Chapter 6

To incorporate natural active ingredients for the development of active films of banana-pseudostem fiber-reinforced potato starch-casein blends

6.1. Introduction

Conventional packaging solutions are becoming increasingly inadequate in today's market, where consumer awareness of sustainability issues is growing rapidly. There is a mounting demand for more environmentally friendly packaging options, driven by the increasing complexity of products, heightened environmental consciousness, and the push towards a circular economy model that aims to reduce the carbon footprint of manufactured goods (Wahab et al., 2023). The packaging industry has seen significant advancements in recent decades in response to these evolving needs and expectations. Innovative solutions, such as biodegradable materials and smart packaging technologies, have emerged and are developing at a rapid pace. These new packaging approaches are designed to address the multifaceted challenges of sustainability, product protection, and consumer expectations in a more holistic and environmentally responsible manner (Huang et al., 2024). Utilizing safe biopolymers and implementing innovative food packaging techniques help maintain packaged food items' quality and safety standards (Xu et al., 2021; Pandita et al., 2024). Therefore, utilizing biopolymer-based food packaging enhances food safety and supports sustainability goals by offering a safer, renewable, and biodegradable substitute for traditional synthetic polymers (Shah et al., 2024). Active packaging is a kind of smart packaging that emits or absorbs substances inside the packaged product to improve the quality and prolong the shelf life of perishable goods. It works by reducing oxygen interactions, moisture uptake or loss, and microbial growth. Essential oils (EOs) are being used extensively as active agents in food packaging to improve food safety and prolong shelf-life (Sun et al., 2020; Fan et al., 2023). They are high in bioactive compounds that possess multiple functional characteristics, including UV radiation, antioxidants, and antimicrobials-blocking abilities (Adilah et al., 2018; Sun et al., 2020). EOs are mostly utilized in film formulations to enhance the barrier qualities of the film matrix when combined with other biopolymers as emulsion-based systems or multilayer coatings (Zhang et al., 2022). EOs have also proven efficient towards a variety of microorganisms, including their eco-friendly and biodegradable properties have made them popular with consumers (Khajeh et al., 2024). Several EOs, such as mint, clove, cinnamon, garlic, ginger, rosemary, or oregano, are incorporated as active agents into biopolymeric films that also enhance the antioxidant activities, which can also prevent oxidation and browning reactions in packaged products (Roy et al., 2022; Anuar et al., 2017). For case, tea tree oil utilized for carrageenan/agar-based films (Roy and Rhim, 2021), *Camellia japonica* oil incorporated in carboxymethyl chitosan/peptide-based films (Liu et al., 2022), including oregano oil used for chitosan/zein-based films (Xue et al., 2021).

While EOs are widely employed as active ingredients in the active packaging industry, their practical application is influenced by key factors like stability, evaporation, and susceptibility to oxidation. Due to their non-polar nature, EOs also contribute hydrophobic characteristics and resistance to water in the films. This chapter focuses on the properties of essential oils (cinnamon and clove) incorporated into the optimal film identified in previous studies, the banana fiber-reinforced starch-casein film. The resulting biofilms were further investigated to assess various properties, including optical characteristics, mechanical strength, structural composition, crystallinity, thermal behavior, and biodegradability, to determine the most effective active film based on the essential oils used.

6.2. Materials and methods

6.2.1. Materials

The potato starch (PS) was extracted based on the procedure outlined in section 3.2.2. Casein was acquired from Zenith India in Guwahati, Assam. The extraction process for banana pseudostem fiber (BPF) is explained in the section. The modification of extracted BPF using ultrasound and cellulase enzyme is described in section. Clove and CNOs (CLO/CNO) were also purchased from Zenith India in Guwahati, Assam.

