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ORIGINAL RESEARCH ARTICLE

Corn Starch-Based Bio-Composite Films Reinforced with Sunflower Seed Hull Powder and Thyme Essential Oil: Enhanced Mechanical, Antioxidant, and Antibacterial Performance for Sustainable Active Food Packaging

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ABSTRACT

Engineering biodegradable sheets with inherent bioactivity is a crucial advancement for next-generation active packaging systems. Corn starch-based bio-composite films were enhanced with sunflower seed hull (SSH) powder as a phenolic-rich reinforcement, glycerol as plasticizer, vinegar as an acidic formulation ingredient and thyme essential oil as a bioactive antimicrobial component, facilitating concurrent antioxidant and antibacterial efficacy. Films were produced using solution casting with differing SSH concentrations (0.25-1g). SSH incorporation improved the physicochemical, mechanical, antioxidant, and antibacterial properties of the developed films. Film thickness progressively increased from 0.024 mm to 0.042 mm in SSHT4, density decreased from 0.78 g/cm^3 to 0.58g/ cm^3 with increasing filler concentration and moisture content reduced from 13.44% to 8.89% in SSHT4, indicating reduced hydrophilicity of the films. Water absorption after 3h immersion reduced from 89.84% to 62.56% in SSHT4, demonstrating reduced water uptake. Tensile strength peaked at 15.94 MPa and elongation at break reached 42.67% in SSHT2 film. SEM- based morphological study demonstrated enhanced dispersion and structural integrity at modest filler loading; ATR-FTIR spectroscopy demonstrated intermolecular interaction and hydrogen bonding between the starch matrix and the SSH component. Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, showed maximum inhibition zones of 14.5± 1.23mm and 12.5±0.62mm, at SSHT2. DPPH antioxidant activity increased from 28.15mg/mL AAE in the control film to 41.19 mg/mL AAE in SSHT2 films. Biodegradability over 14 days ranged from 82.76% to 65.22% at SSHT4. A single, non-replicated visual shelf-life trial using bread suggested delayed spoilage and better quality retention with SSHT2 film packaging, an observation that requires replicated, quantitative validation before being generalized. Because all SSH-containing formulations also contained thyme essential oil while the control contained neither, the antioxidant and antibacterial improvements reported here cannot be attributed to hull reinforcement alone, and reported values represent measurements taken from a single cast film per formulation. Overall, SSH-based bio-composite films demonstrate strong potential as multifunctional, eco-friendly alternatives to traditional food packaging systems.

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1. INTRODUCTION

Plastics remain the dominant material in food packaging due to their flexibility, durability, and cost-effectiveness, but this convenience carries a substantial environmental cost. The packaging sector alone accounts for 50% of global plastic waste output, with food confinement and preservation responsible for 66% of packaging-derived waste (Ncube et al., 2021; Birania et al., 2022). India's per-capita consumption of synthetic polymers (43%) already exceeds the global average (39%), and petroleum-derived plastics continue to dominate worldwide production (Kan and Miller, 2022). This scale of plastic dependence, set against its poor end-of-life fate, underscores the need for biodegradable packaging alternatives.

In response to the escalating environmental danger, research in the packaging business has transitioned towards the production of bio-based polymers, attributed to their biodegradable properties, marking a crucial advancement in the creation of eco-friendly packaging materials (Ncube et al., 2021). Biopolymers demonstrate qualities akin to traditional packaging materials and may be handled using methods such as solution casting, melt mixing, electrospinning, thermo-processing, and extrusion procedures (Yadav et al., 2018). Due to these various advantages, biopolymers generated from renewable biological sources are rapidly being investigated as sustainable alternatives, capable of reducing or completely eliminating reliance on fossil fuel-based packaging materials. Natural polymers encompass carbohydrate-based substances such as chitosan, carrageenan, cellulose, and starch, as well as protein-based materials like gelatin, soy protein, whey protein, and zein; lipids comprise beeswax and fatty acids. (Homthawornchoo et al., 2022).

Concentrating on polysaccharides, starch is regarded as one of the most prevalent, dependable, and economical polymers for film creation. The structural makeup has two primary components: amylose and amylopectin, which account for around 20-30% and 70-80%, respectively. This biopolymer may be extracted from several agricultural sources, including rice, potato, maize, wheat, and sago. Corn possesses a substantial quantity of starch and produces a semi-crystalline biopolymer due to a composition of 20-28% linear amylose and highly branching amylopectin (Hazrol et al., 2021; Roy et al., 2020). Moreover, maize starch generally experiences gelatinization at temperatures between roughly 64.4°C to 77.2°C (Chakraborty et al., 2022). Starch films alone are stated to have inadequate thermoplastic characteristics, mechanical strength, permeability, and fire retardancy (Roy et al., 2020). To address these limitations, starch films may be combined with plasticizers, reinforcing materials, and certain alterations to the chemical structure (Srivastava et al., 2023).

Sunflower seed hulls constitute a significant agro-waste byproduct, accounting for 20-30% of the residual seeds from the oil industry. These agricultural wastes include significant amounts of lignocellulosic components, including cellulose, hemicellulose, and lignin, as well as natural antioxidants and nutritional fibres. Furthermore, it was shown that SSHs possess a substantial phenolic content ranging from 0.7% to 5.4%. The elevated phenolic content resulted in the creation of a starch-based antioxidant film, with chlorogenic acid serving as the principal antioxidant-rich active component. Consequently, this research validated that SSHs function as a powerful reinforcement agent that improves the characteristics of starch-based biofilms using sophisticated valorisation approaches (Tsvetanova et al., 2025; Frolov et al., 2023; De'Nobili et al., 2021; Menzel, 2020). Additionally, plasticizers (non-volatile organic liquids) are extensively utilized additives in the plastic sector. They are integrated to enhance mechanical strength, extensibility, toughness, and processing ease, while crosslinking agents act concurrently to improve the mechanical, functional, and barrier properties of the films. (Hazrol et al., 2021; Zhang et al., 2023).

A prevalent strategy to enhance the efficacy of biofilms is their incorporation into intelligent packaging systems. Active packaging is regarded as an innovative approach designed to prolong the shelf life of food by directly interacting with the food itself. The process entails the integration of an active chemical into the polymer matrix, which affects its antioxidant or antimicrobial properties (Ribeiro-Santos et al., 2017; Menzel, 2020). Antimicrobial agents originate from several sources, with naturally derived ones receiving heightened attention due to growing food safety concerns and customer demands. Numerous studies have documented the increasing interest in naturally sourced antimicrobial compounds (Ju et al., 2019; Sharma et al., 2020). Essential oils (EOs) derived from plants have garnered significant interest for their capacity to preserve fruits, vegetables, meat, fish, dairy products, and baked goods. Essential oils are not utilized directly with food due to their volatile characteristics and vulnerability to destruction from light, oxidation, and elevated temperatures. Consequently, contemporary methods and advancements use essential oils via encapsulation and coatings on liposomes, solid lipid nanoparticles, and packaging materials (Sharma et al., 2021).

The aforementioned methodologies were employed in the current work to develop a corn starch-based bio-composite film for food packaging applications, using sunflower seed husk powder (SSHs) alongside thyme essential oil as an antibacterial agent. Research indicated that milled sunflower seed hulls, when coupled with oat bran or certain minerals, promoted fungal growth, suggesting that sunflower seed hulls likely have little antifungal properties (Tsvetanova et al., 2025). While previous studies have investigated the formulation of bio-composites for food packaging, the particular amalgamation of corn starch, SSH powder, glycerol, vinegar, and thyme essential oil, along with the evaluation of its physicochemical, mechanical, structural, antioxidant, antimicrobial, biodegradation, and food preservation properties, remains insufficiently studied. The primary aim of this study was to develop an eco-friendly and biodegradable bio-composite and to assess its qualities for prospective use in food packaging through visual shelf-life assessment using bread as a model food system. This effort further advanced waste valorisation by using a significant agricultural by-product, specifically sunflower seed hulls (SSHs). It was hypothesised that incorporating SSH powder into the corn starch matrix would progressively improve mechanical strength and reduce water sensitivity by reinforcing the polymer network, while the co-incorporated thyme essential oil would confer antioxidant and antibacterial functionality; formulations combining both components were therefore expected to outperform an unreinforced control on these measured endpoints, without distinguishing the individual contribution of each component.

All ingredients utilized in this investigation were chemically safe, food-grade, and employed without previous processing. A thorough analysis of the films was conducted to assess their efficacy as packaging material.

2. MATERIALS AND METHODS

This section outlines the material used in the experimental procedures followed for the preparation of Corn Starch- based films reinforced with Sunflower seed hull for use in active packaging applications.

2.1 Materials and Chemicals

The bio-composite films were formulated using corn starch as primary biopolymer. Glycerol was employed as a plasticiser, vinegar served as an acidic formulation ingredient, and thyme essential oil was incorporated as an antimicrobial component. Sunflower seed hulls were utilized as the reinforcing material. Sunflower seeds were obtained from local markets in Varanasi, Uttar Pradesh, India and manually dehulled to obtain the hulls. Corn starch (Starch Corn pure, Catalogue No. 58254, CAS 9005-25-8; Batch No. 7108553; Mfg. Date 09/2025) was procured from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai, Maharashtra, India. Glycerol was procured as USP/FCC/EP-grade vegetable glycerin (99.7% purity, produced in Malaysia; Lot No. SN1478, packaged January 2026) from Sharrets Nutritions LLP, Jaipur, Rajasthan, India. Thyme essential oil (100% pure thyme essential oil, Batch No. HeiTHYM, 50 mL) was procured from Heilen Biopharm Pvt. Ltd., Banaskantha, Gujarat, India. Vinegar (non-fruit vinegar prepared from acetic acid and water, Batch No. 301) was procured from U.P. Vinegar Works, Lucknow, Uttar Pradesh, India, and had a measured pH of 2.3.

2.2 Fabrication of Bio-composite films

2.2.1 Preparation of SSHs powder

The raw sunflower seeds were manually dehulled to separate the hulls, which were then thoroughly rinsed with water to eliminate impurities. The cleaned hulls were subjected to drying in a dehydrator at 65°C for 4 hours (De'Nobili et al., 2021), followed by allowing them to cool down to room temperature. Subsequently, the dried hulls were ground into a fine powder and sieved through a 56µm mesh screen to achieve uniform particle size. The resulting powder was then placed in an airtight container for subsequent use.

