Chapter 5

To study the combined film properties of fiber-reinforced potato starch-casein-banana pseudostem and potato starch-casein-bamboo-shoot blended films

5.1. Introduction

The exploration of combined films made from starch, casein, and natural fibers like banana pseudostem along with bamboo shoots represents a significant advancement in biodegradable materials. Utilizing natural plant polymers and fibers as bioplastic reinforcements has increased significantly in recent years. The isolation of fibers from natural sources and the preparation of fiber-reinforced composite films using starch/protein blends have emerged as key areas of research in sustainable packaging. These composite films leverage the unique characteristics of each component to improve mechanical strength, and thermal stability including barrier performance, making them appropriate for various usage, particularly in food packaging (Tafa et al., 2023; Ahmed et al., 2024). Starch serves as a fundamental matrix because of its superior film-forming capabilities and biodegradability (Rammak et al., 2021). Its interaction with casein, a milk-derived protein, enhances mechanical properties, flexibility, and moisture resistance (Fernandes-Guiterrez et al., 2004). Natural additives like banana-pseudostem and bamboo-shoots fibers provide additional benefits. BPF (Banana-pseudostem fiber) enhances structural integrity and water resistance (Taweechat et al., 2021), while BSFs (Bamboo-shoot fiber) reinforce the fibrous structure, increasing tensile strength while maintaining flexibility (Wang and Chen, 2017). Understanding the combined properties of these films is crucial for developing sustainable packaging solutions that meet environmental standards without compromising performance (Khan et al., 2021). The inclusion of cellulose microfibers (CMR) into starch-based films significantly enhances their properties, addressing limitations such as low mechanical strength and improved water vapor permeability (Awang Wahab et al., 2024). Omar et al. (2022) successfully developed biocomposite starch films using modified bamboo cellulose treated with phosphoric acid, demonstrating their potential for food packaging. Similarly, cellulose nanofibers from banana peels improved the mechanical properties of films, including tensile strength, Young's modulus, and water resistance (Pelissari, 2013).

This chapter investigates the mechanical, thermal, including barrier characteristics of starch-casein-banana and starch-casein-bamboo films to assess their viability for real-world applications. Since natural fibers contain cellulose, they are inherently hydrophilic (Dutta and Sit, 2024). However, their incompatibility with some polymeric matrices, high absorption, and poor wettability poses challenges (Rammak et al., 2021). To address these issues, physical treatments are being explored to reduce dependence on petroleum-based products (Taweechat et al., 2021). The primary objective of this chapter is to prepare fiber-reinforced composite films and investigate the combined properties of starch-casein-banana-psuedostem and starch-casein-bamboo shoot fiber composites. This chapter examines the effects of fiber type, fiber content, and processing conditions on film properties while exploring potential synergistic effects between components. By systematically isolating fibers from their natural sources and preparing the corresponding composite films, this research seeks to optimize the performance of these sustainable materials for packaging applications. Ultimately, this study adds to the growing body of knowledge on sustainable products and provides insights into developing novel, eco-friendly composite films. These findings may pave the way for high-performance, biodegradable materials that could replace conventional synthetic plastics, addressing environmental concerns and promoting sustainability.

5.2. Materials and methods

5.2.1. Materials

Potato starch (PS) was extracted following the protocol demonstrated in Section 3.2.2. The best modified PS (HMT), as obtained from Chapter 3, was used for film preparation. Casein was procured from Zenith India, Guwahati, Assam, India, and the best-modified casein (US 30), as obtained in previous chapters, was blended with the modified starch (HMT) for film preparation. Banana pseudostem fiber (BPF) was extracted using the method outlined in Section 4.2.3, while the extraction process for bamboo-shoot fiber (BSF) is detailed in Section 4.2.4.

5.2.2. Chemical and reagents

Analytical grade reagents and chemicals were utilized throughout the study and purchased from Zenith India, Guwahati, Assam.

5.2.3. Ultrasonic modification of fibers

The section has already been explained in Chapter 4 (section 4.2.5).

5.2.4. Enzymatic modification of fibers

The section has already been explained in Chapter 4 (section 4.2.6).

5.2.5. Preparation of PS-casein blended BPF-reinforced film

PS-casein reinforced with BPF and BSF composite film was produced using a solvent casting technique, as per the modified method of Edhirej et al. (2017). PS (5%) composition was put inside a 250 mL conical flask which was kept for stirring in a magnetic stirrer at 90℃ for 15 min at a constant 300 rpm speed to achieve complete gelatinization of the starch. Casein was stirred in a 5% weight aqueous sodium hydroxide solvent at 80℃ for 3 h inside round bottom flask. The determined weight percentage (5 % on a dry weight basis) of banana and BSFs were immersed in DW and stirred separately at a magnetic stirrer at 60℃ for half an hour. Glycerol at the concentration of 40 mL 100 g^{-1} (of dry starch to the solution) was added to avoid film brittleness. The suspension of BPF (treated and untreated) was added to the starch-glycerol water mixture and stirred for 30 min at 90°C using a mechanical stirrer set to 700 rpm. It was then poured onto leveled glass petri plates after the solution became viscous, and kept at 45℃ for 12 h until it gets completely dry. Next, the films were separated from the petri plates by peeling them off and kept in an incubator for conditioning at around 65% RH at 25℃ temperature. The films conditioned were put inside a desiccator (56% RH) for one week at 30℃. The potato starch films reinforced with BPFs were then analyzed for various film properties. The appearance of the composite film is presented in **Fig. 5.1.** and **5.2**. along with film composition shown in **Tables 5.1** and **5.2**.

a) **b)** **c)** **d)** **e)**

Fig. 5.1 Visual appearance of developed BPF-reinforced PS-casein blended film (BPF-PS-CS) (a. N- Native fiber, b. US- ultrasound treated fiber, c. ET- Enzymatic treated fiber, d. USET- ultrasound combined with enzyme treatment, e. ETUS- Enzyme treated with ultrasound treatment)

a) **b)** **c)** **d)** **e)**

Fig. 5.2 Visual appearance of developed BSF-PS-CS BPF-reinforced PS-casein blended film (a. N- Native fiber, b. US- ultrasound treated fiber, c. ET- Enzymatic treated fiber, d. USET- ultrasound combined with enzyme treatment, e. ETUS- Enzyme treated with ultrasound treatment

Table 5.1 Formulation of developed BPF-reinforced PS-casein (BPF-PS-CS) blended films

Sample	PS (% w/v)	Casein (% w/v)	BPF (% wt)	Glycerol (mL)
Native	5	5	5	4
US	5	5	5	4
ET	5	5	5	4
USET	5	5	5	4
ETUS	5	5	5	4

(N- Native fiber, US- ultrasound treated fiber, ET- Enzymatic treated fiber, USET- ultrasound combined with enzyme treatment, ETUS- Enzyme treated with ultrasound treatment)

Table 5.2 Formulation of developed BSF (BSF)-reinforced PS-casein (BSF-PS-CS) blended film

Sample name	PS **(% w/v)**	Casein (wt%)	Glycerol (mL)	BSF (% wt.)
Native	5	5	3	5
US	5	5	3	5
ET	5	5	3	5
USET	5	5	3	5
ETUS	5	5	3	5

(N- Native fiber, US- ultrasound treated fiber, ET- Enzymatic treated fiber, USET- ultrasound combined with enzyme treatment, ETUS- Enzyme treated with ultrasound treatment

5.2.6. Characteristics of developed film

5.2.6.1. Film thickness

The procedure for film thickness has been evaluated as described in the section 3.2.12.1.

5.2.6.2. Film solubility

The protocol for film solubility (%) has been evaluated as per the method explained in detail in the section 3.2.12.1.