6.2.2. Film development

The solvent casting method was used for composite film development (potato starch (PS) + casein (CS) + banana pseudostem fiber (BPF) according to the protocol of

Rawat and Saini, (2024). The compositions of the different film formation solutions are shown in **Table 6.1**. Film development using modified potato starch (PS), modified casein (CS), and modified banana pseudostem fibers (BPF) has been already detailed in (sections 3.2.7; 3.2.8; 4.2.7). Banana pseudostem fibers (5%) were separately mixed in a beaker and homogenized. Clove oil (0.5, 1, and 1.5 %), cinnamon oil (0.5, 1, and 1.5 %), and glycerol (40 mL/100 mL) were included in the solution for the composite film which was then homogenized at 12,000 rpm for 5 mins. The mixture was aired, transferred to the petri plates (150 mm diameter and 10 mm height), and placed in a tray dryer at 45°C for 48 h. After being peeled off, the dry films were allowed to equilibrate for 24 h at 25 °C before further analysis.

Table 6.1 Composition of developed fiber-reinforced active film

Films	Potato starch (PS) (g)	Casein (CS) (g)	Banana pseudostem fibers (BPF) (% wt)	CLO (CLO) (%)	CNO (CLO) (%)	Glycerol (mL)
CNO 0.5	5	5	5	-	0.5	2
CNO 1	5	5	5	-	1	2
CNO 1.5	5	5	5	-	1.5	2
CLO 0.5	5	5	5	0.5	-	2
CLO 1	5	5	5	1	-	2
CLO 1.5	5	5	5	1.5	-	2

6.2.3. Characteristics of Essential oils

6.2.3.1. Antioxidant activity

The antioxidant activity of various concentrations of clove and CNO (0.5, 1, and 1.5%) was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Based on DPPH radical scavenging by EOs, this technique was adapted from the approach with som changes from Dalli et al. (2021). The process began with preparing methanolic solutions of CLO and CNO at concentrations ranging from 1 to 8 mg/mL. For each test, 50 µL of EO was combined with 5 mL of 0.004% DPPH methanolic solution and thoroughly mixed. These prepared mixtures were then left to incubate at room temperature in a dark environment for 30 min. Following the incubation period, the absorbance of each mixture was determined at a wavelength of 517 nm. In this test, methanol was utilized as a blank, and ascorbic acid (vitamin C) was utilized as the reference antioxidant for comparison.

The effectiveness of the essential oils in scavenging DPPH radicals was quantified using equation **(6.1)**, which calculated the percentage of DPPH radicals neutralized by the oils.

$$\text{DPPH radical scavenging activity (\%)} = \frac{Blank\ absorbance - Sample\ absorbance}{Blank\ absorbance} \times 100 \quad (6.1)$$

6.2.3.2.Antimicrobial activity

The essential oils (EOs) antimicrobial characteristics were examined employing the agar disk diffusion process as mentioned by Sarengaowa et al. (2023) on bacterial strains of Gram-negative *E. coli* and the Gram-positive *S. aureus*. This technique involves several steps to assess the oils' ability to inhibit bacterial growth. First, bacterial suspensions were prepared at a concentration of 8 log CFU/mL, and approximately 1 µL of these suspensions was uniformly spread across Luria Bertani Broth, Miller media in petri dishes. Next, small discs measuring 6 mm in diameter were immersed in 5 µL of the EOs being tested. These oil-infused discs were then carefully positioned on the infected culture media's surface. The prepared plates were incubated for 24 h at 37°C. After this incubation time, the effectiveness of the EOs was ascertained by measuring the inhibition's diameter zones that formed around the discs on the agar surface. This method provides a visual and quantifiable means of assessing the antibacterial activity of the EOs against the tested bacterial strains.

6.2.3.3. Total phenolic content (TPC)

The sample's total phenolic content (TPC) was ascertained following Singla et al. (2023) using a modified Folin–Ciocalteu assay, with gallic acid as the standard. Gallic acid is used as the standard in a modified Folin–Ciocalteu test. 100 µL of Folin–Ciocalteu reagent and 1.58 mL of DW were combined with a 20 µL aliquot of supernatant extract. Three hundred microliters of 20% sodium carbonate were added after the mixture had been allowed to react for eight minutes. The samples were vortexed right away and allowed to sit at room temperature for two hours in the dark. Next, a UV-Vis spectrophotometer (Skanit, Thermo-Fisher Scientific) was used to detect absorbance at 765 nm. TPC was represented as milligrams of gallic acid equivalent (GAE) per gram of dry sample weight, and all samples and standards were examined in triplicate.