2.2.2 Synthesis of Bio-composites

Preparation of the bio-composite films was carried out by employing a solution casting technique based on the procedure described by (Quispetera et al., 2021). Corn starch (5g) was added to 50mL of distilled water in a beaker, after which 2.5mL of glycerol,3mL of vinegar, and 0.25mL of thyme essential oil were added to the mixture. To this formulation, sunflower seed hull powder (SSHs) was incorporated at varying concentrations (0.25g,0.5g,0.75g and 1g) while maintaining a constant starch content, as summarised in **Table 1**. The mixture was then heated with continuous stirring at 85$°C$ until a uniform film-forming solution was achieved. The resulting solutions were cast and allowed to dry at ambient temperature for 24 hours. After complete drying, the films were carefully detached and kept for subsequent evaluation. A control film prepared without SSHs was also fabricated under the same condition for comparative analysis. For each formulation, one film-forming solution was prepared and cast as a single sheet; three measurements per test were subsequently taken at different locations on that single sheet and used as technical replicates. The control formulation contained neither SSH powder nor thyme essential oil, whereas every SSH-containing formulation also contained a fixed level of thyme oil (0.25 mL); consequently, this design does not allow the independent contributions of hull reinforcement and thyme oil to be statistically separated, and a dedicated thyme-only formulation was not included in the present study.

Table 1. Formulation Table of the Prepared Bio-composites

Sample	CS (g)	SSHs (g)	Glycerol (ml)	Vinegar (ml)	Thyme Essential Oil (ml)
Control	5	0	2.5	3	-
SSHT1	5	0.25	2.5	3	0.25
SSHT2	5	0.5	2.5	3	0.25
SSHT3	5	0.75	2.5	3	0.25
SSHT4	5	1.00	2.5	3	0.25

2.3 CHARACTERIZATION OF THE BIO-COMPOSITE FILMS

2.3.1 Thickness

Digital micrometre (Mitutoyo Co.,Japan) was used to assess the thickness of each prepared films keeping a zero error of ± 0.01mm. Arbitrary values were taken at three random points of the films and the mean thickness was calculated (Nanda and Singh, 2022).

2.3.2 Density

The density of the developed films was estimated according to the procedures described by Hazrati et al., 2021 based on their mass and volume. Film samples measuring 10× 30mm and weighed using a digital balance. The thickness values were taken from the previously conducted thickness assessment. Volume was calculated using the measured dimensions, and density was then equated using Equation 1. The values were measured in triplicates and the mean value was reported.

ρ=m/v (eqn.1)

Where m= mass (g) and v= volume (cm^3)

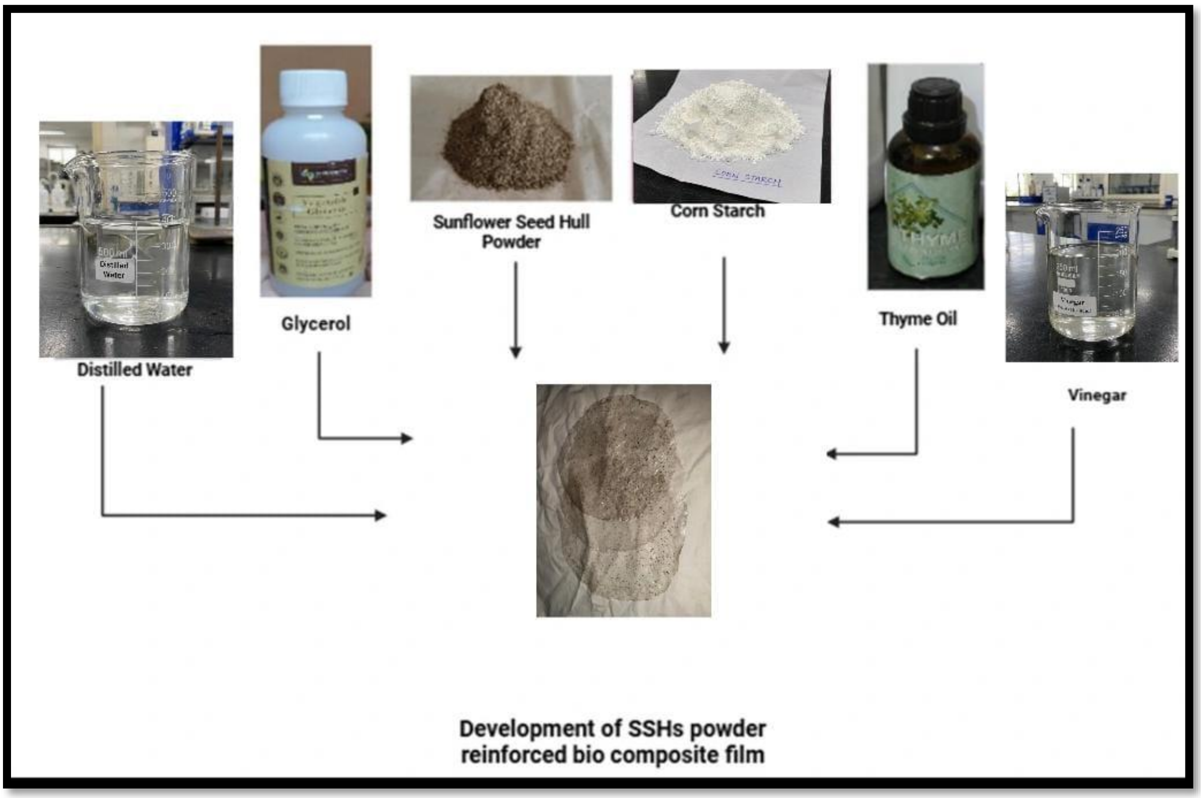


Fig.1. Preparation of bio-composite films from natural ingredients

2.3.3 Moisture content

Moisture content of the films was determined following the method of Biswas et al., 2022 with slight modification. Film samples were cut into 2cm×2cm pieces, and the initial weight of each sample was recorded. The samples were then dried in a hot air oven at 105°C for 24 hours and their final weights was recorded. Equation 2 was used to calculate the % moisture content of the films. The values were recorded in triplicates.

$$\textbf{Moisture content \%} = \frac{Initial\ Weight\ of\ sample - Final\ Weight\ of\ sample\ after\ drying}{Initial\ Weight\ of\ sample} \times 100 \quad \text{(eqn.2)}$$

2.3.4 Water Absorption

Water absorption capacity refers to the ability of the dried films to absorb and retain water. Films with a uniform dimension of 12 mm × 12mm (Wildan et al., 2021) were taken and dried in a hot air oven at 105$°C$ for 3 hours. The initial dry weight of each sample (W_0) was measured before immersion. Subsequently, the dried films were then placed in distilled water for predetermined duration of 30 minutes and 3 hours (Nanda and Singh, 2022). After soaking, the samples were carefully removed and gently wiped with tissue paper to eliminate excess surface water. The final weight after immersion (W_1) was then recorded. The water absorption percentage was determined using equation 3.

$$\textbf{WA\%} = \frac{W_1 - W_0}{W_0} \times 100 \text{ (eqn.3)}$$

Where W_0 = Initial dry weight

W_1 = Final Weight after immersion

2.3.5 Tensile strength

The tensile properties of the prepared bio-composite films, namely tensile strength and elongation at break, were determined using a Universal testing machine (Model INSTRON 5kN) in accordance with ASTM standard D882. Film samples measuring 90 mm × 10 mm were prepared, with a gauge length of 40mm while maintaining a cross-head speed of 3mm/min. Each film sample was analysed in three replicates and the average values were calculated (Suklaw and Ratanakamnuan, 2022). Specimens for all physical and mechanical tests (Sections 2.3.1-2.3.6) were tested under ambient laboratory conditions; controlled relative-humidity/temperature conditioning of specimens prior to testing i.e., 23 degrees C, 50% RH as typically specified for ASTM D882 was not performed or recorded, and this is noted as a limitation of the present study.

2.3.6 Folding Endurance

Folding endurance test is performed to determine the films' flexibility and its physical stability when exposed to processing, handling and packaging application. Each film was cut into a sample size of 4cm×4cm and was manually folded at the same place over and over again till they ruptured. The no. of folds (n) before rupturing was noted which indicated the folding endurance of films (Nanda and Singh, 2022).

2.3.7 Biodegradability Test

Soil burial test was employed to assess the biodegradability of the prepared films. The biodegradability of the films was assessed using soil burial test as described by (Pongputthipat et al., 2023) with some modifications. Film specimen of dimension 3×3 cm was cut and buried 5cm deep inside wet garden soil kept inside plastic containers. The initial weight of the films was recorded as Wi. The films were kept under observation for 14 days and the loss in weight of the film specimens was recorded at every two days. After retrieval from the soil the films were cleaned to remove the adhered soil particles and dried at 40$°C$ until constant weight was achieved. The final weight of the dried films was recorded as Wt. The results were obtained using equation 3:

$$\textbf{\% Weight loss} = \frac{Wi - W_t}{W_i} \times 100 \qquad \text{(eqn.4)}$$

Where W_i = initial weight before burial

W_t = Final weight of films after soil burial

2.3.8 Scanning Electron Microscope (SEM)

Surface morphology of the developed films was examined using scanning electron microscope (Model JSM 6490LV, JEOL,Tokyo, Japan) (Anand et al., 2022). The film samples were cut into 5mm×5mm dimension and was mounted onto SEM specimen stubs and was sputter- coated with a thin layer of platinum. Micrographs were obtained at different magnification 500×,1000×,5500 and 10,000 with a constant accelerating voltage of 15kV. The working distance and current beam were adjusted corresponding to the standard operating protocol of the equipment (Nanda et al., 2024).

2.3.9 ATR-FTIR analysis

The chemical structure of the developed films was analysed using Fourier Transform Infrared (FTIR) spectroscopy in Attenuated Total Reflectance (ATR) mode using an Agilent Cary 630 FTIR spectrometer. The spectra were recorded over a wavenumber range of 4000-650cm^{-1} at a resolution of 16 cm^{-1} with 100 scans for both background and sample. The film samples were directly placed onto the ATR crystal surface with proper contact to ensure accurate spectral acquisition. The obtained spectra were analysed to identify functional groups and evaluate intermolecular interaction within the bio-composite matrix.

2.3.10 Antimicrobial Testing

The antimicrobial activity of the prepared films was performed against gram positive (*S. aureus*) and gram-negative bacteria (*E. coli*) using agar disc diffusion assay (Anand et al., 2022; Aydin and Zorlu, 2022). Initially, nutrient broth was prepared by dissolving 14g of nutrient broth powder in 500 ml distilled water while nutrient agar was prepared by mixing 1.3g nutrient agar in 100mL distilled water. Additionally, the prepared media and the petri dishes were sterilised in an autoclave under standard conditions of 121$°C$, 15psi pressure for 15 minutes. Subsequently, the molten agar medium was poured on petri plates and were allowed to solidify (Nanda et al., 2022). Approximately, 0.1mL of each bacterial strains were inoculated into the nutrient broth (Contessa et al., 2021) and incubated at 37$°C$.The bacterial suspension was then diluted using sterile saline solution to 1× 10 to the power 8 CFU/mL and was uniformly spread over the nutrient agar plates. (Aydin and Zorlu et al., 2022). The films were cut in circular disc of 5mm diameter and was placed aseptically over the nutrient agar-bacterial suspension plates. Two control setups were also maintained; negative control (plain film without thyme oil) and positive control (Ampicillin 32 µg/mL for *E. coli* and Vancomycin 2 µg/mL for *S. aureus*. Thereafter the inoculated agar wells were maintained at 4$°C$ for 1 hour followed by aerobic incubation at 37$°C$ for 24 hours. (Anand et al., 2022). The antibacterial efficacy of the prepared films was evaluated by measuring the diameter of the inhibition zone formed around the film disc, and the values were recorded in millimetres (Singh et al., 2026).

