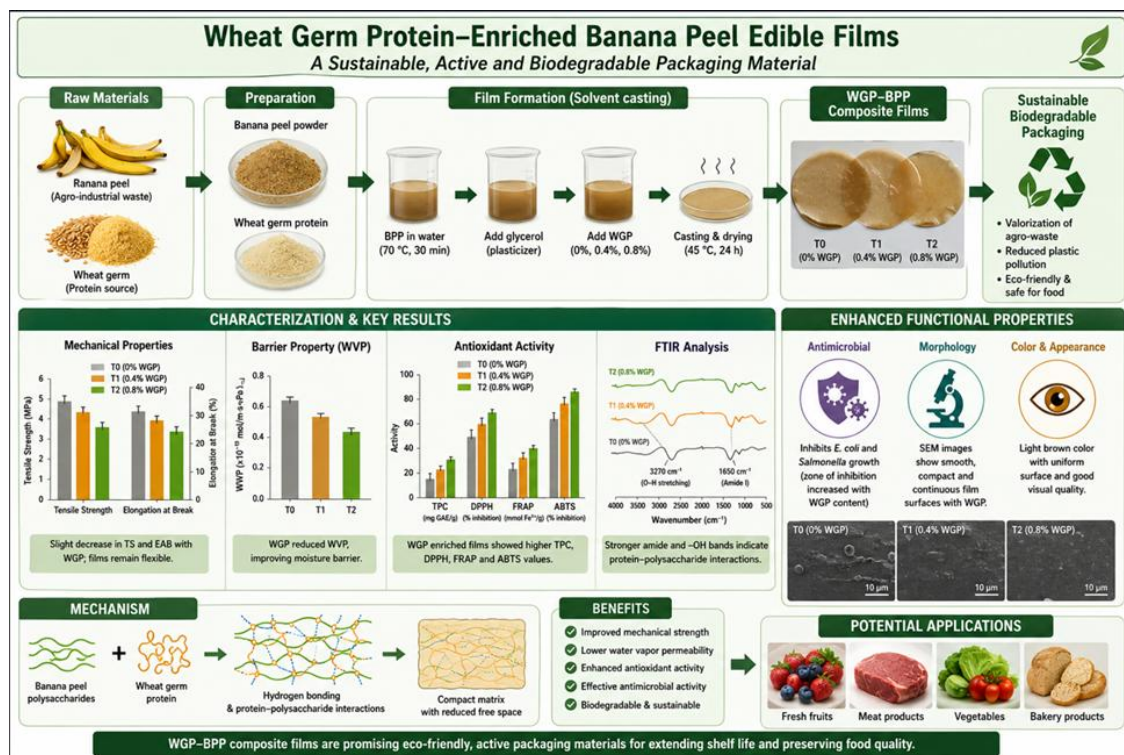
The idea of the present study was to formulate and evaluate edible biodegradable antimicrobial films from banana peel powder (BPP) with different concentration of wheat germ protein (WGP). Their physicochemical antioxidant mechanical and antimicrobial properties were studied and evaluated to investigate their potential application as sustainable alternatives to synthetic packaging materials.

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2. Materials and Methods

2.1 Materials

The juicy bananas (*Musa* spp.) and wheat grains were bought from local markets of Faisalabad, Pakistan. Ethanol, glycerol, hexane and clove oil were purchased as analytical grade reagents from Merck, Germany. Distilled water was used for preparing all the solutions.

2.2 Preparation of Banana Peel Powder (BPP)

Peels of bananas were washed, cut into 3 mm slices, dried in an oven at 60 °C for 6 h until constant weight, in a convective dryer (Gander MTN, USA). The dried material was ground to fine powder and kept in airtight containers at room temperature for use in further studies (Salazar et al., 2021).

2.3 Extraction of Wheat Germ Protein (WGP)

The wheat germ was separated from tempered wheat (14% moisture) with a Quadrant Senior Mill (C.W. Brabender, Germany). The defatting of germ was carried out using a Soxhlet apparatus with hexane for 24 h according to the method of Zhu et al. (2006). The defatted germ was air dried and suspended in distilled water in a 1:16 w/v ratio, adjusted the pH to 10, and stirred it for 1 h at 60 °C. The supernatant after centrifugation (8000 rpm for 15 min) was acidified to pH 4.5 with 1 M HCl to precipitate the proteins. The precipitate was washed, neutralized, and freeze-dried to obtain WGP powder (Sun and Ren, 2015).