6.2.4. Characteristics of films

6.2.4.1. Thickness

The thickness of the developed film has been evaluated according to the procedure outlined in Section 3.2.12.1.

6.2.4.2. Film solubility

Film solubility has been evaluated by the method detailed in Section 3.2.12.1.

6.2.4.3. Optical properties

Film opacity has been determined as explained in Section 3.2.12.4.

6.2.4.4. Water vapor permeability (WVP)

Barrier properties of the film as water vapor permeability (WVP) have been measured as detailed in the section 3.2.12.2.

6.2.4.5. Film color

Film color has been evaluated as described in section 3.2.12.3.

6.2.4.6. Fourier transform infrared spectroscopy (FTIR)

This spectral analysis aimed to provide insights into the chemical bonds and interactions that may have formed during the reinforcement and the active incorporation into the starch-casein fiber-reinforced film, helping to elucidate the structure and behavior of the composite material. The FTIR spectra of active film samples were analyzed employing an FTIR spectrometer (IMPACT 410, Nicolet, USA). The analysis covered the wavenumber range of 4000–500 cm^{-1}, with spectra recorded at a 4 cm^{-1} resolution following Chutia and Mahanta, (2021) method.

6.2.4.7. Mechanical properties

Elongation at break (EAB%) and tensile strength (TS) were assessed using the procedure outlined in section 3.2.12.5.

6.2.4.8. Sealing properties

Sealing strength has been determined as per the method described in section 3.2.12.6.

6.2.4.9. Thermal properties

The thermal characteristics of the film have been evaluated as per the protocol described in section 3.2.12.7.

6.2.4.10. Antioxidant activity

The antioxidant capacity of clove and cinnamon (EOs) incorporated banana pseudostem fiber-reinforced PS-casein blended films with varying EO content (0.5, 1, and 1.5%) was assessed utilizing the method of DPPH radical scavenging ability (Adilah et al., 2018). This test measures the transformation of DPPH from purple to clear, demonstrating its capacity to donate electrons. The test involved soaking 10 mL of pure ethanol with roughly 0.10 g of each film sample and shaking for 10 min to extract the antioxidants. Then, 3 mL of this film extract was combined with 1 mL of 0.1 mM ethanolic DPPH solution. The mix was kept for incubation at ambient temperature in darkness for 30 min. Using a spectrophotometer, the absorbance of the mixture was measured at 517 nm, with ethanol serving as the blank solution. The scavenging action of DPPH radicals of developed active film was determined in triplicate using the equation below.

$$\text{DPPH radical scavenging activity \%} = \frac{A_{DPPH} - sample}{A_{DPPH}} \times 100 \qquad (6.2)$$

In the above equation, A_{DPPH} = absorbance of the DPPH solution, and A_{sample} = absorbance of the film sample extract.

6.2.4.11. Antimicrobial properties

Huang et al. (2024) methodology with few modifications has been adopted for determining the antimicrobial properties of the developed banana fiber-reinforced PS-casein blended films. The antibacterial effectiveness of the films was tested for two kind of foodborne pathogens: the Gram-negative *E. coli* and the Gram-positive *S. aureus*. Culturing and diluting the bacterial broth achieved an optical density (OD600) of approximately 0.5. A small piece of film was then incubated with 200 µL of this bacterial solution at 37°C for half an hour. Following incubation, the mixture was diluted using sterile saline. After shaking the resulting bacterial solution, a 50 µL sample was spread onto a nutrient agar plate. This inoculated plate was then cultured at 37°C for a full day (24 h). Quantification of the inhibition zone was done thereby assessing the films' capacity to inhibit these pathogenic bacteria.