2.3.11 Antioxidant Activity

The antioxidant potential of the prepared films was evaluated using DPPH (2,2- diphenyl-1 picrylhydrazyl) radical scavenging assay (Singh et al., 2025). The method was adapted from Sadeghi et al., 2025 and performed at single concentration using standard spectrophotometric approach with slight modifications. Film samples (25 mg) were dispersed in 5mL of distilled water and vortexed for 4 minutes to achieve uniform extraction of the active compounds. An aliquot of this extract (2.8 mL) was reacted with 0.2 mL of 1 mM DPPH solution prepared in methanol. This mixture was thoroughly mixed and kept in dark for 30 minutes at room temperature prior to analysis. Absorbance was recorded at 515 nm using UV- Visible spectrophotometer (Sadeghi et al., 2025). A control was prepared using methanol in place of sample extract, while methanol without DPPH served as blank sample. A calibration curve was generated using ascorbic acid as reference sample, and the antioxidant capacity of the sample was expressed as mg/mL ascorbic acid equivalent (AAE) based on the extract concentration. The percentage of radical scavenging activity was determined using the following equation:

$$\text{Antioxidant activity \%} = \frac{A_b - A_s}{A_b} \times 100$$

Where,

A_b represents absorbance of control and A_s represents absorbance of sample.

2.4 preliminary visual shelf-life study

A preliminary visual shelf- life assessment was performed to examine the practical suitability of the fabricated bio-composite films for use in active food packaging. Fresh bread slices of roughly equal dimensions were chosen as a representative food model and categorized into three experimental groups: (i) bread left unwrapped as a control system, (ii) bread enclosed in the control film sample, (iii) bread slice packaged using the SSHT2 film. All samples were maintained under room conditions for total of 20 days. Observable changes in physical attributes such as appearance, surface texture, drying and mold visibility were recorded at defined time intervals on days 0,5,10,15 and 20. The rate of the quality degradation across the different packaging type film was then compared to determine the effectiveness of the developed films in maintaining bread quality over the storage duration,

2.5 Statistical analysis

Experimental data are presented as mean± standard deviation where applicable. Statistical analyses were conducted using IBM SPSS Statistics (Version 32) and GraphPad Prism (Version 11). Differences among the formulations across physicochemical and mechanical properties were assessed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post hoc test. Antibacterial activity data were evaluated using one-way ANOVA with Dunnett's multiple comparison test, with the control group as the reference group. A probability value of $p<0.05$ was considered statistically significant in all analyses. It should be noted that the reported n=3 for each formulation corresponds to three measurements taken at different locations on a single cast film (technical replicates) rather than independently prepared and cast films. ANOVA outcomes are therefore reported to summarise the measured data and should be interpreted as descriptive of within-film variability rather than as confirmatory inference across independently manufactured batches; findings should be treated as preliminary until confirmed using independently cast replicate films.

3. RESULTS AND DISCUSSION

This section discusses and analyses the characteristics and properties of the bio-composites prepared at different hull powder concentration.

3.1 Fabrication of bio-composite films

The prepared films were easily removed after drying the corn starch- glycerol solution. However, an increased concentration of the reinforcement material (Sunflower seed hull powder) showed reduced translucency and increased brittleness (Nanda &Singh, 2022).

3.2 Thickness

The incorporation of the reinforcement material led to a progressive increase in the thickness of the films varying slightly. The thickness of the films ranged from 0.024±0.003 mm (Control) to 0.042±0.002mm (SSHT4). Statistical analysis confirmed that film thickness was significantly influenced by SSH concentration ($F= 21.560$, $p< 0.001$). This enhancement in the thickness may be attributed to presence of more solid content and high intermolecular interactions within the polymer matrixes. The difference in the thickness may also be associated due to viscosity of the film forming solution and the drying nature of the films. A similar trend has been reported by Oluwasina et al., 2021; Neupane et al., 2025 which also concluded that thickness is directly proportional to increasing reinforcement material.

Table 2. Thickness and Density of developed bio-composites reinforced with SSH powder. Values are presented as mean ± SD (n=3). Means sharing different superscript letters within a column are significantly different according to Tukey's HSD post hoc test ($p< 0.05$).

Film Formulation Code	Thickness (mm)	Density (g/cm^3)
C	0.024± 0.002^{a}	0.78 ± 0.05^{a}
SSHT1	0.027 ± 0.001^{ab}	0.74 ± 0.03^{ab}
SSHT2	0.029 ±0.002^{b}	0.69 ± 0.02^{bc}
SSHT3	0.035 ± 0.001^{bc}	0.63 ± 0.03^{c}
SSHT4	0.042 ± 0.002^{c}	0.58 ± 0.02^{d}

3.3 Density

The present study shows that control film possessed a density of 0.78±0.05 while the film with maximum thickness showed 0.58±0.02. The observed variation in density among the formulations was statistically significant, as confirmed by one-way ANOVA ($F(4,10) =19.206$, $p<0.001$). Pairwise comparisons using Tukey's HSD further demonstrated significant differences between selected treatment groups ($p< 0.05$). This data is supported by the previous study of Nanda & Singh, 2022 which strongly establishes the relationship between density and compactness. Density, also known as the volumetric mass is defined as the mass per unit volume the density of the film is influenced by the filler concentration and distribution inside the matrix of the polymer. This variation in the density is attributed due to the filler and matrix interaction. Higher the filler concentration more rigid will be the interfacial bonding. Consequently, a high interfacial bonding results in filling of the internal voids with the matrix thereby leading to a compact structure (Nanda & Singh, 2022). Similar trend of inverse relationship between thickness and density has been reported by Hazrati et al., 2021; Neupane et al., 2025.

3.4 Moisture Content

The moisture content of the developed CS-based films decreased progressively with increasing concentration of sunflower seed hull powder. The value of the moisture analysis was recorded as 13.44 ±0.37, 11.24±0.22, 10.63±0.26, 9.89±0.23, 8.89±0.34 for C, SSHT1, SSHT2, SSHT3 and SSHT4 respectively. One-way ANOVA revealed a significant effect of formulation on moisture content ($F(4.10) =100.916$,

$p < 0.001$). Tukey's HSD post hoc test indicated significant differences among the formulations ($p<0.05$). Starch molecules are characterised by presence of numerous hydroxyl (-OH) group, which contribute to their hydrophilic nature and facilitate interaction with water molecules. Consequently, neat starch generally exhibits higher moisture content (Amaraweera et al., 2021). However, incorporation of reinforcing materials has been reported to reduce moisture uptake in starch- based (Roslan and Tukiran , 2022). In the present study, addition of sunflower seed hull (SSH) fibres likely contributed to a reduction in the moisture content by limiting the availability of the hydrophilic sites and restriction water interaction within the matrix. A comparable decreasing trend in moisture content has been reported by Désiré et al., 2021 where increasing the microcrystalline cellulose in starch- based films resulted in a reduction in moisture from 15.97% to 11.43%. Similarly, in corn- starch based bio-composite films, incorporation of nature kenaf fibres has been shown to lower the amount of water retained in the films (Hazrol et al., 2022). Consistent findings have also been documented in previous studies (Iffath et al., 2025; Srivastava et al., 2023; Priya et al., 2021; Nanda & Singh, 2022).

Table 3. Moisture content and water absorption (%) of bio-composite films. Values are expressed as mean± SD (n=3). Different superscript letters within the same column indicate significant differences (Tukey's HSD post-hoc, $p< 0.05$).

Composite films	Moisture content (%)	Water absorption (%)	
		30 minutes	3 hours
CONTROL	13.44± 0.37^{a}	82.27±2.69^{a}	89.84±2.69^{a}
SSHT1	11.24±0.22^{b}	76.22±2.58^{ab}	81.94±2.70^{b}
SSHT2	10.63±0.26^{bc}	69.44±2.91^{bc}	76.93±1.61^{bc}
SSHT3	9.89±0.23^{c}	64.17±2.69^{cd}	69.96±2.46^{c}
SSHT4	8.89±0.34^{d}	58.00±2.65^{d}	62.56±3.31^{d}

3.5 Water absorption

Water absorption is an important property of biodegradable packaging films, as higher water uptake may adversely affect the stability and shelf life of packaged food products. **Table 3**. presents the results which demonstrated that the control starch film exhibited the highest water absorption, whereas incorporation of sunflower seed hull powder resulted in a progressive reduction in the water uptake. After 30 minutes, water absorption values decreased from 82.27%, 76.22%, 69.44%, 64.17% to 58.00% for C, SSHT1, SSHT2, SSHT3 and SSHT4 respectively. A similar decreasing trend was observed after 3h of immersion, with the control film showing the maximum water absorption (89.84%). One-way ANOVA revealed significant differences among the film formulations for water absorption for 30 min ($F(4,10) = 37.64$, $p< 0.001$) and for 3h ($F(4,10) = 48.85$, $p< 0.001$). Tukey's HSD post hoc analysis further confirmed significant differences between the formulations ($p<0.05$). The higher water absorption of the control film may be associated with the hydrophilic nature of the starch and the presence of free hydroxyl groups within the polymer matrix. Comparable findings were documented by Pradiza et al., 2025, who noted greater water uptake in pure starch and PVA /starch films. The reduction in water absorption observed after incorporation of SSH may be related to the filler-induced reduction in matrix free volume and restriction of water diffusion pathways. Oulwasina et al., 2021 similarly reported that filler materials reduced water penetration by occupying microvoids within the polymer network, thereby decreasing hydrophilicity and improving barrier characteristics.

3.6 Tensile testing & Elongation at break

The mechanical behaviour of the prepared films showed a composition- dependent biphasic trend, wherein both tensile strength (TS) and elongation at break (EAB) increased progressively with filler incorporation up to 0.5g (SSHT2), followed by a gradual decline at higher concentrations. The tensile strength increased from $12.90 \pm 0.65\ MPa$ (control) to $14.28 \pm 0.54\ MPa$ (SSHT1) and then attained a maximum peak of $15.94 \pm 0.48\ MPa$ (SSHT2). Beyond this concentration, a reduction in TS was observed, with values decreasing to $15.32 \pm 0.32\ MPa$ (SSHT3) and $13.74 \pm 0.73\ MPa$ (SSHT4). A comparable pattern was noted for elongation at break. The EAB increased progressively from $29.90\ \pm 1.59\%$(control) to $36.06 \pm 2.29\%$ reaching peak value of $42.67 \pm 2.23\%$ and subsequently declined at higher filler concentration (SSHT3 and SSHT4) to $40.95 \pm 2.04\%$ and $24.08 \pm 2.42\%$ respectively. One-way ANOVA revealed that the film formulation had a significant effect on both TS ($F= 13.834$, $p< 0.001$) and EAB ($F= 39.330$, $p< 0.001$). Tukey's HSD post-hoc analysis further confirmed significant differences among several formulation, indicating that the mechanical performance of the films was strongly influenced by SSH concentration.