2.4 Antioxidant Activity of WGP

Total phenolic content (TPC) was determined by the Folin Ciocalteu method and reported in mg GAE/g of dry sample as the marker of antioxidant activity of WGP. Free radical scavenging activities (DPPH) and antioxidant activity (ABTS and FRAP) were

determined using assays developed by Vu et al. (2018).

2.5 Preparation of Edible Films

The solvent casting method (Kumar et al., 2022) was used for the manufacturing of the edible films. Two grams of banana peel powder was dispersed in 100 mL distilled water then stirred and heated at 70 °C for 30 min and then a plasticizer (glycerol) of 4 mL was added, followed by incorporation of WGP at 0% (T0), 0.4% (T1) and 0.8% (T2) concentration. The mixtures were homogenized degassed and poured into Petri dishes. The films were first conditioned for 24 h at 45 °C and 25 °C and 50% RH, respectively, before further testing.

2.6 Film Characterization

2.6.1 Mechanical Properties

A texture analyzer (TA) was used to assess the tensile strength (TS) film thickness, elongation at break (EAB), and water vapor permeability (WVP) according to ASTM D882-10.XT Plus, UK). Film samples were cut into rectangular pieces (usually 10 mm × 100 mm) and conditioned at 25 ± 2 °C and 50 ± 5% R.H. for 48 h before the measurement for mechanical testing. The initial grip separation and crosshead speed were 50 mm and 50 mm min⁻¹, individually. The stress–strain curve from the tensile test was used to determine the TS and EAB. The thickness of the film was measured at five random locations with a digital micrometer; the readings were then averaged and used for calculations. This procedure follows reported procedures for characterization of biodegradable or edible films (Jiang et al., 2020).

2.6.2 FTIR Analysis:

The functional group interactions in BPP and WGP were identified in the range of 4000-500 cm⁻¹ by using Fourier Transform Infrared Spectroscopy (FTIR) (Bruker Tensor 27, Germany).

2.6.3 Surface Morphology:

The surface uniformity and compactness were studied under the Scanning Electron Microscope (SEM, JEOL JSM-6380A, Japan) after gold coating, to examine the film microstructure.

2.6.4 Microbial Analysis:

The total plate count (TPC), yeast and mold count, and the detection of *Escherichia coli* (*E. coli*) and *Salmonella* were conducted using standard microbiological methods as described by Mokbel and Hashinaga (2005). Samples were serially diluted in sterile peptone water and then plated on Plate Count Agar (PCA) and incubated at 37 ± 1 °C for 48 h as per TPC. Potato Dextrose Agar (PDA) was used to determine the yeast and mold count and it was incubated at 28 ± 1 °C for 3–5 days. The detection of *E. coli* was performed using MacConkey Agar and *Salmonella* detection was performed after pre-enrichment in buffered peptone water (BPW) using Xylose Lysine Deoxycholate (XLD) Agar. Colony counts are reported as Log CFU/g sample.

2.6.5 Color Measurement:

Color parameters (L*, a*, and b*) of the films were determined using a Hunter Lab colorimeter to evaluate their visual attributes. Prior to measurement the instrument was calibrated with a standard white and black tile. Film samples were placed on a white

background and measurements were taken at three different points for each film. The L^* value represents the lightness (0 = black, 100 = white), the a^* value the red – green axis (positive = red, negative = green), and the b^* value the yellow, blue axis (positive = yellow, negative = blue). The total colour difference (ΔE^*) was obtained from the following equation:

$$\Delta E^* = (L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2$$

L_0^* , a_0^* , and b_0^* are the colour parameters of a standard white background. This method is based on the standard colourimetric method outlined by Hassan et al. (2018).

2.7 Statistical Analysis

Each experiment was repeated three times and the results are reported as mean $\pm$ SD. The one-way analysis of variance (ANOVA) was used to find significant differences at the 0.05 level followed by Tukey's test (Version 25.0 SPSS).

3. Results and Discussion

3.1 Mechanical Properties

The mechanical performance of edible films is important for the application in food packaging affecting the strength integrity and handling properties of the films. For this study the tensile strength (TS) and elongation at break (EAB) of the banana peel powder (BPP) based films showed significant effect with the addition of wheat germ protein (WGP). The control film (T0) exhibited the highest TS value (5.0 ± 0.02 MPa) while TS decreased to 4.6 ± 0.04 MPa and 4.0 ± 0.07 MPa for T1 (0.4% WGP) and T2 (0.8% WGP) respectively. As the WGP content increased, the EAB slightly decreased, which suggested that the more rigid the matrix the less flexible it was, but the stronger the protein polysaccharide interactions within the matrix.