5.2.6.3. Film color

The process for film color has been determined as per the method explained in section 3.2.12.3.

5.2.6.4.Optical properties

The procedure for determination of the optical properties of the film has been determined as explained in section 3.2.12.4.

5.2.6.5. Mechanical properties

Tensile strength (TS) and percent elongation at break (% EAB) determination for the developed film have been evaluated as per the method described in the section 3.2.12.5.

5.2.6.6.Water vapor permeability (WVP)

The barrier properties of the developed film are measured as water vapor permeability (WVP), described in section 3.2.12.2.

5.2.6.7.Sealing properties

The sealing strength of the developed film was evaluated as described in the section 3.2.12.6.

5.2.6.8.Thermo-gravimetric analysis

The thermal properties of the developed film have been determined by the process described in section 3.2.12.7.

5.2.6.9.Biodegradability

The procedure for the examination of biodegradability of developed film have been done by the method described in section 3.2.12.8.

5.2.6.10. Statistical Analysis

SPSS statistical software (version 26, SAS Institute Inc., Cary, NC, USA) was employed to obtain the experimental values indicated as mean ± standard deviation. One-way (ANOVA) variance along with Duncan's multiple range test (DMRT) with a probability ($p<0.05$) were considered for measuring statistical differences.

5.3. Results and discussions

5.3.1. Film thickness

Tables 5.3 and **5.4** present the thickness values of fiber-reinforced starch-casein-based blended film. The thickness of developed films plays a significant role in determining their mechanical properties, barrier qualities, and general effectiveness as packing materials. Uniform thickness with no significant differences between the developed film was observed due to the same proportions of components used in the preparation of the film. The thickness of the developed film varied from 0.35 to 0.37 mm (BPF-reinforced film) and 0.30 to 0.33 mm (BSF-reinforced film). Tan et al. (2017) observed nearly uniform thicknesses of approximately 0.35 mm in a blend comprising waste ramie and cotton fibers along with a starch polymer. Compared with pure starch and casein films measuring 0.27 mm in thickness (outlined in Chapter 3), films incorporating native and modified fibers (Banana pseudostem and bamboo shoot) exhibited increased thickness, with variations dependent on adding fibers.

5.3.2. Opacity

The opacity results of the BPF and BSF-reinforced PS-casein blended film (BPF-PS-CS and BSF-PS-CS) gives details on the size of added matter scattered throughout the matrix of starch (Dutta and Sit, 2023) shown in **Tables 5.3** and **5.4**. If the scattered element within the starch possesses a bigger particle size, it fills the starch matrix's pores, leading to a decreased light route, which increases the opacity of the biofilm (Oluwasina et al., 2019). The opacity of the packaging film affects the consumer acceptability of food materials as it is an important criterion that protects light-sensitive food items from UV and light radiation (Kumar et al., 2022). The sequence of opacity (mm^{-1}) ($p<0.05$) was 2.86>2.72>2.40>1.87 (BPF-PS-CS blended film), 2.54>2.12>1.94>1.43 (BSF-PS-CS blended film). Film opacity improved with ultrasonication and cellulase enzyme treatment reinforcement of BPF and BSF. Hemicellulose and lignin in fibers could increase the light reflection and thus improve the optical properties (Nie, 2023). Kumar et al. (2022) showed similar trends, where the cellulose nanofibrils improved the optical properties of the gelatin film. According to these findings, opacity was not consistently affected by the addition of low amounts of ultrasonic or enzymatically treated fibers, most likely because the total amount of added fibers kept minimal. However, the difference in opacity values

obtained for the used treatment may be due to many factors like biofilm density, nature of starch, properties of BPF, etc. The opacity of the packaging film affects the consumer acceptability of food materials as it is an important criterion that protects light-sensitive food items from UV and light radiation (Kumar et al., 2022). The ultrasonication can affect the opacity of films by reducing particle size, altering the internal structure, reducing film thickness, and creating a more cohesive structure. These changes collectively contribute to improved transparency and reduced opacity, which is crucial for packaging applications (Oluwasina et al., 2019). Ultrasound-treated pea-protein isolate films, with enhanced solubility, exhibit reduced opacity, resulting in fewer non-dissolved particles in the film-forming solution. The improved solubility due to ultrasonication of pea-protein isolate films leads to unfolded side chains capable of associating through enhanced covalent bonding and hydrophobic interactions, thereby contributing to stronger TS (Cheng and Cui, 2021). Moreover, smooth and uniform surfaces developed on the developed films (**Fig. 4.1**: Appearance of the films) contribute to reducing scattering, while uniform dispersions of ultrasound-treated fibers further reduce the overall opacity. Also, the reduction in opacity for the treatment of BSFs with cellulase was observed by modifying the structure and properties of cellulose fibers, thereby increasing the exposure of hydroxyl groups and creating more compact and homogeneous structures (Raghuwanshi and Garnier, 2019). The enzymatic treatment of cellulose can reveal hydroxyl groups through its etching action, thereby increasing the reactivity. Thus, BSF-reinforced films more suitable for applications where transparency is desirable, such as food packaging, where consumers may prefer some visibility of the contents. In contrast, BPF-reinforced films, with their higher opacity, are advantageous for applications where light blocking is beneficial.

Table 5.3 Characteristics of BPF-reinforced PS-casein (BPF-PS-CS) blended films

Sample	Thickness (mm)	Opacity (mm^{-1})	L*	a*	b*	WVP (g $mm/m^2/h/kPa$)	Solubility (%)
US	0.35 ± 0.01^{a}	2.40 ± 0.12^{b}	48.90 ± 0.72^{b}	0.213 ± 0.01^{c}	20.26 ± 0.98^{c}	0.031 ± 0.001^{b}	17.449 ± 0.87^{a}
ET	0.342 ± 0.02^{a}	1.87 ± 0.09^{c}	49.43 ± 2.11^{a}	0.43 ± 0.02^{a}	20.49 ± 0.86^{a}	0.038 ± 0.001^{a}	16.982 ± 0.84^{c}
USET	0.37 ± 0.01^{a}	2.86 ± 0.14^{a}	51.21 ± 2.38^{a}	0.37 ± 0.01^{b}	20.43 ± 0.81^{b}	0.0234 ± 0.001^{c}	15.56 ± 0.77^{c}
ETUS	0.36 ± 0.02^{a}	2.72 ± 0.13^{ab}	50.89 ± 2.21^{a}	0.41 ± 0.01^{a}	20.28 ± 0.10^{c}	0.142 ± 0.007^{d}	17.632 ± 0.88^{b}

Means depicted by different superscript small letters within the column are significantly different ($p<0.05$). (N- Native fiber, US- ultrasound treated fiber, ET- Enzymatic treated fiber, USET- ultrasound combined with enzyme treatment, ETUS- Enzyme treated with ultrasound treatment).

Table 5.4 Characteristics of BSF-reinforced PS-casein (BSF-PS-CS) blended film

Sample	Thickness (mm)	WVP (g $mm/m^2/h/kPa$)	Solubility (%)	L*	a*	b*	Opacity (mm^{-1})
US	0.314 ± 0.101^{a}	0.153 ± 0.007^{b}	18.24 ± 0.90^{a}	45.90 ± 0.82^{b}	0.233 ± 0.11^{d}	20.13 ± 0.14^{bc}	2.54 ± 0.12^{a}
ET	0.33 ± 0.01^{a}	0.163 ± 0.008^{b}	16.43 ± 0.82^{b}	45.43 ± 2.14^{b}	1.33 ± 0.04^{b}	20.30 ± 0.16^{bc}	1.94 ± 0.09^{c}
USET	0.326 ± 0.02^{a}	0.142 ± 0.007^{c}	16.95 ± 0.81^{b}	46.21 ± 2.51^{b}	1.73 ± 0.22^{a}	21.73 ± 0.11^{bc}	1.43 ± 0.07^{d}
ETUS	0.30 ± 0.01^{a}	0.121 ± 0.006^{bc}	15.23 ± 0.72^{c}	55.89 ± 2.23^{a}	1.01 ± 0.54^{c}	28.14 ± 0.10^{a}	2.12 ± 0.10^{b}

Means depicted by different superscript small letters within the column are significantly different ($p<0.05$). (N- Native fiber, US- ultrasound treated fiber, ET- Enzymatic treated fiber, USET- ultrasound combined with enzyme treatment, ETUS- Enzyme treated with ultrasound treatment).