The observed improvement in flexibility at lower filler concentration (0.25g, 0.5g) can be attributed to the uniform distribution of SSH fibres in the starch matrix, allowing adequate chain mobility resulting in improved flexibility (Wildan and Lubis, 2021). However, at higher filler concentration, the reduction in TS and EAB may be associated due to limited adhesion between the phases and restricted polymer chain movement due to reduce flexibility and failure (Wildan and Lubis, 2021; Srivastava et al., 2023). Similar trend has been reported in the previous studies. For instance, Kusmono et al., 2021 observed that chitosan/CNC bio-composite films exhibited an increase in TS and elongation at break up to an optimum CNC concentration (4%) followed by decline at higher loadings. Likewise, Mahardika et al., 2021 reported that PVA-based bio-composite reinforced with durian peel fibres showed increase in TS from 24MPa to 37 MPa along with an improvement in elongation at break (79%) up to 6 wt% addition of natural fibre. In addition, Srivastava et al., 2023 demonstrated that rice husk fibre reinforced films exhibited enhanced mechanical properties up to an optimum fibre content (20%), beyond which both TS and EAB declined. Overall, the result indicated that optimal filler incorporation is essential for enhancing mechanical performance without compromising flexibility. It should be noted that the observed density and strength trends may also be influenced by concurrent variation in film thickness, moisture content, and the fixed thyme oil loading across formulations, as well as by casting heterogeneity inherent to the single-film-per-formulation design; these interacting factors cannot be fully isolated from the effect of SSH concentration alone based on the present dataset.

Table 4. TS and EAB of bio-composite films containing different concentration of sunflower seed hull (SSH) powder. Values are presented as mean± SD (n=3). Different superscript letters in the same column indicate significant differences (Tukey's HSD, p< 0.05).

Film Formulation Code	Tensile strength (MPa)	% of Elongation at break
C	12.90 ± 0.65^{a}	29.90 ± 1.59^{b}
SSHT1	14.28 ± 0.54^{bc}	36.06 ± 2.29^{c}
SSHT2	15.94 ± 0.48^{d}	42.67 ± 2.23^{d}
SSHT3	15.32 ± 0.32^{cd}	40.95 ± 2.04^{d}
SSHT4	13.74 ± 0.73^{ab}	24.08 ± 2.42^{a}

3.7 Folding Endurance

Folding endurance represents the ability of the film to withstand repeated bending at the same location before breaking (Aldawsari et al., 2023). This parameter provides insight into the flexibility and handling performance of the film (Nanda & Singh, 2022). In the present investigation, the control film exhibited the highest folding endurance (20.33 ± 2.08^{c}) indicating greater flexibility. However, the incorporation of SSH content led to a noticeable decline in folding endurance. Among the modified samples, SSHT2 demonstrated maximum folding endurance (17.67 ± 2.51^{bc}), followed by SSHT1 (14.67 ± 2.51^{b}) and SSHT3 (13.33 ± 2.08^{b}) and SSHT4 showed very low endurance (6 ± 2.00^{a}), indicating brittle nature. The observed differences in the folding endurance among the formulations were statistically significant, as evidenced by one-way ANOVA ($F(4,10) = 17.469$, $p<0.001$). This reduction in the folding endurance can be linked to the increased filler concentration, which restricts the mobility of polymer chains and reduces flexibility. Despite this, SSHT2 exhibited comparatively improved folding endurance among the formulated film, which may be attributed due to more uniform dispersion and stronger interaction between SSH and CS matrix. Comparable findings have been reported by Nanda & Singh, 2022 where an increase in the coconut fibre and corn cob concentration led to decreased flexibility of the films.

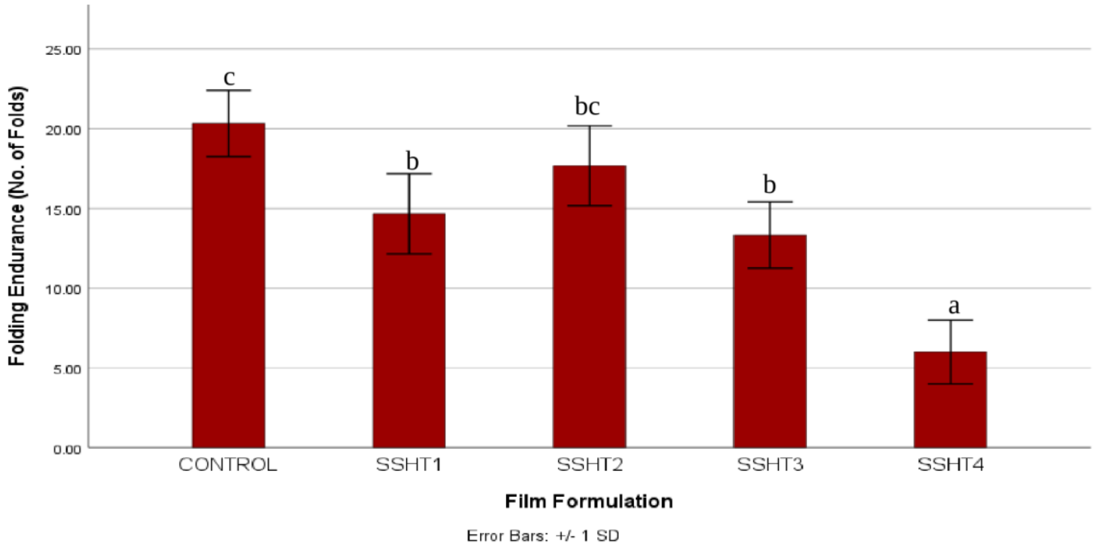


Fig.2. Evaluation of Folding Endurance of CS film and SSH-based film formulation, expressed as folding cycles (n), indicating flexibility of the films. Values are presented as mean ± standard deviation (n=3). Different letters above bars indicate statistically significant differences among formulations according to Tukey's HSD post hoc test (p<0.05).

3.8 Biodegradability test

Biodegradability was examined through a 14-day soil burial test, and the percentage weight loss was recorded at 2-day intervals. The test results revealed that control films possessed highest percentage of weight loss (82.76%) at the end of 14th day showing rapid degradation due to absence of the reinforcement material. A decreasing trend in weight loss was observed for filler- based films, with SSHT1, SSHT2, SSHT3, SSHT4 showing a % weight loss of 74.19%, 74.07%, 69.70% and 65.22% respectively. All the films showed visual cracks and fragmentation after burial indicating progressive degradation. **Figure 3** shows the graphical representation of the bio-composites degraded over 14 days. Similar, trends have been reported by Othman et al., 2023; Srivastava et al., 2023; Malekzadeh et al., 2024; Suwanpratap et al., 2026, where biodegradability reduced upon increasing the filler concentration. This can be attributed due to addition of filler material which leads to formation of more compact and torturous structure due to enhanced internal arrangement and composition. This newly formed network hampers moisture penetration and reduces microbial and enzymatic action responsible for degradation. Owing to the observational nature of the biodegradability assessment, formal statistical comparison was not conducted for this parameter.

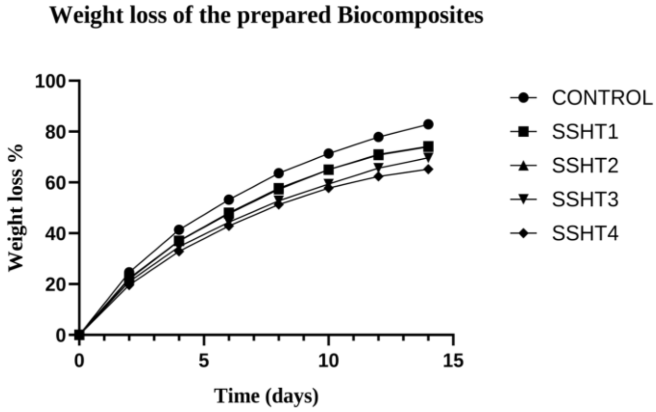


Fig.3. Graph showing % degradation of the bio-composites over 14 days soil burial biodegradability test. Weight loss recorded at 2-day interval each.

3.9 Scanning electron microscope

SEM analysis revealed the internal morphology and constituent distribution influencing the mechanical and barrier properties (Iffath et al., 2025). Micrographs revealed that the control film exhibited smooth, uniform and homogenous matrix, without noticeable defects, indicating good film- forming ability. However, some agglomerated starch granules were also visualised. With incorporation of SSH hull powder, a reduction in the smoothness was observed. The SSHT2 film showed slightly rough surface with uniform distribution of the SSH particles within the matrix indicating good interfacial bonding. This enhanced interaction may be attributed to presence of hydrogen bonding between the starch matrix and the functional group of the filler (Ramesh et al., 2024). No visual defects and cracks were observed at this concentration. However, SSHT3 exhibited highly discontinuous and irregular surface with visible agglomeration, micro voids and cracks, suggesting weak interfacial adhesion due to increased filler loading. structural deformities with cracks and micro voids suggesting poor film forming ability. These findings indicate that reinforcement material improves structural integrity and film forming property only up to a certain concentration after which structural deformities start to dominate. The results are consistent with the studies of Srivastava et al., 2023 who reported that the surface smoothness and uniformity declined after addition of fibre to the starch matrix. Similarly, Medina-Jaramillo et al., 2025 observed uniform and homogenous surface films at moderate concentration which declined due to presence of agglomeration at higher concentration. These SEM observations are qualitative; no quantitative image analysis (e.g., pore/void area fraction) was performed, and the interfacial bonding described above is an interpretation of surface appearance rather than a directly measured mechanical or chemical property.