The findings were consistent with the results of studies conducted by Zhang et al. (2023) and Didar et al. (2023) which showed that with the increase in protein content in the biopolymer matrix, intermolecular hydrogen bonding increases which makes the matrix more rigid and less elastic. This moderate decrease in TS here indicates efficient crosslinking of both BPP polysaccharides and WGP molecules to give rise to a tighter network with improved structural integrity. The same results were obtained for wheat protein films also mixed with fruit residues (Garavand et al., 2022).

In conclusion the mechanical results showed that partial inclusion of WGP (up to 0.4%) results in better balance between mechanical properties and it can be used for light food packaging applications.

Treatments	Film Thickness (mm)	Film Solubility (%)	Tensile Strength (MPa)	Elongation at Break (%)	Water Vapor Permeability (10^{-10} mol/m $\cdot$ s $\cdot$ Pa)
T ₀	0.50 $\pm$ 0.002	20 $\pm$ 0.1	5.0 $\pm$ 0.02	25 $\pm$ 0.1	0.5 $\pm$ 0.002
T ₁	0.10 $\pm$ 0.005	19 $\pm$ 0.15	4.5 $\pm$ 0.05	24 $\pm$ 0.1	0.4 $\pm$ 0.007
T ₂	0.15 $\pm$ 0.002	18 $\pm$ 0.1	4.0 $\pm$ 0.07	23 $\pm$ 0.1	0.3 $\pm$ 0.01

Table 1. Effect of WGP incorporation on the physical mechanical and barrier properties of BPP-based edible films

3.2 Water Vapor Permeability (WVP)

Water vapor permeability (WVP) is one of the key parameters to consider when evaluating the moisture barrier capacity of a film. Gradual improvement in the water vapor barrier of the films was obtained with the addition of WGP. The control film exhibited the highest WVP ($3.8 \times 10^{-9} \text{ g}\cdot\text{m}/\text{m}^2\cdot\text{s}\cdot\text{Pa}$), while T1 and T2 showed lower values of 3.4×10^{-9} and $3.1 \times 10^{-9} \text{ g}\cdot\text{m}/\text{m}^2\cdot\text{s}\cdot\text{Pa}$, respectively.

This decrease in WVP was likely due to the increased density of protein–polysaccharide network, which decreased free spaces and water diffusion. Kurek and Ščetar (2017) reported similar results using gelatin-based films and Rossi et al. (2024) in composite protein fiber matrices. Also the water interaction of some of the hydrophobic residues in WGP may have helped to reduce the overall water affinity of the film (Boukid et al., 2018). The improved moisture barrier properties increase the potential of the film as a semi-moist and dry food edible packaging material.

3.3 Antioxidant Activity

The antioxidant property of edible films was greatly improved as the WGP concentration increased. Total phenolic content (TPC) increased from $0.52 \pm 0.03 \text{ mg GAE/g}$ (control) to $0.8 \pm 0.02 \text{ mg GAE/g}$ in the 0.8% WGP film. Likewise, the DPPH radical scavenging activity was enhanced from $36 \pm 0.4\%$ in T0 to $55 \pm 0.2\%$ in T2, and the corresponding FRAP values were enhanced. This improvement has been attributed to the bioactive peptides and phenolic compounds that are naturally found in wheat germ, which are beneficial to free radical neutralization (Garavand et al., 2022). Moreover banana peel has considerable polyphenol and flavonoids which can synergize with WGP to enhance the antioxidant capacity (Vu et al., 2018; Hikal et al., 2022). The obtained results are in line with the work of Didar et al. (2023), which showed that the incorporation of plant extracts into a protein based film results in films with greater antioxidant activity as a result of the presence of active hydroxyl and carbonyl groups. The antioxidant film can be a crucial component in the active packaging system, which is used to retard the oxidation of lipids and preserves the freshness of the food during storage.

3.4 Antimicrobial Activity

Antimicrobial assays showed that the WGP-enriched films were effective in inhibiting the growth of the common foodborne pathogens such as *E. coli* and *Salmonella*. Inhibition zones were larger for higher concentration of protein, and the largest effect was observed with the 0.8% WGP film. This may correspond to the bioactive peptides and phenolic compounds present in WGP which cause disruption of microbial cell membranes, and the natural antimicrobial properties of banana peel, which include tannins and polyphenols (Hikal et al., 2022).

Some studies have shown that films with plant protein content exhibit better antimicrobial activity, which is attributed to the presence of phenolic-protein complexes in the film (Rossi et al., 2024; Garavand et al., 2022). The results showed that the obtained films are both passive (barrier) and active (antimicrobial) to be desirable for food preservation applications.