5.3.3. Film solubility

Tables 5.3 and **5.4** present solubility data for PS-CS blended films reinforced with either BPF/BSF under different treatments: The solubility values ($p<0.05$) for BPF-PS-CS films are: US: 17.449%, ET-16.982%, US+ET-15.56%, ET+US-17.632%. Ultrasound combined (US) with cellulase treatment (ET) resulted in the lowest solubility, indicating enhanced water resistance, and also effect of ultrasound may counteract the enzyme treatment in terms of solubility. The solubility values for BSF-PS-CS are US-18.24%, Enzyme-treated (ET)-16.43 %, Ultrasound+ Enzyme-treated (USET): 16.95%, (ETUS, enzyme treatment+ ultrasound treatment)- 15.23 ± 0.72%. The lowest solubility was achieved with the ET+US treatment, suggesting that enzymatic treatment followed by ultrasound treatment significantly improved water resistance. In both cases, enzyme treatment alone (ET) or combined with ultrasound (USET for banana pseudostem, ETUS for bamboo-shoot) led to reduced solubility, with BPFs achieving slightly better results overall. Specifically, ETUS treatment in BSFs yielded the lowest solubility (15.23 ± 0.72%), which suggests better compatibility between bamboo fibers and the starch-casein matrix. Ultrasound treatment reduced solubility in both types of fibers, but it was more effective in combination with enzyme treatment for BPFs, while it appeared less effective in the combined treatment for bamboo shoot fibers. Studies have shown that enzymatic treatments improve the compatibility amongst natural fibers and polymer matrices by breaking down surface impurities and exposing more bonding sites, thereby enhancing water resistance. Similarly, ultrasound treatment improves fiber dispersion within the matrix, improving mechanical and water barrier properties. Enzymatic and ultrasonic treatments on natural fibers reduced water solubility in starch-based films by enhancing fiber-matrix adhesion and minimizing water uptake pathways (Sifuentes-Nieves et al., 2023; Li et al., 2022). Banana and BSFs for film preparation are attributed to their complex chemical composition with high lignin and crystalline cellulose content, structural characteristics that resist solvent penetration, and variable effects of processing methods that can either enhance or degrade fiber properties while maintaining fiber integrity (Nguyen et al., 2022; Ai et al., 2022; Chen et al., 2022; Low et al., 2022). Ultrasound enhances mass transfer rates, allowing solvents to penetrate more effectively into the fiber matrix. Enzymatic treatment thus involves using specific enzymes (such as cellulase and hemicellulose) to selectively degrade components of the fiber. Enzymes target hemicellulose and lignin, which are responsible for the hydrophobic nature of fibers. By breaking down these components, the overall solubility of the fiber increases, allowing for

better integration into the film matrix (Li et al., 2022). The combination of ultrasound and enzyme treatment thus effectively enhances the solubility of fiber-reinforced films by improving fiber accessibility, facilitating selective degradation of non-cellulosic components, and promoting a more uniform dispersion within the film matrix. This approach not only improves mechanical properties but also maintains biodegradability, making it a promising method for developing high-performance biocomposite materials suitable for various applications, particularly in sustainable packaging solutions which are explained in further sections.

5.3.4. Film color

The color of the biopolymeric films can be a significant factor in consumer perception of product quality. In packaging, appearance is often the first attribute that customers notice, and color can influence their decision-making process. The color analysis results reveal the color characteristics of both treated and untreated BPF/BSF-reinforced PS-CS films. The developed film exhibits a yellowish tint due to the presence of casein. **Tables 5.3** and **5.4** present the color parameters L*, a*, and b* of BPF-reinforced and BSF-reinforced PS-CS blended film. In **Table 5.3**., which details the BPF-PS-CS blended composites, the L* parameter measures lightness, with higher values indicating lighter colors. The untreated native BPF composite exhibits the highest L* value (51.2%), indicating a lighter color, whereas the USET-treated composite has the lowest L* value (48.9%), suggesting a darker color. The a* and b* parameters, representing redness-greenness and yellowness-blueness respectively, vary across treatments (ET, US, USET, ETUS), reflecting shifts in composite coloration. In **Table 5.4**, which examines BSF-PS-CS composites, the native BSF composite has the highest L* value (45.9%), also indicating a lighter color compared to treated composites. The ET-treated BSF composite has the lowest L* value (45.4%), pointing to a darker appearance. Similar to BPF composites, a* and b* values differ across treatments, highlighting color shifts in redness and yellowness. Comparatively, these variations in color parameters between banana pseudostem and bamboo shoot fiber-reinforced composites stem from inherent differences in fiber composition and structure; for instance, BPFs have higher cellulose and lower lignin content than bamboo-shoot fibers (Alawar et al., 2009; Reddy and Yang, 2005). The combined treatments (USET and ETUS) seem to impact the color parameters more significantly than individual treatments, suggesting that synergistic effects may alter fiber surface properties and enhance interactions with the polymer matrix, resulting in more

noticeable color changes. Additionally, fiber-matrix interactions, influenced by interfacial adhesion and light reflection, further affect color characteristics (Ramaraj, 2007), with treatment variations altering these interactions and thus the observed color properties. As the fiber treatment is combined with cellulase and ultrasound, the film matrix becomes more compact, resulting in a lighter appearance. Ultrasonic treatment combined with enzymatic hydrolysis can effectively soften the fiber's molecular structure. These modifications facilitate the binding of substrates to enzymes, hence enhancing the efficiency of enzymatic hydrolysis (Vera et al., 2020). Thus, the color in US +ET-treated BSF films was more defined with more + values of b* and a*. Singha et al. (2023) obtained similar results for the color parameters, showing positive b* values, a comparative trend for lightness, and a* values upon the incorporation of sugarcane bagasse fiber upon sweet lime peel- and PVOH-based biodegradable films.

5.3.5. Mechanical properties

The produced film's mechanical characteristics were represented by its elongation at break (EAB%) and tensile strength (TS) represented in **Table 5.5**. Reinforcement with BPF and BSFs and modifications of PS-CS blended films with ultrasound and cellulase enzyme increased the mechanical properties of the films ($p<0.05$). The adhesion between fibers and matrix is improved due to reinforcement which enhances the film's mechanical attributes of developed composite film compared to PS and casein film alone. For BPF-PS-CS blended films, untreated samples showed the lowest mechanical characteristics, with TS of 1.53 MPa and EAB % of 1.43%. Ultrasonic treatment (US) led to significant improvements, increasing TS by 247% to 5.31 MPa and EAB to 3.26%, while the combination of ultrasound and enzyme treatment (USET) provided the best performance, with a TS of 6.86 MPa, representing a 348% increase over the untreated sample. Enzyme treatment alone (ET) resulted in a moderate TS improvement to 3.31 MPa. Cellulases enhance compatibility between incompatible polymer blends within composite films by modifying surface properties or chemical structures, thereby promoting better interfacial interactions and overall film integrity (Zwawi, 2021). In comparison, BSF-PS-CS blended films displayed a more uniform distribution of mechanical properties across treatments, with gradual improvements and less dramatic variation. The peak TS of 5.65 ± 0.28 MPa indicated good structural integrity but was generally lower than that of BPF-PS-CS blended films with USET treatment. Interface adhesion between BPFs and the matrix was enhanced under USET treatment, improving stress transfer efficiency, while BSFs