3.10 ATR-FTIR analysis

The FTIR spectra of the control and SSHT2 films exhibited characteristic absorption bands of starch-based films. In the control film, the broad absorption around 3200-3400 $cm^{-1}$was attributed to O-H stretching vibrations of hydroxyl groups present in starch, glycerol, and water which is in agreement with the findings of Wardak et al., 2021. Peaks observed near 2920-2935 cm^{-1} corresponded to C-H stretching vibration, while the bands between 1150 and 950 cm^{-1} were associated with C-O and C-O-C stretching vibrations characteristic of starch molecules, as also reported by Nanda and Singh, 2022. The SSHT2 film showed similar absorption bands, indicating that starch remained the primary matrix component. However, slight broadening of the peak around 3300-3375 cm^{-1} was observed, which may be associated with the presence of additional groups corresponding to lignocellulosic components of sunflower seed hull powder. Similar hydroxyl stretching behaviour in sunflower seed shell materials was reported by Hayoun et al., 2021. Slight intensity changes in the fingerprint region further suggested interactions between starch and cellulose- rich sunflower seed hull powder. The absence of major new peaks indicated that incorporation of sunflower seed hull powder did not significantly alter the chemical structure of the starch matrix, and the interaction mainly occurred due to hydrogen bonding and van der Waal's interactions, which probably occurs between filler components and the polymer matrix (Islam et al., 2026). These FTIR interpretations are based on single spectra per formulation rather than replicated spectral acquisitions, and the peak shifts described should be regarded as suggestive of interaction rather than definitive proof of new hydrogen bonding; complementary structural or thermal evidence (e.g., XRD, DSC) was not obtained in this study.

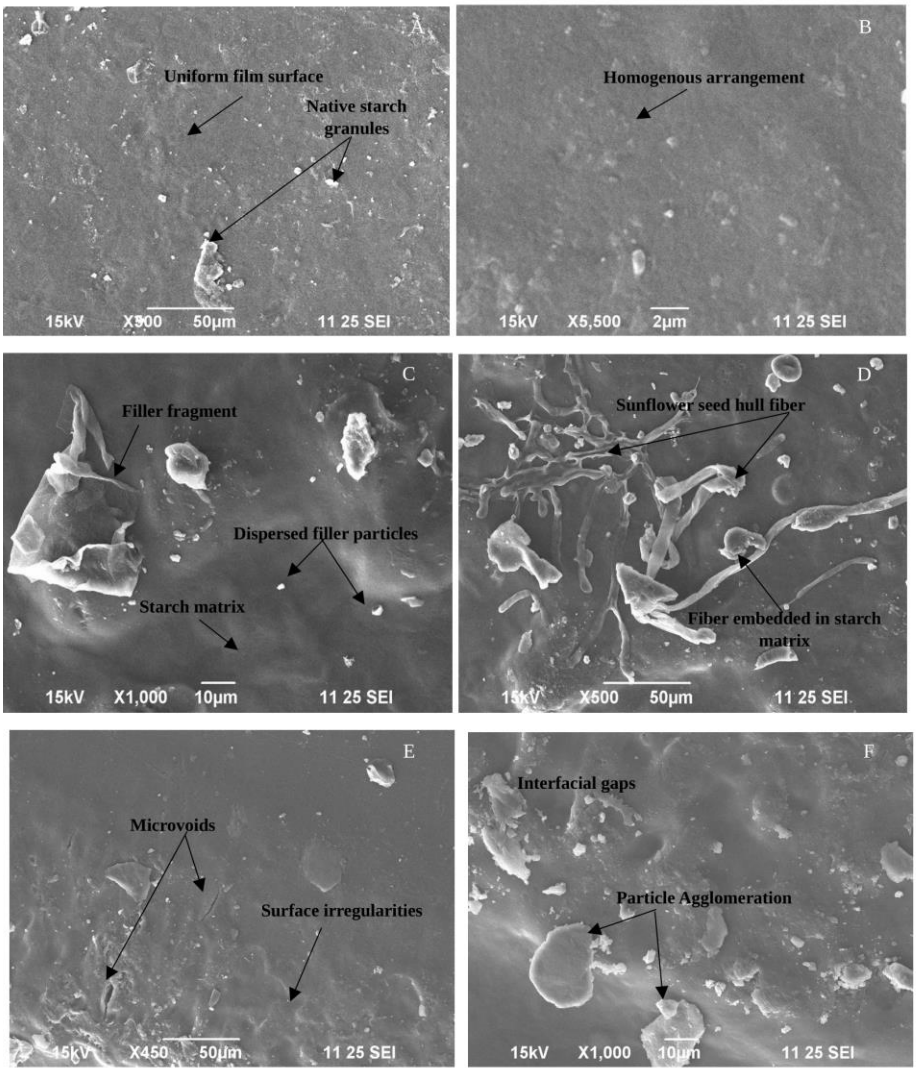


Fig.4. SEM micrographs illustrating surface morphology of developed bio-composite films:(A-B) Control film showing homogenous arrangement and smooth film surface, (C-D) SSHT2 film showing dispersed filler particles and fibre embedded within the starch matrix, (E-F) SSHT3 film showing particle agglomeration, micro voids and surface irregularities.

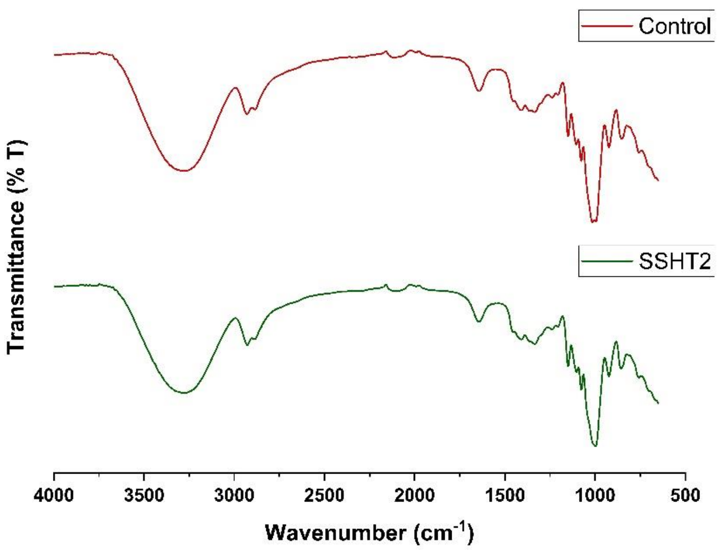


Fig. 5. FTIR spectra of control film and SSHT2 bio-composite film

3.11 Antimicrobial Activity

Antimicrobial properties play a highly significant role in food packaging, especially active packaging applications (Merino et al., 2023). In the present study antibacterial activity of the developed bio-composites were analysed through disc diffusion assay against *Staphylococcus aureus* (Gram-Positive) and *Escherichia coli* (Gram Negative). Thyme essential oil (0.25ml) was incorporated as the active antimicrobial agent, and its effectiveness was evaluated at varying filler concentration. The observed zone of inhibition patterns are shown in **Figure 6.**

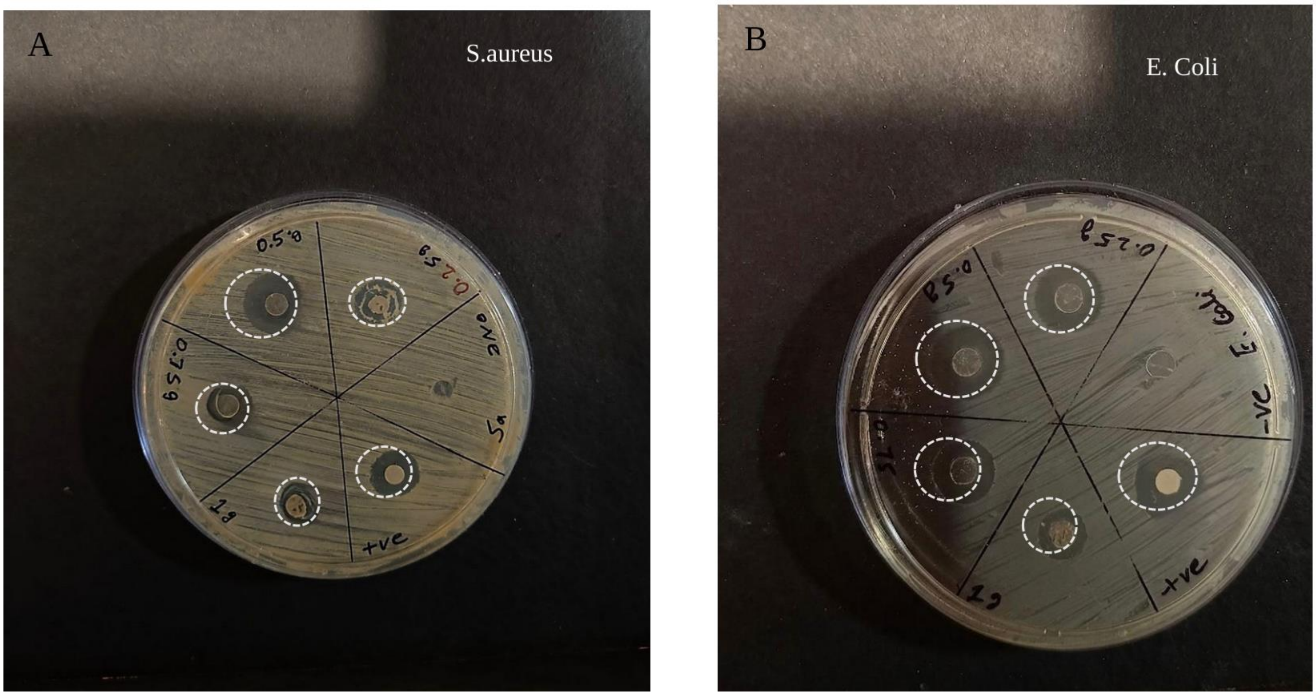


Fig. 6. Zone of inhibition formed by prepared bio-composites using Agar disc- diffusion method (A) *S. aureus* (B) *E. coli*

A distinct, non-linear inhibition pattern was obtained which can be attributed to the concentration gradient created by filler loadings at different concentration. The diameter of inhibition zone increased from 0.25g to reach a maximum at 0.5g, followed by gradual decrease at higher concentration of 0.75g and 1g. The inhibition zone is listed in **Table 5**. A comparable trend was reported by Castro et al., 2024 where incorporation of bentonite clay and essential oil filler content caused enhancement in the antimicrobial activity up to an optimal filler loading. Beyond this threshold, excessive filler concentration caused a decline in the zone of inhibition. This reduction was explained by the aggregation and overlapping of filler particles which hinders the diffusion of the active compounds from the essential oil, thereby limiting its interaction with the bacterial cell wall. Consequently, higher filler concentration caused a decrease in the antimicrobial activity. The use of thyme essential oil in this study is supported by Vasile et al., 2017 who reported its potent antimicrobial efficacy, with notably greater inhibition zone against S. aureus ($64.7\pm1.2mm$) compared to E. coli ($35.5\pm4.5mm$), indicating its enhanced activity towards Gram-Positive Bacteria. In addition, the present results are reinforced by the work of Syafiq et al., 2021 showing that essential- oil based films are more effective against Gram-Positive strains compared to Gram Negative strains. This difference arises due to structural variation in cell wall where the Gram-Negative bacteria contain outer thick lipopolysaccharide layer, thereby limiting the penetration of active compounds from the antimicrobial agents. Similar observation reported by Nanda and Singh, 2022 and Tongnuanchan and Benjakul, 2014 further confirm less susceptibility of Gram- Negative strains.