6.2.4.12. Release behavior

The release behavior of the best EO incorporated from the PS-CS blended BSF films was evaluated in food simulants adapting the protocol explained by Fan et al. (2022) and adopting few alterations. Liquids called food simulants are used to replicate actual food conditions. 3% acetic acid was used as an acidic food simulant, while DW was used as an aqueous/high-moisture food simulant, and 95% ethanol as a fatty food simulant. For 6 h, the simulant medium was slowly swirled at 50 rpm and 27°C. A UV-Vis spectrophotometer (Skanit, ThermoFisher Scientific, USA) was used to measure the release of EO after taking about 3 mL of the sample solution at each time interval. The procedure further involved cutting the film into 30 mm × 30 mm square and immersing it in 50 mL of the simulant solution. This was maintained at 25°C for 96 h. Absorbance were measured at specific intervals (0, 1, 2, 4, 6, 12, 24, 48, and 96 h) against banana fiber-reinforced PS film without incorporation of EOs. A UV-Vis spectrophotometer (Skanit, ThermoFisher Scientific, USA) was used to assess the amount of EOs released from the film at 287 nm. The % of EOs released was then formulated by the below equation:

$$\text{Release percentage} = M_t/M \times 100 \qquad (10)$$

In the equation, the variable Mt represents the quantity of EO that has been released from the film at a specific time t (measured in h). M denotes the initial concentration of EO present in the films before the release process begins.

6.2.4.13. Biodegradability

The biodegradability test of the films was evaluated as described in the section 3.2.12.8.

6.2.5. Statistical Analysis

SPSS statistical software (version 26, SAS Institute Inc., Cary, NC, USA) was employed for determination of the experimental data 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 determining statistical differences.

6.3. Results and discussion

6.3.1. Antioxidant activity

Fig 6.1 shows the antioxidant activity of two essential oils (EOs) - CLO and CNO. From the table, CLO has an antioxidant activity of 34.94% while CNO has an antioxidant activity of 37.594%. This indicates that CNO has a higher antioxidant activity compared to CLO. The higher the percentage, the stronger the antioxidant properties of the essential oil. CNO demonstrated superior antioxidant activity in various assays compared to other essential oils, particularly in scavenging DPPH radicals and inhibiting lipid peroxidation (Liu et al., 2023) (Eldahshan et al., 2023). Johari and Khan (2022) also evaluated the EOs antioxidant capacity from basil, lemongrass, cinnamon, and black Pepper using ABTS, DPPH, and FRAP assays. All plant samples exhibited strong free radical scavenging activity, indicating their potential as scavenging free radicals. In studies comparing CNO with other extracts, it was found that while extracts from cinnamon bark exhibited higher antioxidant activity in some cases (e.g., lower IC50 values), the essential oil still maintained significant antioxidant properties (Brodowska et al., 2016; Shahid et al., 2018).