provided more consistent fiber-matrix bonding overall (Bian et al., 2020). Ultrasonic treatment improved fiber dispersion in both films, leading to more uniform stress distribution and increased mechanical properties (Wang et al., 2020). For practical applications, the high TS of USET-treated BPF films suits heavy-duty packaging, while BSF films with moderate TS are appropriate for regular packaging needs, and high EAB films support flexible packaging applications (Fazeli and Simao, 2019). Also, for agricultural uses, these films are suitable for mulch films, seed coatings, and temporary protective covers. Compared with commercial materials, our bio-based films achieved TS values up to 6.86 MPa, reaching 60-85% of the performance of low-density polyethylene (LDPE) and offering a 2 to 3 times improvement over pure starch films. Thus, the enhanced mechanical properties could be associated with several reasons. Firstly, the use of casein could lead to improved protein cross-linking during the modification process, resulting in firmer films. These findings aligned with the notion that crosslinking induced more densely packed protein networks in the blend films, resulting in a significant decrease in water vapor permeability (WVP) (Wang et al., 2017). Both the plasticizer content and the nature of the fiber addition significantly impacted the casein-based composite films' mechanical characteristics, with glycerol content having a more pronounced influence (Yang et al., 2020). Additionally, the uniform thickness of films obtained through dual-modified fiber-reinforced PS-casein films also contributed to the enhanced mechanical properties, as discussed earlier. Further, lower solubility and lower WVP of BPF-reinforced PS-casein blended films showed suitable potential for packaging applications combined with ultrasound and cellulase treatments. The elevated levels of TS observed in this research are comparable to the study reported by Jumaidin et al. (2020) for sugarcane bagasse-reinforced biodegradable potato starch composites. Consequently, through modification of natural fibers using US and ET, the fibers still maintain their reinforcement responsibility by enhancing mechanical properties. Increasing the duration of treatment for fibers used resulted in a reduction of the interfibrillar zone, which facilitated more effective impurities elimination. Consequently, a fibrillary arrangement parallel to the direction of load application was achieved. This enhancement positively impacted the mechanical attributes of the bamboo fiber-reinforced composites (Ramesh et al., 2021). Supporting literature, such as Wang et al. (2018), Chen et al. (2022), and Rodriguez et al. (2024), corroborates our findings, showing similar TS improvements (200-300%) with fiber reinforcement and ultrasonic treatments. Also, results are in agreement with Kang et al. (2023) who reported increased mechanical properties of pullulan/oat protein/ nisin

composite film using ultrasonic treatment, and Liu et al. (2021) on maize starch/stearic acid/ sodium carboxymethyl cellulose composite film.

5.3.6. Sealing properties

The heat-sealing process induced melting by usage of heat that promoted the interfacial interactions between the contact surfaces give the sealed film enough seal strength (Nilsuwan et al., 2018). All the developed film samples were thermally sealable due to the uniform thicknesses obtained. **Table 5.5** presented the sealability values of developed BPF-reinforced and BSF-reinforced PS-CS blended films that depicted significant variations ($p<0.05$). The sealing strength of the fiber-reinforced film was enhanced due to the inherent tendency of the sealability of biopolymer and the use of glycerol. Plasticizers are used engaged with polymer chains, creating hydrogen bonds and aiding in the reassembly of polymer chains through heat application (Lim et al., 2020). The analysis of sealing properties shows that BSF PS-casein blended films have a sealing strength range of 2.09-2.84 MPa, with the highest sealing strength recorded at 2.84 ± 0.14 MPa, and a sealing efficiency range of 50.11-79.9%, reaching a maximum of 79.9 ± 3.04%. In comparison, BPF PS-CS blended films exhibit a wider sealing strength range of 0.91-4.68 MPa, with a peak value of 4.68 ± 0.20 MPa, and a sealing efficiency range of 41.99-89.23%, achieving a maximum of 89.23 ± 4.08%. While BPF films demonstrate a higher maximum sealing strength and efficiency, they also show greater variability in strength (0.91-4.68 MPa) compared to the more consistent range observed in bamboo-shoot films (2.09-2.84 MPa). In terms of sealing efficiency, BPF-PS-CS films achieve a higher maximum but with substantial variation across samples, whereas bamboo-shoot films maintain good, though slightly lower, efficiency values. The findings align with published research, such as Magalhães et al. (2019), who reported sealing strength ranges of 2-5 MPa for bio-based films, and Gupta and Nayak, (2015) who observed sealing efficiencies of 40-85% in starch-based composites. Bangar et al. (2023) also documented similar variations in properties due to the effects of natural fibers. Because of the change, the sealing strength has increased of the developed films. The mechanical characteristics of the bio-composite film were significantly impacted by the ultrasonic amplitude and duration (Nie et al., 2018). It has promoted increased contact interactions between the two opposing surfaces, giving the sealed film enough seal strength (Nilsuwan et al., 2018). Since starch had great adhesion and BPFs had also formed a strong hydrogen connection with the polymer matrix to create heat-sealed films (Orsuwan and Sothornvit, 2018).

Table 5.5. Mechanical properties of the developed fiber-reinforced PS-casein blended film

Films	**BPF-PS-CS blended film**				**BSF-PS-CS blended film**			
	Tensile strength (TS) (MPa)	**Elongation at break (%) (EAB)**	**Sealing strength (MPa)**	**Sealing efficiency (%)**	**Tensile strength (TS) (MPa)**	**Elongation at break (%) (EAB)**	**Sealing strength (MPa)**	**Sealing efficiency (%)**
N	1.53±0.07^{d}	1.43±0.07^{d}	0.91±0.04^{d}	59.47±2.97^{d}	3.04±0.15^{d}	1.03±0.05^{e}	2.43±0.12^{c}	79.9±3.04^{a}
US	5.31±0.25^{b}	3.26±0.16^{a}	2.23± 0.11^{c}	41.99±2.04^{e}	4.34±0.20^{b}	2.63±0.13^{c}	2.65±0.13^{b}	61.05±3.00^{b}
ET	3.31±0.16^{c}	2.62±0.13^{c}	2.33± 0.10^{c}	70.39±3.05^{b}	4.17±0.19^{c}	2.29±0.11^{d}	2.09±0.10^{d}	50.11±2.50^{c}
USET	6.86±0.33^{a}	2.76±0.12^{b}	4.68 ±0.20^{a}	68.22±3.40^{c}	5.65±0.28^{a}	3.14±0.15^{a}	2.84±0.14^{a}	50.26±2.50^{c}
ETUS	3.25±0.16^{c}	2.64±0.12^{c}	2.90 ± 0.14^{b}	89.23±4.08^{a}	4.42±0.22^{b}	2.86±0.14^{b}	2.77±0.13^{a}	62.66±3.10^{b}

Means depicted by different superscript small letters within the column are significantly different ($p<0.05$). (N- Native fiber, US- ultrasound treated fiber, ET- Enzymatic treated fiber, USET- ultrasound combined with enzyme treatment, ETUS- Enzyme treated with ultrasound treatment).