Overall, the study demonstrated that SSHT2 film exhibited the maximum antibacterial activity against both the strains of bacteria. In the present study, SSHT2 film showed highest antibacterial activity against both the strains of bacteria. The experimental results are presented graphically in **Figure 7**, where A shows antibacterial activity against *S. aureus* and B shows antibacterial activity against *E. coli.*

3.12 Antioxidant Analysis

The antioxidant potential of the developed film was determined using DPPH assay. The results revealed that the film incorporated with sunflower hulls exhibited a higher antioxidant activity (41.19 mg/mL AAE) compared to control film sample (28.15 mg/mL AAE), indicating a clear improvement in radical scavenging efficiency due to addition of sunflower seed hulls. The regression analysis (y= -0.0305x + 2.1007; R^2= 0.9623) demonstrates a strong inverse relationship between concentration and absorbance, confirming that increasing the concentration leads to decrease in absorbance, thereby indicating effective free radical scavenging activity. This enhancement is primarily associated with the rich phenolic composition of the sunflower seed hulls. As reported by Menzel et al., 2019 these hulls contain significant amount of phenolic compounds ranging from 40-86 mg per 100 g dry weight with chlorogenic acid identified as the major constituent. Moreover, films prepared using sunflower hull extracts have shown improved antioxidant performance owing to these phenolic substances (Menzel, 2020). For instance, Wu et al., 2021 reported that cellulose- based films showed enhanced antioxidant effect upon addition of oregano essential oil through pickering emulsion due to presence of phenolic compounds. Similarly, the incorporation of methylene blue (MB) and Vitamin C into polymer matrices resulted in a marked increase in the antioxidant behaviour (Sadeghi et al., 2025). Overall, the results confirm that incorporation of sunflower seed hulls effectively improve the antioxidant functionality of the starch-based films, making them suitable for active packaging applications. Across the measured endpoints, SSHT2 (0.5 g SSH) was selected as the formulation for further evaluation on the basis of a simple multi-criteria comparison: it produced the highest tensile strength (15.94 MPa) and elongation at break (42.67%) among all formulations, the largest antibacterial inhibition zones against both S. aureus and E. coli, and the highest antioxidant activity (41.19 mg/mL AAE), while higher SSH loadings (SSHT3, SSHT4) showed declining mechanical performance despite further reductions in water absorption and density. SSHT2 was therefore judged the best overall balance of mechanical, antibacterial, and antioxidant performance among the tested formulations; this comparison was made post hoc on the measured means rather than via a prespecified weighted scoring rule, and is noted as such.

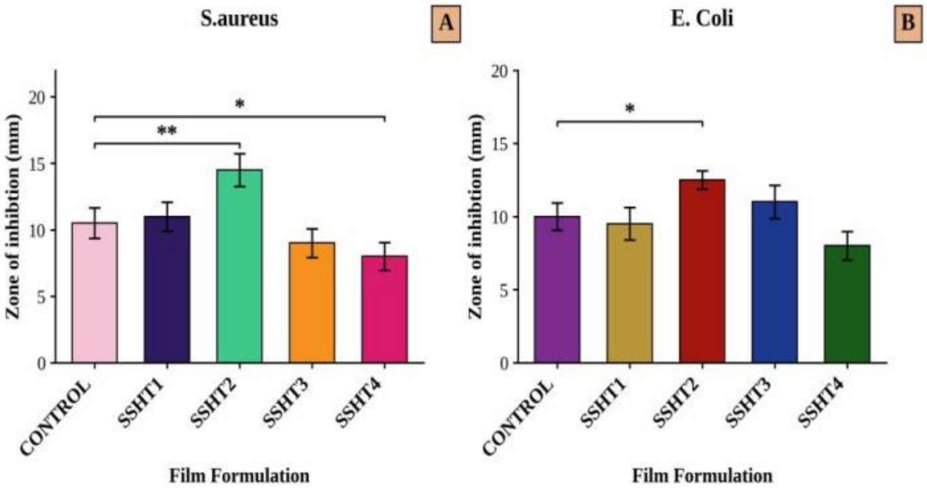


Fig.7. Antibacterial activity of different film formulations against S. aureus (A) and E. coli (B) measured by zone of inhibition (mm). Data are presented as mean± standard deviation (n=3). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. Statistically significant differences are denoted by (*) where $p < 0.05$ and (**) where $p < 0.01$ (Nanda & Singh, 2022).

Table 5. Zone of inhibition of prepared films at different filler loadings

Film Formulation (g)	Thyme Essential Oil (ml)	*S. aureus* (mm)	*E. coli* (mm)
SSHT1 (0.25g)	0.25ml	11 ± 1.09	9.5 ±1.11
SSHT2 (0.5g)	0.25ml	14.5 ±1.23	12.5 ± 0.62
SSHT3 (0.75g)	0.25ml	9 ± 1.07	11.0 ± 1.13
SSHT4(1g)	0.25ml	8 ± 1.04	8.0 ± 0.98
Positive control (Vancomycin for *S. aureus*; Ampicillin for *E. coli*)	-	10.5 ± 1.14	10.0 ± 0.94
Negative control (plain film without thyme oil)	-	0	0

3.13 Visual shelf-life evaluation of bread

Table 6 summarizes the visual quality changes observed in bread samples across the storage period. Among all the tested samples, unwrapped bread deteriorated most quickly, showing progressive surface dehydration and increased firmness, and visible mold development as storage time extended. By the 20th day, these samples had spoiled to a degree that rendered it unfit for consumption. Bread enclosed in the control film showed moderately better preservation outcomes. The film helped limit moisture evaporation and slowed mold development to a certain degree, though significant quality decline was still evident over extended storage. Bread packaged using SSHT2 film, however, retained a noticeable fresher look and better structural quality throughout the observation period. Fungal colonisation was visibly postponed, and the bread remained within acceptable quality standards for a longer period compared to both other groups. This trial was a single, non-replicated, visually assessed run without controlled humidity/temperature, standardized inoculation, blinding, or quantitative microbial counts, and should be treated as an illustrative demonstration rather than evidence of shelf-life extension. With that caveat, the apparently enhanced preservation performance may be tentatively associated with the antioxidant and antimicrobial actions of thyme essential oil and hull phenolics within the SSHT2 film matrix, though this cannot be statistically confirmed from the present data. Prior research has documented that thyme essential oil exhibits notable antifungal activity against a range of food spoilage fungi and molds, among them *Rhizopus* species (Mota et al.,2012) and both *Aspergillus*, and *Penicillium* species (Hernández-Figueroa et al., 2025). These findings highlight the promising role of this developed film as an eco-friendly active packaging solution.

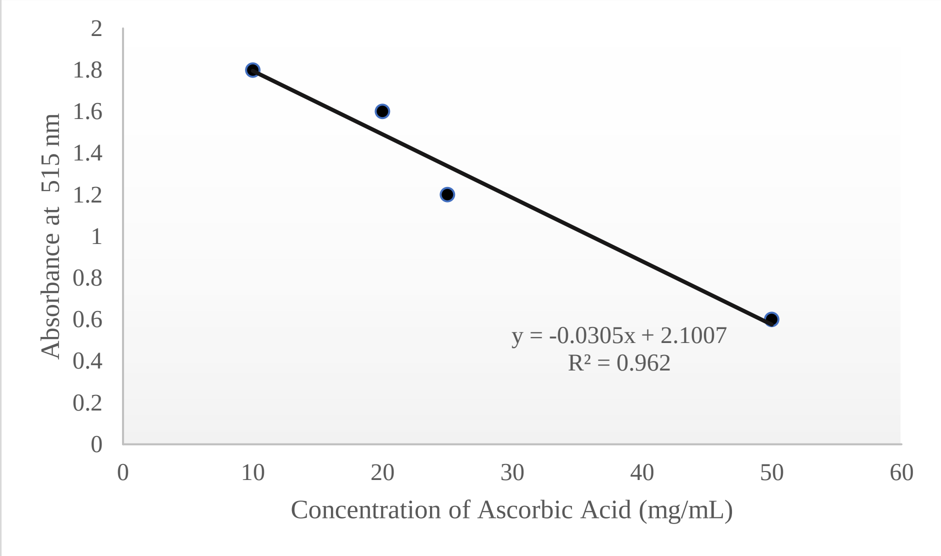
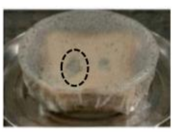


Fig. 8. Standard calibration plot of ascorbic acid showing the decrease in absorbance at 515 nm with increasing concentration (mg/mL) in the DPPH assay. The linear regression demonstrates strong correlation ($R^2 = 0.962$).

Table 6. Visual assessment of bread quality and spoilage under different packaging conditions during storage.

Storage Period	Unwrapped Bread	Bread wrapped with Control film	Bread wrapped with SSHT2 film
Day 0	Fresh look, soft and smooth texture, no signs of spoilage	Fresh look with smooth, soft surface and no spoilage signs	Fresh appearance, soft texture, no visible spoilage
Day 5	Surface drying and increased firmness noted	Texture softness and overall appearance maintained	Fresh appearance and soft texture preserved
Day 10	Considerable surface drying and early fungal development observed.	Minor surface drying and stiffening with no notable fungal presence	Fresh appearance with no visual fungal development
Day 15	Dark and firm texture with clearly visible fungal colonies	Moderate moisture loss, early spoilage signs, and mild texture degradation	Slight surface moisture loss with overall acceptable appearance
Day 20	Total spoilage resulting from extensive fungal growth	Visible spoilage with established fungal colonies	Spoilage onset delayed with initial appearance of tiny fungal colonies with comparatively superior appearance and texture preservation

Table 7. Photographic representation of spoilage progression in unwrapped bread, control- film-wrapped bread, and SSHT2 film-wrapped bread samples monitored at Day 0,5,10,15, and 20 during ambient storage

Sample	Day 0	Day 5	Day 10	Day 15	Day 20
Unwrapped Bread slice					
Bread Wrapped in Control film					
Bread wrapped in SSHT2 film					

4. STUDY LIMITATIONS

Although the developed sunflower seed hull reinforced bio-composite films demonstrated promising results, certain limitations must be acknowledged. Detailed raw material characterization and X-ray diffraction (XRD) analysis could not be performed due to time constraints and difficulties in converting the developed film materials into a suitable powdered form required for XRD analysis. Thermal stability and heat seal ability of the films were also not evaluated, as these analyses require specialized equipment and additional time resources that were unavailable within the scope of study. Biodegradability assessment was limited to a single experimental run due to the time-intensive nature of the soil-burial test; replication of this parameter in future studies is recommended for statistical validation. Additionally, the films were fabricated using laboratory-scale solution casting technique, which may limit direct industrial scalability. Furthermore, the food packaging evaluation was restricted to bread as a sole food model, and only short- term shelf-life assessment was conducted; long- term studies across diverse food systems would better validate the film's preservation efficacy. Antifungal activity of the films was not assessed, which remains an important aspect for comprehensive food packaging applications. Additionally, each formulation in this study was represented by a single cast film, with reported n=3 values corresponding to repeated measurements at different locations on that film rather than independently prepared and cast replicate films; the statistical comparisons reported here should therefore be regarded as descriptive rather than fully confirmatory, and future work should employ independently cast batches per formulation. The experimental design also did not include a thyme-oil-only control (without SSH), so the individual contributions of hull reinforcement and thyme essential oil to the observed antioxidant and antibacterial improvements could not be statistically separated in this study; a factorial SSH × thyme design is recommended for future work.