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Parameters	Wheat Germ Protein
TPC	0.8 ± 0.2
DPPH	55 ± 0.2
FRAP	0.7 ± 0.3
ABTS	80 ± 5

Table 2: Bioactive properties and antioxidant capacity of Wheat Germ Protein (WGP).

3.5 Structural and Morphological Analysis

FTIR spectra showed successful interactions between WGP and BPP molecules. The presence of hydrogen bonding and protein polysaccharide association was suggested by peaks at 3270 cm⁻¹ (O–H stretching) and 1650 cm⁻¹ (amide I). Amide bands were stronger in the WGP films than in the film without WGP, indicating the protein aggregation and linking (Zhu et al., 2006; Sun & Ren, 2015).

The SEM images showed that the surfaces of the WGP-incorporated formulations were smooth, continuous, and compact while the control showed some small pores and cracks. These morphological changes agree with the ones found by Pelissari et al. (2021) and Rossi et al. (2024), which reported the improvement in film homogeneity and structural density due to the incorporation of protein from plants. The structure of the WGP-blended films was found to be very compact, which correlates with their excellent mechanical and barrier properties.

3.6 Color and Visual Appearance

Color measurements revealed that all films had a light brown color with slight decrease in lightness (L*) in the presence of WGP contributed by the natural pigment of wheat germ. The a* (red-green) and b* (yellow-blue) values rose slightly suggesting better visual richness. Similar colour change of PEFs was observed by Abedinia et al. (2020). The uniformity of color and smoothness of texture insures compatibility of BPP and WGP as composite materials.

Parameters	a*	b*	L*
T0	2.3 ± 0.01^a	6.5 ± 0.08	46 ± 0.8^a
T1	1.8 ± 0.02^a	5.9 ± 0.07	45 ± 0.6^b
T2	1.5 ± 0.02^{ab}	5.6 ± 0.02	43.6 ± 0.7^b

Table 3. Effect of WGP incorporation on the color parameters (L*, a*, b*) of the developed films.

3.7 Sensory Evaluation

The overall the different advances in preparing the wheat germ protein (WGP) films and banana peel (BP) films together improved the functional properties of composite films. As reported by Zhang et al. (2022) that addition of protein polysaccharide films containing wheat derived proteins increased the antioxidant activity the improved

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antioxidant activity observed upon the addition of WGP was attributed to bioactive peptides and phenolic compounds that act as radical scavengers. The antimicrobial activity of the films also showed improved antimicrobial properties that are in agreement with the results of Rossi et al. (2024) indicating that the protein polysaccharide composites with plant protein derived from these composites inhibit bacterial proliferation by interacting with bacterial cell membranes and by reducing the proliferation of microbes on food surfaces.

On the other hand it was noted that there was an enhancement in the barrier properties, including a decrease in water vapor permeability and moisture sensitivity after introducing WGP. Didar et al. (2023) reported that the barrier properties were improved and they concluded that this was due to the creation of a denser, hydrogen-bonded polymeric matrix between protein and polysaccharide chains. In the meantime the films were still mechanically acceptable similar to the results of Garavand et al. (2022) who demonstrated that the incorporation of plant proteins into polysaccharide based films resulted in an increase in intermolecular cohesion and consequently in tensile strength without decreasing elongation at break. In conclusion the results obtained in this study support the assertion that WGP and BPP are complementary biomaterials for the development of functional food packaging which can be potentially applied in sustainable and active food packaging.

Conclusion

The aim of present work was to develop biodegradable edible films as alternative to conventional food packaging using banana peel powder (BPP) and wheat germ protein (WGP) as an alternative edible film material. The addition of 0.4% WGP resulted in improvement of film compactness and mechanical strength as well as barrier properties. Additionally synergistic interactions between phenolic compounds and protein functionalities increased antioxidant and antimicrobial activities. The FTIR and SEM studies confirmed the extensive intermolecular bonding and uniform morphology. Agro industrial byproducts are valorized like banana peel and wheat germ that contributes to the circular bioeconomy and promotes the ecofriendly materials for active packaging. The WGP enriched films efficiently inhibit the growth of microorganisms and oxidation processes which suggests they could be used for the preservation of perishable foods. Optimization of formulations feasibility of large scale production, and introduction of natural additives to achieve better antimicrobial activity are the areas where further research is warranted. In conclusion BPP&WGP composite films provide a promising guidance for the development of next generation packaging materials that are biodegradable and functional, sustainable, thereby mitigating plastic waste and preserving food quality.

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