5.3.7. Water vapor permeability (WVP)

Maintaining the WVP of the biodegradable films helps preserve items by draining off excess water produced during the post-ripening process, which delays the growth of bacteria that cause spoiling (Kang et al., 2023). Barrier properties are crucial in packaging materials as they play a vital role in maintaining the quality and shelf life of the packaged items. The barrier characteristics of biodegradable films are determined by factors such as the composition of the film, the processing techniques used, and the specific application requirements. The barrier qualities of the film have been enhanced, leading to reduced penetration of moisture. The barrier property (WVP) results of the developed film are shown in **Table 5.4**. The WVP of banana-pseudostem and bamboo-shoot fiber-reinforcement PS-casein blended films is decreased sufficiently significantly ($p<0.05$) compared to PS and casein film due to existing cellulosic nanofibers in the matrix. Cellulose present in the fiber acts as a hindrance for water vapor that creates an indirect way for water molecules passage (Fazeli et al., 2018). The comparative analysis of water vapor permeability (WVP) shows that BPF-PS-CS films exhibit WVP values ranging from 0.0234 to 0.142 g mm/m²/h/kPa, while BSF-PS-CS films range from 0.121 to 0.163 g mm/m²/h/kPa. For BPF-PS-CS films, the best WVP performance is achieved with the USET treatment (0.0234), followed by US (0.031g mm/m²/h/kPa), ET (0.038 g mm/m²/h/kPa), and ETUS (0.142g mm/m²/h/kPa), indicating a clear treatment hierarchy: USET > US > ET > ETUS. In contrast, BSF-PS-CS films display less pronounced differences across treatments, with the ETUS treatment providing the best WVP (0.121 g mm/m²/h/kPa) and ET the highest (0.163 g mm/m²/h/kPa). Generally, BPF-reinforced films show lower WVP values than BSF-reinforced films, which have more consistent but higher WVP values across treatments. The treatment effects are more significant in BPF reinforced films, highlighting different optimal treatment sequences for each fiber type. The reinforcement of modified BPFs made the film more rigid with sufficient resistance to moisture across the film. It showed that the modification of BPFs has improved potato starch-casein and BPF interaction across the matrix. Since starch is hydrophilic modified BPF reinforcement enhanced the WVP of the film through greater crystallinity of BPF thus retarding the water uptake (Nie, 2023). The decreasing water vapor and oxygen transmittance values for bio-nanocomposite films made from wheat starch reinforced with cellulose nanocrystals demonstrated penetration into the matrix (Montero et al., 2021). The findings imply that natural fibers may enhance TS without impairing the film's capacity to

function as a water barrier. Because of these characteristics, the films are therefore better suited for storing food (Li et al., 2022). The decrease in WVP of composite films is thought to result from the existence of structured dispersed fiber particle layers securely embedded within the polymer matrices (Khalil et al., 2018). This compels water vapor traversing the film to navigate a convoluted pathway by the polymer matrix enveloping the fiber particles, consequently elongating the actual distance for diffusion. The composite reinforced film developed possesses a tight structure and excellent water vapor permeabilities, which makes it an ideal material for product packaging. Hence, the developed composite film can be marketed for use in the packaging sector. The findings imply that adding natural fibers might enhance TS without impairing the film's capacity to serve as a barrier against water. Because of these features, the films, therefore, become extra suited for food preservation (Li et al., 2022). When comparing the water vapor permeability of both films, BPF-reinforced films demonstrate superior barrier properties with lower WVP values (minimum 0.0234 g mm/m²/h/kPa) compared to BSF-reinforced films (minimum 0.121 g mm/m²/h/kPa). This significant difference aligns with findings from Lauer et al. (2020) and Stark et al. (2021), who obtained similar trends in natural fiber-reinforced biopolymer films. Interestingly, the treatment sequence affects each fiber type differently - USET treatment provides optimal results for BPF films, while ETUS works best for bamboo shoot films. These findings align with Preetha et al. (2019), who observed that natural fibers require specific treatment optimizations to achieve optimal barrier properties. The overall WVP ranges (0.0234-0.163 g mm/m²/h/kPa) are within acceptable limits for many food packaging applications, though BPF films show more promising potential for moisture-sensitive products due to their lower WVP values.

5.3.8. Thermal properties

Thermal degradation is an important criterion of developing a film to understand the limits of processing, modification or operating temperatures. The PS starch-based films' thermal resilience reinforced with native and modified BPF/BSF by ultrasound and cellulase enzyme is determined by thermogravimetry (TG) and differential thermogravimetry curves (DTG) as shown in **Fig. 5.3**. **Table 5.6.** presented the degradation intervals of BPF/BSF (native and modified) obtained from the TGA plot. Generally, composite materials are thermally stable compared to single bio-polymeric materials (Azevedo et al., 2020). In the thermo-gravimetric curves of modified developed

films three stages of mass loss were observed. The main stage of decomposition occurred in the temperature range of 252–386 ℃ for native film with 77.82% of degradation. This can be attributed to thermal decomposition of starch, loss of hydroxyl groups and depolymerization of cellulose (Wang et al., 2020). In case of treated BPF-reinforced film, initial mass loss occurred about 73.1 ℃ to 127.8 ℃ with 5% degradation. 2nd stage of mass loss took place around 207.5 ℃ to 252 ℃. Final stage of mass change was from 303.2 ℃ to 506 ℃ referred to degradation of cellulose particles. Initial stage of TGA curve of developed film showed minimum mass loss (about 12%) due to moisture loss from the internal area of film (Azevedo et al., 2020). The removal of hemicellulose resulted in a fibrous structure, thereby making the moisture loss easier and increasing the resistance of the BPFs (Priyadarshana et al., 2022). Mass decomposition around 2nd stage in the developed film showed 50% to 60% that depicted thermal expansion due to de-polymerization of cellulose and glycerol decomposition. The last stage of degradation in the developed film could be attributed to lignin decomposition (Fazeli and Simao, 2019). The results showed that reinforcement of BPFs enhanced the composite thermal stability. The improvement is added due to increase in the amount of cellulosic matter in BPFs that possessed greater thermal stability compared to starch (Azevedo et al., 2020). As the ultrasonication and enzyme treatment combined, it retarded the further mass loss in the developed film indicating the firmness of the fibrous matrix and providing higher stability of the crystalline structure of BPFs (Ullah et al., 2018). The addition of plant fibers resulted in an increase in the required decomposition temperature and an increase in the final weight residue (Yusuf et al., 2019). Hence, better thermal stability can be achieved with the reinforcement of BPF modified by ultrasound and cellulase enzyme. For BSF-PS-CS blended film there were three primary phases to the degradation of BSF-PS-CS, with the majority of the thermal breakdown taking place between 200°C and 500°C. Film's initial mass loss took place at a temperature of around 110°C, (primarily due to water evaporation from the BSF). Subsequently, between 230°C and 280°C, sufficient reduction in molecular weight (16.04% to 19.10%) was observed in BSF-PS-CS films (native and US), predominantly attributed to hydrolysis. Upon incorporation of BSF, the developed film's thermal stability was greatly improved. With a final residual mass of 2.07%, the breakdown of the polysaccharides of cellulose and hemicellulose in BSF and the rupture of the glycosidic link are responsible for the majority of the weight loss. The modified film, on the other hand, displays a slower rate of breakdown, a longer decomposition time and temperature (219–417°C), and a larger residual mass loss (73.70 to 98.93%) compared