5. CONCLUSION

The incorporation of sunflower seed hull powder into corn-starch bio-composite films, combined with thyme essential oil, yields multifunctional active packaging materials with enhanced mechanical, antioxidant, and antibacterial properties. The SSHT2 formulation emerged as the best-performing composition among those tested, showing favourable trends across the evaluated parameters, including a preliminary, single-run indication of delayed bread spoilage in the exploratory visual shelf-life trial. These findings indicate that SSH-based bio-composite films hold potential as sustainable and bioactive alternatives to conventional food packaging; confirming this potential will require independently replicated casting batches, a thyme-only control to isolate the contribution of hull reinforcement, and additional performance data (e.g., water-vapor/oxygen permeability, migration and food-contact safety testing) beyond the laboratory-prototype stage represented here. Future investigation should explore antifungal evaluation, thermal characterization, long-term shelf-life studies across varied food products, and scale-up feasibility to advance the commercial applicability of these films.

CONFLICT OF INTEREST

The authors declared no potential conflict of interest with respect to the authorship and publication.

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ETHICS APPROVAL

Not applicable. No ethical approvals were required for this study as it did not involve human or animal subjects.

CONSENT TO PARTICIPATE

Not applicable. This research did not involve human participants.

PERMISSION TO REPRODUCE MATERIAL

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DATA AND MATERIAL AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

DECLARATION OF GENERATIVE AI IN SCIENTIFIC WRITING

This is to certify that the research presented in this manuscript has been conducted solely by the authors without the assistance of generative AI tools in any stage of scientific writing, including the conception, drafting, revising, and editing of the manuscript. All analyses, interpretations, and conclusions are the original work of the authors, derived from the referenced studies and methodologies employed. No AI tools were used to generate text, images, or data interpretations for this submission.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Ishita Ghosh: Methodology, Writing – original draft and software, Alka Nanda: Writing – review, editing and software and Neetu Singh: Supervision and Validation.

REFERENCES

Aldawsari, H.M., Kotta, S., Asfour, H.Z., Vattamkandathil, S., Elfaky, M.A., Ashri, L.Y. and Badr-Eldin, S.M., 2023. Developmen t and evaluation of quercetin enriched bentonite-reinforced starch-gelatin based bioplastic with antimicrobial property. *Saudi Pharmaceutical Journal*, *31*(12), p.101861. https://doi.org/10.1016/j.jsps.2023.101861

Amaraweera, S.M., Gunathilake, C., Gunawardene, O.H., Fernando, N.M., Wanninayaka, D.B., Dassanayake, R.S., Rajapaksha, S.M., Manamperi, A., Fernando, C.A., Kulatunga, A.K. and Manipura, A., 2021. Development of starch-based materials using current modification techniques and their applications: A review. *Molecules*, *26*(22), p.6880. https://doi.org/10.3390/molecules26226880

Anand, S., Rajinikanth, P.S., Arya, D.K., Pandey, P., Gupta, R.K., Sankhwar, R. and Chidambaram, K., 2022. Multifunctional biomimetic nanofibrous scaffold loaded with asiaticoside for rapid diabetic wound healing. *Pharmaceutics*, *14*(2), p.273. https://doi.org/10.3390/pharmaceutics14020273

Aydin, G. and Zorlu, E.B., 2022. Characterisation and antibacterial properties of novel biodegradable films based on alginate and roselle (Hibiscus sabdariffa L.) extract. *Waste and biomass valorization*, *13*(6), pp.2991-3002.

Birania, S., Kumar, S., Kumar, N., Attkan, A.K., Panghal, A., Rohilla, P. and Kumar, R., 2022. Advances in development of biodegradable food packaging material from agricultural and agro-industry waste. *Journal of Food Process Engineering*, *45*(1), p.e13930. https://doi.org/10.1111/jfpe.13930

Biswas, A., Ahmed, T., Rana, M.R., Hoque, M.M., Ahmed, M.F., Sharma, M., Sridhar, K., Ara, R. and Stephen Inbaraj, B., 2023. Fabrication and characterization of ZnO nanoparticles-based bio-composite films prepared using carboxymethyl cellulose, taro mucilage, and black cumin seed oil for evaluation of antioxidant and antimicrobial activities. *Agronomy*, *13*(1), p.147. https://doi.org/10.3390/agronomy13010147

Castro, D., Podshivalov, A., Ponomareva, A. and Zhilenkov, A., 2024. Study of the reinforcing effect and antibacterial activity of edible films based on a mixture of chitosan/cassava starch filled with bentonite particles with intercalated ginger essential oil. *Polymers*, *16*(17), p.2531. https://doi.org/10.3390/polym16172531

Chakraborty, I., N, P., Mal, S.S., Paul, U.C., Rahman, M.H. and Mazumder, N., 2022. An insight into the gelatinization properties influencing the modified starches used in food industry: A review. *Food and Bioprocess Technology*, *15*(6), pp.1195-1223.

Contessa, C.R., de Souza, N.B., Gonçalo, G.B., de Moura, C.M., da Rosa, G.S. and Moraes, C.C., 2021. Development of active packaging based on agar-agar incorporated with bacteriocin of Lactobacillus sakei. *Biomolecules*, *11*(12), p.1869. https://doi.org/10.3390/biom11121869

De'Nobili, M.D., Bernhardt, D.C., Basanta, M.F. and Rojas, A.M., 2021. Sunflower (Helianthus annuus L.) seed hull waste: Composition, antioxidant activity, and filler performance in pectin-based film composites. *Frontiers in Nutrition*, *8*, p.777214. https://doi.org/10.3389/fnut.2021.777214

Désiré, A.Y., Charlemagne, N., Claver, K.D., Achille, T.F. and Marianne, S., 2021. Starch-based edible films of improved cassava varieties Yavo and TMS reinforced with microcrystalline cellulose. *Heliyon*, *7*(4). https://doi.org/10.1016/j.heliyon.2021.e06612

Frolov, S.M., Silantiev, A.S., Sadykov, I.A., Smetanyuk, V.A., Frolov, F.S., Hasiak, Y.K., Dudareva, T.V., Bekeshev, V.G., Grishin, M.V., Golubev, E.K. and Baimukhambetova, D., 2023. Composition and textural characteristics of char powders produced by thermomechanical processing of sunflower seed husks. *Powders*, *2*(3), pp.624-638. https://doi.org/10.3390/powders2030039

Hayoun, B., Bourouina-Bacha, S. and Pazos, M., 2021. *Production of modified sunflowers seed shells for the removal of bisphenol A. RSC Adv 11: 3516–3533* https://doi.org/10.1039/D0RA09137E

Hazrati, K.Z., Sapuan, S.M., Zuhri, M.Y.M. and Jumaidin, R., 2021. Preparation and characterization of starch-based bio-composite films reinforced by Dioscorea hispida fibers. *Journal of Materials Research and Technology*, *15*, pp.1342-1355. https://doi.org/10.1016/j.jmrt.2021.09.003

Hazrol, M.D., Sapuan, S.M., Zainudin, E.S., Wahab, N.I.A. and Ilyas, R.A., 2022. Effect of kenaf fibre as reinforcing fillers in corn starch-based bio-composite film. *Polymers*, *14*(8), p.1590. https://doi.org/10.3390/polym14081590

Hazrol, M.D., Sapuan, S.M., Zainudin, E.S., Zuhri, M.Y.M. and Abdul Wahab, N.I., 2021. Corn starch (Zea mays) biopolymer plastic reaction in combination with sorbitol and glycerol. *Polymers*, *13*(2), p.242. https://doi.org/10.3390/polym13020242

Hernández-Figueroa, R.H., López-Malo, A., Ramírez-Corona, N. and Mani-López, E., 2025. Estimation of the Antifungal Threshold of Thyme Essential Oil for Bread Preservation, Ensuring Consumer Acceptance and Product Quality. *Foods*, *14*(20), p.3549. https://doi.org/10.3390/foods14203549

Homthawornchoo, W., Kaewprachu, P., Pinijsuwan, S., Romruen, O. and Rawdkuen, S., 2022. Enhancing the UV-light barrier, thermal stability, tensile strength, and antimicrobial properties of rice starch–gelatin composite films through the incorporation of zinc oxide nanoparticles. *Polymers*, *14*(12), p.2505. https://doi.org/10.3390/polym14122505

Iffath, R., Ara, R., Ahmed, T. and Biswas, A., 2025. Fabrication and characterization of waste eggshell microparticles reinforced biodegradable composite packaging films enriched with pectin and orange peel essential oil. *Applied Food Research*, *5*(1), p.100735. https://doi.org/10.1016/j.afres.2025.100735

Islam, M., Sinha, A.S.K. and Prasad, K., 2026. PLA/starch bi-layer films reinforced with rice straw cellulose nanofibers and functionalized with organosolv–lignin nanoparticles and grapefruit bioactives for shelf life extension of green grapes. *Sustainable Food Technology*, 4, pp.752-771. https://doi.org/10.1039/D5FB00561B

Jiang, B., Yu, J. and Liu, Y., 2020. The environmental impact of plastic waste. *Journal of Environmental & Earth Sciences*, *2*(2).

Ju, J., Chen, X., Xie, Y., Yu, H., Guo, Y., Cheng, Y., Qian, H. and Yao, W., 2019. Application of essential oil as a sustained release preparation in food packaging. *Trends in Food Science & Technology*, *92*, pp.22-32. https://doi.org/10.1016/j.tifs.2019.08.005

Kan, M. and Miller, S.A., 2022. Environmental impacts of plastic packaging of food products. *Resources, Conservation and Recycling*, *180*, p.106156. https://doi.org/10.1016/j.resconrec.2022.106156

Mahardika, M., Asrofi, M., Amelia, D., Syafri, E., Mavinkere Rangappa, S. and Siengchin, S., 2021, September. Tensile strength and moisture resistance properties of bio-composite films based on polyvinyl alcohol (PVA) with cellulose as reinforcement from durian peel fibers. In *E3S Web of Conferences* (Vol. 302, p. 02001). EDP Sciences. https://doi.org/10.1051/e3sconf/202130202001

Malekzadeh, E., Tatari, A. and Dehghani Firouzabadi, M., 2024. Effects of biodegradation of starch-nanocellulose films incorporated with black tea extract on soil quality. *Scientific Reports*, *14*(1), p.18817.