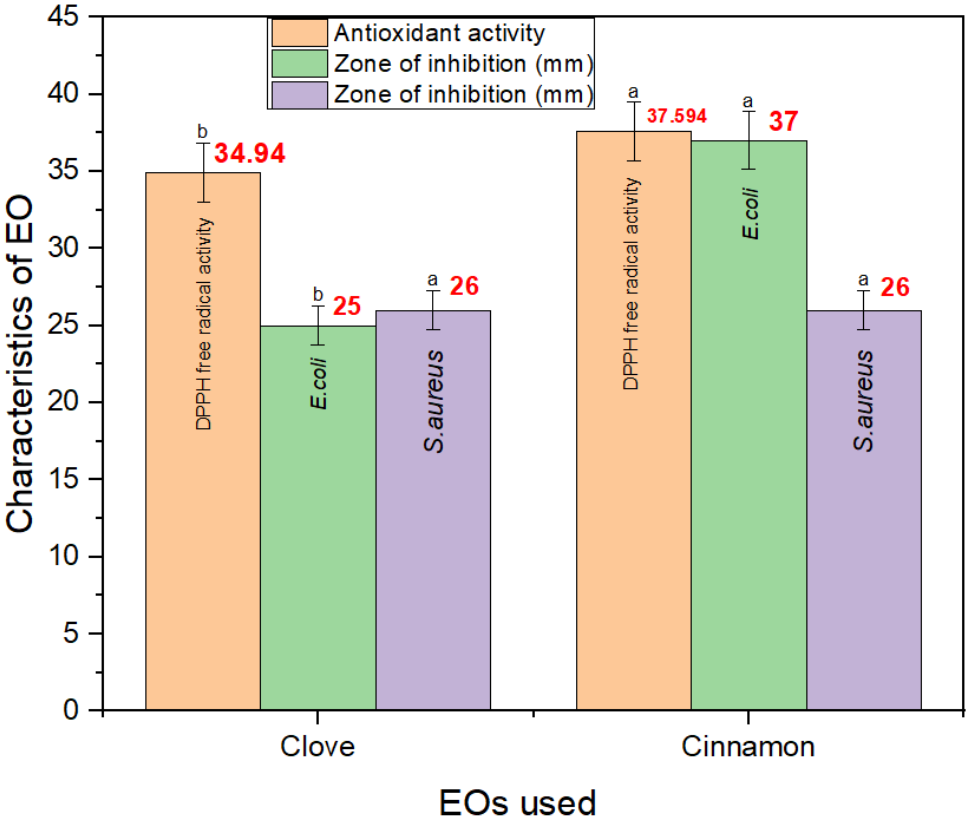

Fig. 6.1 Characteristics of Essential oils (EO) used for film preparation

6.3.2. Antimicrobial property

Fig. 6.1 presents data on the zone of inhibition (in mm) for two EOs utilized for film development, CLO and CNO, against two microorganisms: *E. coli* and *S. aureus*. **Fig. 6.2** indicates the notable zone of inhibition formed by two microorganisms. Cinnamon EO (CNO) demonstrates stronger antimicrobial effects, with 37 mm, and 26 mm inhibition zones towards *E. coli* and *S. aureus* than clove essential oil (CLO). These findings suggest that Cinnamon EO has a higher antimicrobial and antioxidant activity than Clove EO. Combining these bioactive properties suggests potential applications for these EO in active food packaging, where they could enhance the product's protective qualities. Studies have shown that incorporating cinnamon EO into composite materials can effectively create antimicrobial sachets, increasing release efficiency and microbial inhibition (Fahma et al., 2020). This aligns with other research (Khan et al., 2024; Guimarães et al., 2019; Azizkhani et al., 2021), which has also noted the significant antibacterial efficacy of essential oils against pathogens like *S. aureus*, and *E. coli*. It is known that EOs exert their antimicrobial effects by disrupting bacterial cell membranes, inhibiting enzyme activity, and interfering with metabolic processes, ultimately leading to bacterial cell death or growth inhibition. Their bioactive compounds penetrate and accumulate within the phospholipid bilayer of bacterial cell membranes, disrupting structural integrity and impacting cellular metabolism, ultimately leading to cell death (Chouhan et al., 2017). These properties underscore the potential for EOs in developing natural and effective antimicrobial solutions in food preservation and other applications.

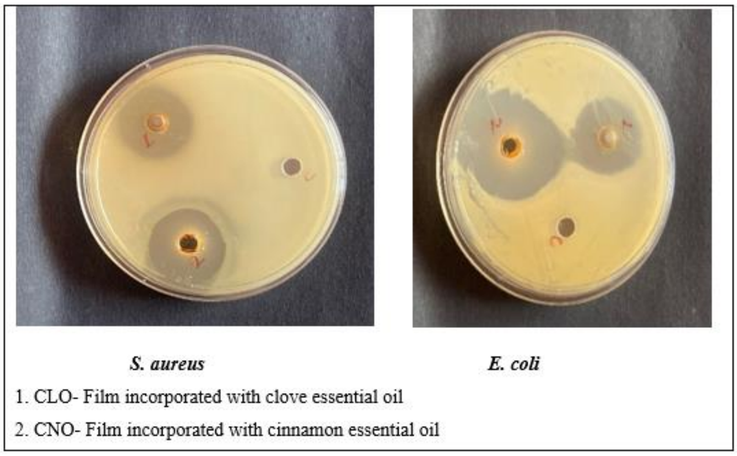

Fig. 6.2 Antimicrobial activity of EOs used for film preparation against *S. aureus* and *E. coli*