with native BSF-PS-CS film (58.73%). The removal of components as stated by FTIR results such as hemicellulose and lignin through ultrasound and cellulase treatment, resulted in improved fiber thermal stability, aligning with findings from previous research on banana-fiber reinforced starch films (Dutta and Sit, 2024). The aforementioned trend aligns with previous reports on bamboo fiber thermal characteristics by Liu et al. (2021) and Ramesh et al. (2021). Results showed that bamboo composites outperformed kenaf fiber reinforced composites in terms of thermal stability. and regenerated bamboo fiber composites. The TGA analysis of the composites revealed a shift in the maximum degradation temperature to a higher level than that of the casein, as reported by Dutta and Sit (2023), suggesting slightly higher thermal stability. By disrupting the hydrogen bonds that bind cellulose molecules to nearby crystalline regions, ultrasonication reduces the amount of amorphous cellulose, enhances the degree of crystallinity, and facilitates the hydrolysis of the amorphous region (Zwawi, 2021). Furthermore, increased surface roughness observed in SEM results has enhanced the interfacial bonding with casein. Enzyme treatments have also been found to effectively increase thermal stability by extending the temperature range and raising the degradation temperature, making the fibers more resistant to thermal decomposition. Studies by Kowalczyk et al. (2011) found that treating hemp with pectin-methyl-esterase (PME), xylanase, and xylanase + cellulase enhanced thermal degradation by eliminating hemicelluloses. Consequently, the synergistic effect of BSF-reinforced casein film has led to improved thermal stability (Lv et al., 2011). Furthermore, studies demonstrate that the crystallinity of the reinforcements and matrix in composite materials directly affects their thermal stability since a high degree of crystallinity increases their heat resistance (Chin et al., 2020). The results of FTIR and XRD indicate that BSF has improved crystallinity. Therefore, the modification of natural fibers has improved the thermal resistance where the improved thermal stability observed by banana-pseudostem fibers reinforced films compared with BSF-reinforced starch-casein films.

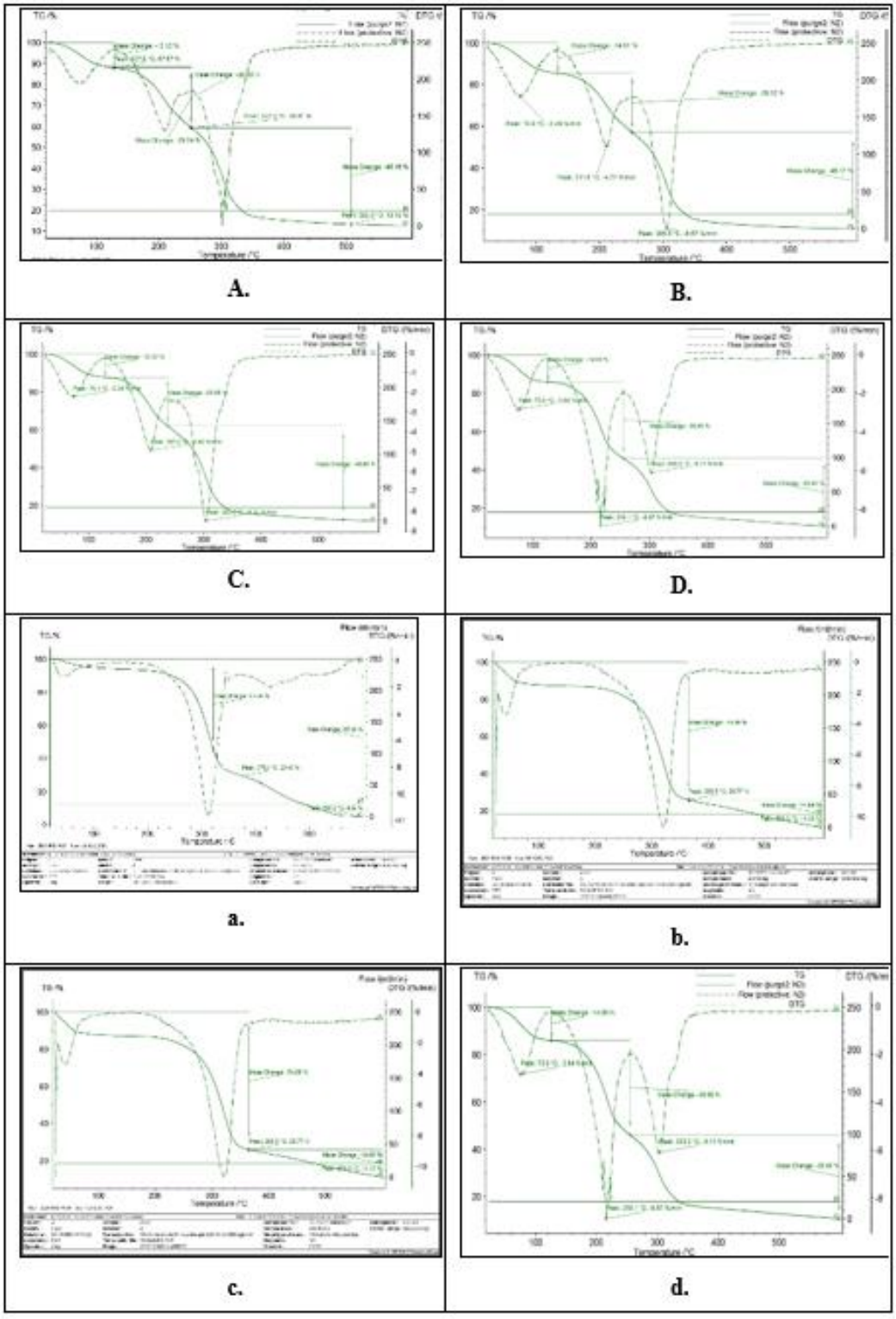

Fig. 5.3 TG/DTG thermograph of modified Banana-fiber-reinforced starch-casein films (BPF-PS-CS=A, B, C, and D) BSF (BSF-reinforced starch-casein films (BSF-PS-CS= a, b, c, and d), (A, a= US- ultrasound treated fiber, B, B= ET- Enzymatic treated fiber, C, c= USET- ultrasound combined with enzyme treatment, D, d= ETUS- Enzyme treated with ultrasound treatment)

Table 5.6. Degradation intervals of BPF/BSF (native and modified) obtained from TGA plot

Stage	Type of degradation	Temp range (°C)	Loss of mass (%)				
			Native	US	ET	USET	ETUS
1	Moisture loss	<100	8.53	3.61	-	-	-
2	Degradation of extractable material	100-250	16.04	19.10	28.97	-	-
3	Hemicellulose degradation	250-350	-	-	-	-	-
4	Cellulose and lignin degradation	350-500	-	58.23	-	-	73.70
5	Lignin degradation	>500	58.73	58.23	56.20	81.28	98.93

(N- Native fiber, US- ultrasound treated fiber, ET- Enzymatic treated fiber, USET- ultrasound combined with enzyme treatment, ETUS- Enzyme treated with ultrasound treatment).

5.3.9. Biodegradability

To demonstrate the eco-friendly nature of the produced films, their biodegradability underwent analysis through simulated soil experiments. The change in mass of the developed BPF-PS-CS blended films exposed to soil decomposition is presented in **Fig. 5.4**. Developed films depicted sufficient weight loss with an extended period because of the degradation of the biologically oxidizable materials leading to variations in structural integrity (Indumathi et al., 2019) which can be seen in **Fig. 5.5**. At the end of 6 weeks, almost 80% of the developed film was completely degraded. The native film was completely biodegraded within 4 weeks which may be because starch-casein has a hydrophilic character (Hosseini et al., 2023). About 50% of developed films got decomposed by 30 days. Starch-based films were easily biodegraded in a week (Dutta and Sit, 2022) but due to fiber reinforcement, films were degraded to 100% by the end of 6 weeks. The enzyme treatment may also lead to gradual biodegradation for a long duration. The mass changes before and after soil degradation are depicted in **Fig. 5.5.** which has proposed an effective decomposition of developed films. Ultrasound and enzyme treatment might have initiated water absorption promoting degradation to 100% mass loss