Medina-Jaramillo, C., Coelho-Leandro, G., Ayala-Valencia, G. and López-Córdoba, A., 2025. Starch-based bio-composites reinforced with cellulose nanofibers from potato peel byproducts. *Scientific Reports*, *15*(1), p.38412. https://doi.org/10.1038/s41598-025-22303-9

Menzel, C., 2020. Improvement of starch films for food packaging through a three-principle approach: Antioxidants, cross-linking and reinforcement. *Carbohydrate Polymers*, *250*, p.116828. https://doi.org/10.1016/j.carbpol.2020.116828

Menzel, C., González-Martínez, C., Chiralt, A. and Vilaplana, F., 2019. Antioxidant starch films containing sunflower hull extracts. *Carbohydrate Polymers*, *214*, pp.142-151. https://doi.org/10.1016/j.carbpol.2019.03.039

Merino, D., Bellassi, P., Paul, U.C., Morelli, L. and Athanassiou, A., 2023. Assessment of chitosan/pectin-rich vegetable waste composites for the active packaging of dry foods. *Food Hydrocolloids*, *139*, p.108580. https://doi.org/10.1016/j.foodhyd.2023.108580

Mota, K.S.D.L., Pereira, F.D.O., De Oliveira, W.A., Lima, I.O. and Lima, E.D.O., 2012. Antifungal activity of Thymus vulgaris L. essential oil and its constituent phytochemicals against Rhizopus oryzae: interaction with ergosterol. *Molecules*, *17*(12), pp.14418-14433. https://doi.org/10.3390/molecules171214418

Nanda, A. and Singh, N., 2022. Preparation and antimicrobial activity of corn cob and coir reinforced biodegradable starch bio-composite films for food packaging application. *Asian Food Science Journal*, *21*(7), pp.53-66.

Nanda, A., Pandey, P., Rajinikanth, P.S. and Singh, N., 2024. Revolution of nanotechnology in food packaging: Harnessing electrospun zein nanofibers for improved preservation-A review. *International journal of biological macromolecules*, *260*, p.129416. https://doi.org/10.1016/j.ijbiomac.2024.129416

Ncube, L.K., Ude, A.U., Ogunmuyiwa, E.N., Zulkifli, R. and Beas, I.N., 2021. An overview of plastic waste generation and management in food packaging industries. *Recycling*, *6*(1), p.12. https://doi.org/10.3390/recycling6010012

Neupane, S., Poudel, S., Ghimire, A. and Shakya, S., 2025. Enhanced mechanical performance and biodegradability in filler-reinforced potato biopolymers for sustainable packaging. *Materials Today Communications*, p.114081. https://doi.org/10.1016/j.mtcomm.2025.114081

Oluwasina, O.O., Akinyele, B.P., Olusegun, S.J., Oluwasina, O.O. and Mohallem, N.D., 2021. Evaluation of the effects of additives on the properties of starch-based bioplastic film. *SN Applied Sciences*, *3*(4), p.421.

Othman, S.H., Ronzi, N.D.A., Shapi'i, R.A., Dun, M., Ariffin, S.H. and Mohammed, M.A.P., 2023. Biodegradability of starch nanocomposite films containing different concentrations of chitosan nanoparticles in compost and planting soils. *Coatings*, *13*(4), p.777. https://doi.org/10.3390/coatings13040777

Pongputthipat, W., Ruksakulpiwat, Y. and Chumsamrong, P., 2023. Development of biodegradable bio-composite films from poly (lactic acid), natural rubber and rice straw. *Polymer bulletin*, *80*(9), pp.10289-10307.

Pradiza, R.R., Junus, S., Asrofi, M. and Ilyas, R.A., 2025. Moisture characteristics of bio-composites from PVA/cassava starch reinforced by lemon peel fiber. *Journal of Fibers and Polymer Composites*, *4*(1), pp.1-10. https://doi.org/10.55043/jfpc.v4i1.218

Priya, N.V., Vinitha, U.G. and Sundaram, M.M., 2021. Preparation of chitosan-based antimicrobial active food packaging film incorporated with Plectranthus amboinicus essential oil. *Biocatalysis and Agricultural Biotechnology*, *34*, p.102021. https://doi.org/10.1016/j.bcab.2021.102021

Quispetera, C.A.O., Olivera, C.A.C., Flores, J.W.V., Alfaro, E.G.B. and Flores, Y.V., 2021. Bioplastic made from Manihot esculenta (Cassava) and Ficus benjamina as an ecological alternative for food products. *Chemical Engineering Transactions*, *87*, pp.67-72. https://doi.org/10.3303/CET2187012

Ramesh, T., Saravanakumar, S.S., Balavairavan, B. and Senthamaraikannan, P., 2024. Development and characterization of a polylactic acid/sesame husk powder–based bio-composite film for packaging application. *Waste and Biomass Valorization*, *15*(5), pp.2845-2856.

Ribeiro-Santos, R., Andrade, M., de Melo, N.R. and Sanches-Silva, A., 2017. Use of essential oils in active food packaging: Recent advances and future trends. *Trends in food science & technology*, *61*, pp.132-140. https://doi.org/10.1016/j.tifs.2016.11.021

Roslan, R. and Tukiran, N.A., 2022. Review on the characterization of starch bio-composite films in packaging application. *Food Research (Malaysia)*, *6*(4), pp.1-15.

Roy, K., Thory, R., Sinhmar, A., Pathera, A.K. and Nain, V., 2020. Development and characterization of nano starch-based composite films from mung bean (Vigna radiata). *International journal of biological macromolecules*, *144*, pp.242-251. https://doi.org/10.1016/j.ijbiomac.2019.12.113

Sadeghi, S., Pirsa, S., Asefi, N. and Gharekhani, M., 2025. Preparation and characterization of biodegradable cellulose nanofiber films modified with methylene blue and vitamin C for detecting oxidants. *Scientific Reports*, *15*(1), p.23041. https://doi.org/10.1038/s41598-025-07566-6

Sharma, S., Barkauskaite, S., Duffy, B., Jaiswal, A.K. and Jaiswal, S., 2020. Characterization and antimicrobial activity of biodegradable active packaging enriched with clove and thyme essential oil for food packaging application. *Foods*, *9*(8), p.1117. https://doi.org/10.3390/foods9081117

Sharma, S., Barkauskaite, S., Jaiswal, A.K. and Jaiswal, S., 2021. Essential oils as additives in active food packaging. *Food chemistry*, *343*, p.128403. https://doi.org/10.1016/j.foodchem.2020.128403

Singh, N., Nanda, A., Singh, A. and Singh, T., 2026. Cutting-Edge Antimicrobials in Food Packaging Applications. In *Biotechnological Solutions for a Sustainable Future: From Soil Health to Industrial Applications* (pp. 225-253). Singapore: Springer Nature Singapore.

Singh, P., Singh, N., Daniel, M. and Nanda, A., 2025. Functional Edible Films Enriched with Antioxidants: Innovations in Food Packaging and Safety. *Journal of Advances in Food Science & Technology*, *12*(3), pp.15-31.

Srivastava, V., Singh, S. and Das, D., 2023. Rice husk fiber-reinforced starch antimicrobial bio-composite film for active food packaging. *Journal of Cleaner Production*, *421*, p.138525. https://doi.org/10.1016/j.jclepro.2023.138525

Suklaw, N. and Ratanakamnuan, U., 2022, January. Mechanical properties and biodegradability of starch-based bio-composite films reinforced with microcrystalline cellulose from rice embryo. In *Journal of Physics: Conference Series* (Vol. 2175, No. 1, p. 012034). IOP Publishing. https://doi.org/10.1088/1742-6596/2175/1/012034

Suwanprateep, S., Angsuseranee, N. and Piriyayon, S., 2026. Optimizing biodegradable starch composite films reinforced with oil palm cellulose: A structure–property–degradability approach for sustainable packaging. *Next Materials*, *11*, p.101602. https://doi.org/10.1016/j.nxmate.2026.101602

Syafiq, R., Sapuan, S.M. and Zuhri, M.R.M., 2021. Antimicrobial activity, physical, mechanical and barrier properties of sugar palm based nanocellulose/starch bio-composite films incorporated with cinnamon essential oil. *Journal of Materials Research and Technology*, *11*, pp.144-157. https://doi.org/10.1016/j.jmrt.2020.12.091

Tongnuanchan, P. and Benjakul, S., 2014. Essential oils: extraction, bioactivities, and their uses for food preservation. *Journal of food science*, *79*(7), pp.R1231-R1249. https://doi.org/10.1111/1750-3841.12492

Tsvetanova, F., Naydenova, G. and Boyadzhieva, S., 2025. Sunflower seed hulls and meal—A waste with diverse biotechnological benefits. *Biomass*, *5*(3), p.47. https://doi.org/10.3390/biomass5030047

Vasile, C., Sivertsvik, M., Miteluţ, A.C., Brebu, M.A., Stoleru, E., Rosnes, J.T., Tănase, E.E., Khan, W., Pamfil, D., Cornea, C.P. and Irimia, A., 2017. Comparative analysis of the composition and active property evaluation of certain essential oils to assess their potential applications in active food packaging. *Materials*, *10*(1), p.45. https://doi.org/10.3390/ma10010045

Wardak, M.H., Kingwascharapong, P., Aryan, S., Tanaka, F. and Tanaka, F., 2021. Preparation and characterization of corn starch-based film: Effect of citric acid or sunflower oil and its combination. *Journal of Food Measurement and Characterization*, *15*(2), pp.1907-1915.

Wildan, M.W. and Lubis, F.I., 2021. Fabrication and characterization of chitosan/cellulose nanocrystal/glycerol bio-composite films. *Polymers*, *13*(7), p.1096. https://doi.org/10.3390/polym13071096

Wu, M., Zhou, Z., Yang, J., Zhang, M., Cai, F. and Lu, P., 2021. ZnO nanoparticles stabilized oregano essential oil Pickering emulsion for functional cellulose nanofibrils packaging films with antimicrobial and antioxidant activity. *International Journal of Biological Macromolecules*, *190*, pp.433-440. https://doi.org/10.1016/j.ijbiomac.2021.08.210

Yadav, A., Mangaraj, S., Singh, R.M.N.K., Kumar, N. and Arora, S., 2018. Biopolymers as packaging material in food and allied industry. *Int. J. Chem. Stud*, *6*(2), pp.2411-2418.

Zhang, W., Roy, S., Assadpour, E., Cong, X. and Jafari, S.M., 2023. Cross-linked biopolymeric films by citric acid for food packaging and preservation. *Advances in Colloid and Interface Science*, *314*, p.102886. https://doi.org/10.1016/j.cis.2023.102886