6.3.3. Total phenolic count

Table 6.2 presents the TPC of EOs (clove and cinnamon). The table provides the total phenolic content of clove and CNOs (EOs), measured in terms of gallic acid equivalents (GAE) per 100 mL. The data shows that CLO has a significantly higher phenolic content (104.47 mg/100 mL) compared to CNO (9.334 mg/100 mL). This difference suggests that CLO contains more phenolic substances, that are known for its antioxidant, antimicrobial, and preservative properties, making it potentially more effective for applications in bioactive films. In comparing these results with published research, studies confirm that CLO generally has a high concentration of phenolic compounds, particularly eugenol, which is responsible for its strong antioxidant and antimicrobial activities (Kumar et al., 2022). This high phenolic content makes CLO an effective ingredient for enhancing the functional characteristics of packaging films, like extending shelf life and preventing microbial growth. CNO, while also beneficial, typically has a lower phenolic content compared to CLO, with cinnamaldehyde being its main active compound. Although lower in phenolics, CNO still exhibits antioxidant and antimicrobial properties but may be less potent in comparison to CLO (Tomaino et al., 2005). This comparison aligns with the findings in the table, where CLO's higher phenolic content suggests it may be more suitable for applications requiring strong antioxidant effects, while CNO can still contribute to bioactivity but with less intensity. Incorporating these EOs into biofilms could enhance the preservation qualities of packaging, with CLO potentially offering superior efficacy in films due to its higher phenolic content**.**

Table 6.2 Total phenolic content of EOs used

Sample	**TPC (mg GAE/100 mL)**
Clove EO	104.47± 4.98[a]
Cinnamon EO	9.334± 0.44[b]

6.3.4. Characteristics of films

6.3.4.1. Determination of thickness

Table 6.3 describes the thicknesses of the previously developed film now incorporated with varied concentrations of the active component used (0.5%, 1%, 1.5%) CNO and CLO, showing significant variations ($p>0.05$). The thickness values varied from the observed are 0.353 mm, to 0.377 mm, respectively, indicating a slight increase in thickness as the concentration of CLO rises. This gradual increase in thickness could be attributed to a few factors. First, adding more EO to the material likely introduces the additional volume, causing a minimal growth in the structure and thus a marginal increase in thickness. Additionally, higher concentrations of EO may enhance interactions within the matrix, possibly leading to minor structural adjustments that result in a thicker material. CNO might also affect the density or flexibility of the banana-fiber-reinforced PS-casein composites, as essential oils can alter the packing of molecules within the material, leading to a less compact arrangement. Previous research has shown that incorporating essential oils into films often leads to slight changes in physical properties, including thickness, due to the oil’s impact on the film matrix (Rawat and Saini, 2024). Fahma et al. (2020) and Azizkhani et al. (2021) found that the addition of essential oils such as cinnamon and clove led to marginal increases in thickness, which were attributed to the oils creating additional volume within the polymer matrix. These findings suggest that EOs, by interacting with the film matrix, may slightly expand or disrupt the compactness of the material's structure, leading to a minor but measurable increase in thickness. Moreover, the results align with findings by Khan et al. (2024), which indicate that essential oils, when added in higher concentrations, contribute to changes in the molecular arrangement within the film, often affecting density and flexibility. This effect can lead to a less compact structure, resulting in increased thickness, as seen with the highest concentration of 1.5% CNO in this table. However, these thickness variations are typically minor, suggesting that essential oils can be added to films without significantly compromising their structural integrity—an advantageous property for applications in active packaging, where maintaining physical characteristics alongside antimicrobial properties is essential.