of developed films. The cavitation and super-mixing effects of ultrasonic processing may have accelerated the pace of degradation of the composite film by creating a smoother surface that made the film more prone to deterioration (Liu et al., 2019). Biodegradability was observed through the soil burial test method. The developed film samples lost their integrity when buried until 32 days of decomposition. For BSF-PS-CS films, the changes occurred throughout the burial period, and the end-life is shown in **Fig. 5.6**. showing the film's end life in 3 weeks. Additionally, the percentage of mass loss throughout the degradation period is shown in a line graph (**Fig. 5.7**). The process revealed that after 2 weeks of decomposition, the developed film had become hard and crisp, which caused it to break easily when touched. This is due to the use of a biopolymer for film preparation, which had an excellent degrading nature over time. After 14 days of the burial period, both the ultrasound and enzyme-treated films underwent complete degradation leading to their gradual breakdown and decomposition as the incubation time extended. The high cellulose, hemicellulose, and xylem contents of BSF resulted in higher pyrolysis-induced porosity in the produced films, which caused the composites to break down quickly and cause weight loss. These findings collectively demonstrate the favorable biodegradability of the manufactured films, highlighting their environmental friendliness (Dirpan et al., 2023). The TGA curve, which discusses the rate and degree of weight loss, also indicates efficient biodegradation, which breaks down the polymer matrix into smaller, more easily digested fragments that microorganisms are more likely to eat. This outcome suggests that the film continues to be thermally resistant at high temperatures. Thus, as per the results, fiber-reinforced PS-casein blended-based films are easily biodegradable making them effective from an environmental point of view. They decompose naturally in the environment without leaving behind harmful residues, helping to tackle the global plastic waste issue (Gupta et al., 2022). When buried in soil, the banana and BSF composites exhibited enhanced thermal stability with not much difference in term of biodegradation rate among samples.at elevated temperatures compared with those reinforced with kenaf fiber and hybrid composites (Ramesh et al., 2021). This enhancement is attributable to the lower levels of hemicellulose present in BSF. Additionally, moisture absorption by fibers from the soil tends to increase. Similar outcomes have been documented by Yang et al. (2019) regarding the mechanical and biodegradable characteristics of starch/polypropylene biodegradable composites reinforced with bamboo fiber and by Yusof et al. (2019) for treated bamboo fiber-reinforced tapioca starch composite. The

composite exhibited improved flexural properties and biodegradation, evidenced by a distinct decrease in weight loss over time.

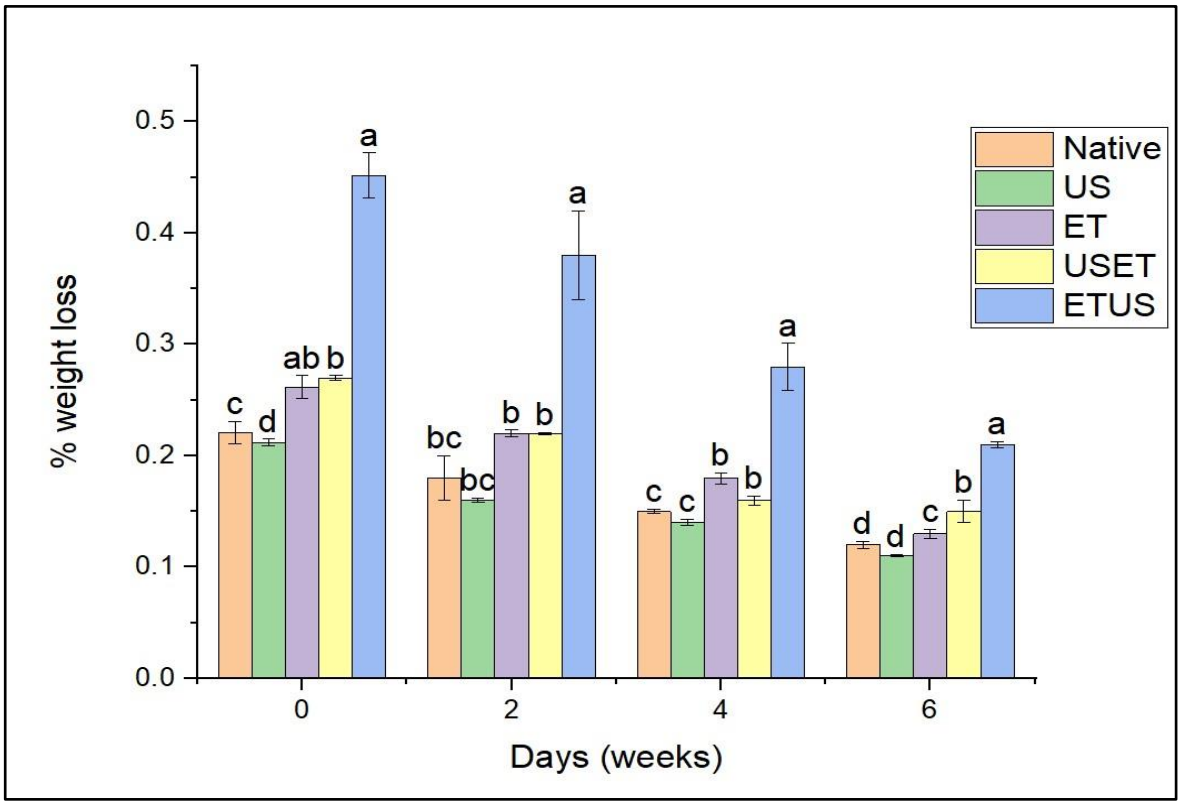

Fig. 5.4 Soil degradation of developed BPF-PS-CS blended films. Data presented as different letters on the bar diagram show the significant differences ($p<0.05$). (N- Native fiber, US- ultrasound treated fiber, ET- Enzymatic treated fiber, USET- ultrasound combined with enzyme treatment, ETUS- Enzyme treated with ultrasound treatment)

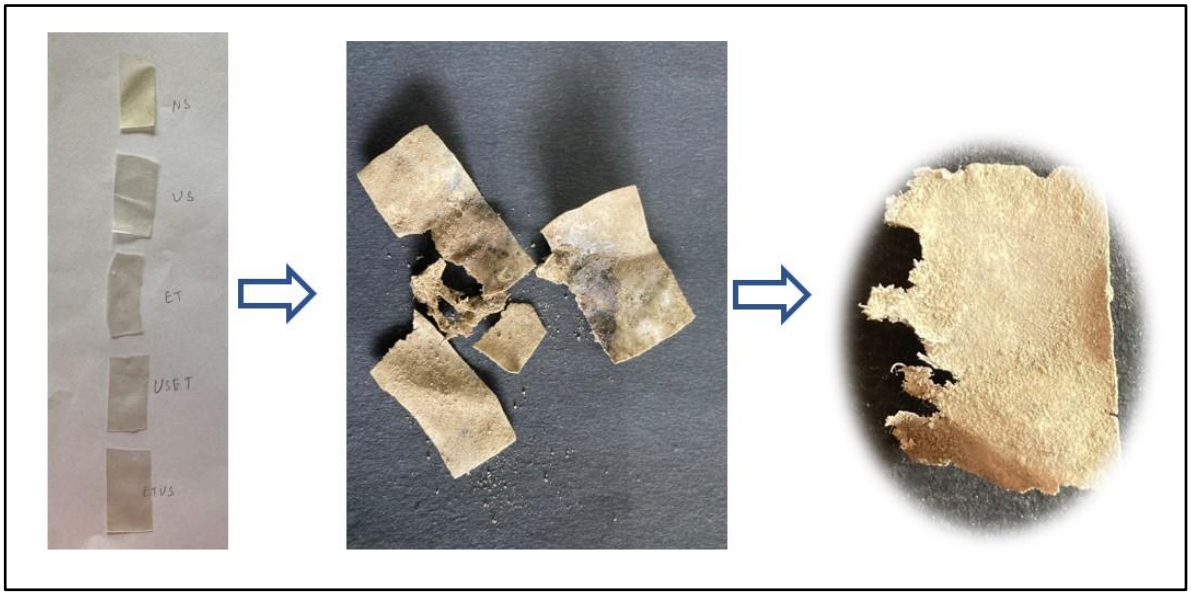

Fig. 5.5 Degradation of developed BSF-PS-CS blended films in 6 weeks

a) Developed film, b) and c) mass loss in developed film in subsequent days of soil-burial test

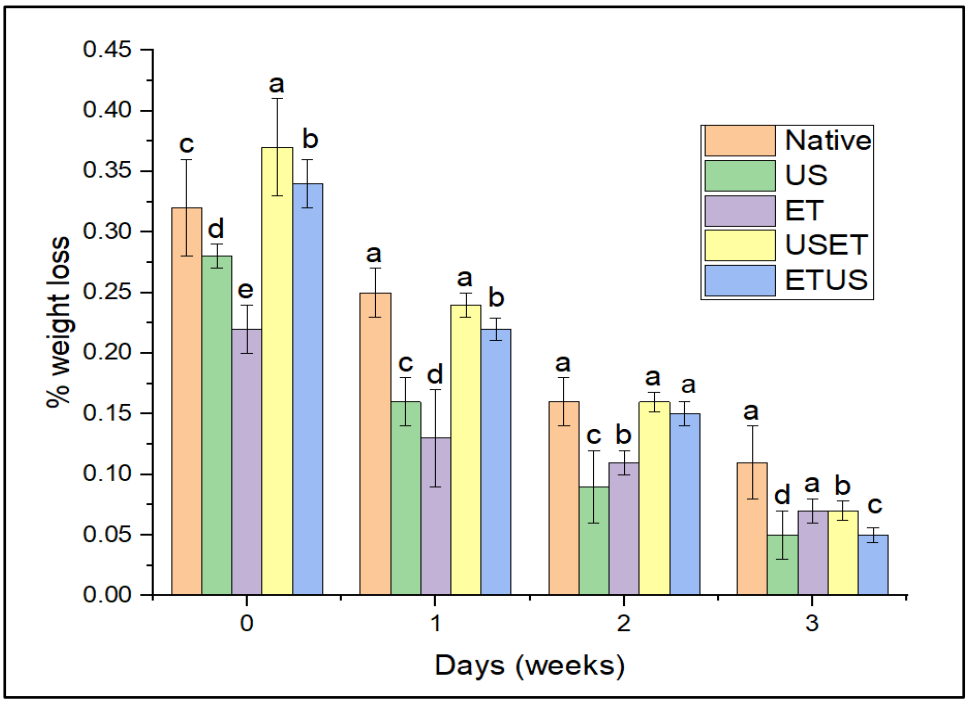

Fig. 5.6 Soil degradation of BSF-PS-CS blended films. Means depicted by different superscript small letters within the column are significantly different ($p<0.05$). (N- Native fiber, US- ultrasound treated fiber, ET- Enzymatic treated fiber, USET- ultrasound combined with enzyme treatment, ETUS- Enzyme treated with ultrasound treatment)

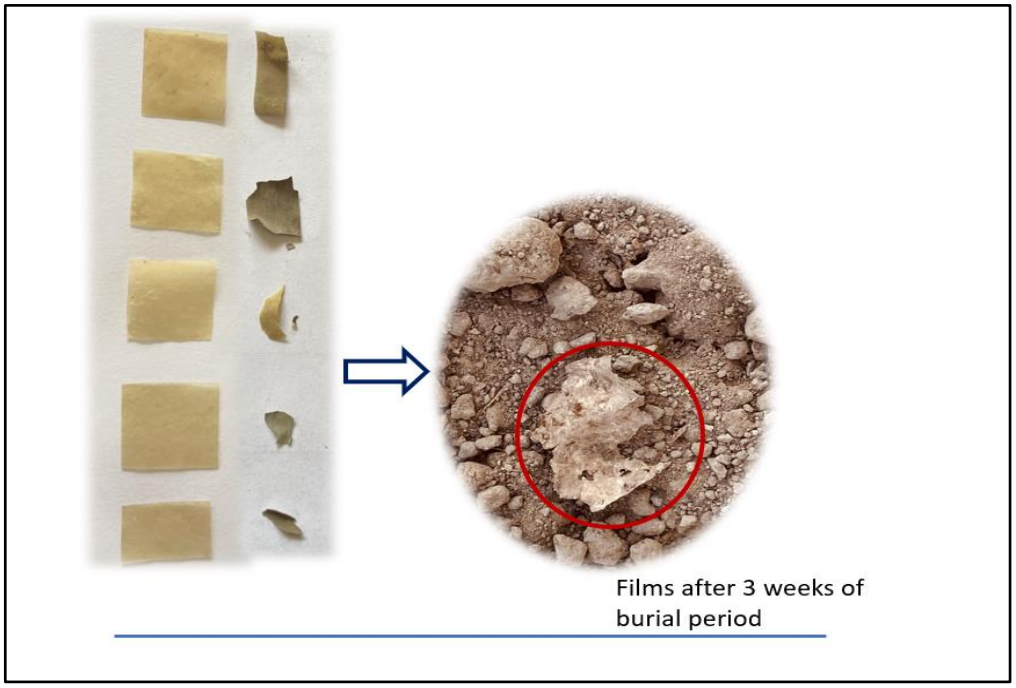

Fig. 5.7 Degradation of developed BSF-PS-CS blended film in 3 weeks showing developed film and mass loss in subsequent days of soil-burial test

5.4. Conclusion

The chapter found that the combination of starch, casein, BPF (Banana pseudostem fiber), and BSF (bamboo-shoot fiber) resulted in composite films with improved barrier and mechanical qualities, making them suitable for sustainable packaged usage. It provided evidence that composite films made from starch (PS), casein (CS) blends reinforced with BPF, and BSF can serve as a good substitute for traditional synthetic polymers in various applications. The combination of ultrasound (US) and cellulase treatment (ET) improved the solubility of fiber-reinforced films, particularly in the context of biocomposite materials. The developed films showed effective sealing ability and reduced water vapor permeability (WVP) due to the presence of cellulosic nanofibers in the matrix. The WVP of BPF-PS-CS and BSF-PS-CS blended films was significantly decreased compared to PS and casein film. The treatment sequence affected each fiber type differently, with USET treatment providing optimal results for BPF films and ETUS working best for bamboo shoot films. The tensile strength (TS) increased with the addition of BPF to the films, while BSFs provided more consistent fiber-matrix bonding. Also, adding fibers enhanced the films' thermal resilience. The film continued to be thermally resistant at high temperatures. The results showed that the developed films are easily biodegradable, making them effective from an environmental point of view. Overall, banana pseudostem fiber (BPF) reinforced films have demonstrated superior performance compared to bamboo shoot fiber (BSF)-reinforced PS-casein films, upon ultrasound and enzyme modifications showing higher TS, lower water vapor permeability (WVP), reduced solubility, and enhanced thermal stability, creating highly appropriate for packaging usage. The combination of ultrasound and enzyme treatment (USET) was the most effective for banana pseudostem fiber (BPF) films, while enzyme followed by ultrasound treatment (ETUS) worked best for bamboo shoot fiber (BSF) films. However, USET-treated BPF-PS-CS films showed the highest TS and reduced water vapor permeability (WVP), making them superior overall. BPF-reinforced PS-CS blended films exhibited higher TS, lower WVP, reduced solubility, and enhanced thermal stability compared to BSF-reinforced films. These characteristics make them the best option for packaging applications, particularly heavy-duty packaging. Consequently, BPFs as reinforcement have been selected in this chapter as the preferred material for further investigation in the following chapter.

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