Table 6.3 Characteristics of developed active PS-CS blended film reinforced with BPF

Parameters	**Units**	**0.5% CNO**	**1% CNO**	**1.5% CNO**	**0.5 CLO**	**1%CLO**	**1.5% CLO**
Thickness	**mm**	0.353±0.01[c]	0.354±0.01[c]	0.362±0.01[b]	0.352±0.01[c]	0.361±0.01[b]	0.377±0.01[a]
Opacity	**mm^{-1}**	0.88±0.04[d]	0.543±0.02[e]	0.469±0.02[f]	0.96±0.04[c]	1.10±0.05[b]	1.32±0.05[a]
L*		88.25±4.40[a]	86.24±4.30[b]	72.37±3.60[c]	64.32±3.20[cd]	66.35±3.30[d]	60.52±3.00[e]
a*		-0.64±0.03[d]	-0.15±0.007[e]	-0.16±0.006[e]	1.45±0.07[b]	1.22±0.06[c]	1.64±0.08[a]
b*		15.51±1.25[b]	12.12±0.60[c]	11.74±0.08[d]	12.12±0.60[d]	16.12±0.78[a]	8.32±0.40

L*: Lightness; a*: Redness; b*: Yellowness. Values are given as mean ± standard error (SE). a, b, c, d: Different letter subscripts in the same column indicate a statistically significant difference *($p < 0.05$)*, CNO- Cinnamon EO, CLO- Clove EO

6.3.4.2. Film color

Table 6.3 presents color measurements, lightness (L*), green-to-red (a*), and blue-to-yellow (b*), including total color difference (ΔE), for banana fiber-reinforced PS-casein blended film incorporated with increasing concentrations of cinnamon and clove (EO). The appearance of the active film has been shown in **Fig. 6.3** compared with individual PS, casein, and banana-pseudostem-reinforced films. As the concentration of EO rises, significant variations ($p<0.05$) are noticed in these colorimetric parameters, which are often associated with the natural coloration and chemical composition of CNO. The lightness decreases from 88.25 to 72.37 with higher EO concentrations, indicating that the material becomes darker as more CNO is added. This reduction in L* could be attributed to the natural color of CNO, imparting a yellow-brown hue, and reducing the lightness of the material (Ghasemlou et al., 2013). The a* values shift from slightly negative (-0.64 and -0.15) to a positive red tone (16.15) as EO concentration increases. This shift towards red can be attributed to the cinnamaldehyde and other phenolic compounds in CNO, which give it a reddish tint that becomes more pronounced at higher concentrations (He et al., 2021). The *b* values decreased from 25.51 to 1.74, suggesting a reduction in yellow tones. The high initial b* values may reflect the initial yellowish hue, which becomes less intense as the red tint (represented by increasing a* values) dominates with higher EO levels (Hosseini et al., 2009). The ΔE values range widely, from 9.85 to 33.12, showing that the material undergoes significant color changes with increasing EO concentrations. High ΔE values indicate substantial visual differences, likely due to the combined changes in L*, a*, and b*, consistent with findings in other studies on essential oils' impact on film color (Atares et al., 2016). These changes indicate that the addition of Cinnamon and CLO made the films less bright and more opaque. The overall effect of adding CNO to the developed composite film matrix was the production of films that were less dark and more transparent. Wu et al. (2017) observed that adding CNO to biofilms caused a decline in lightness and an increase in red tint, due to the natural pigments and phenolic compounds in the EO. Similarly, He et al. (2021) found that incorporating EOs such as cinnamon caused color shifts in films due to the oils' natural colors, with higher concentrations leading to more intense red tones. Color parameters are crucial in the production of packaging materials because they influence both the functionality and the aesthetic appeal of the packaging. These recent studies confirm that CNO can effectively alter the color properties of films, which may be useful for creating visually distinctive packaging materials. However, the color changes could also be a drawback in applications where minimal color alteration is preferred. Thus, the impact of essential oils on color should be considered carefully in the layout of bio-based packaging substances.

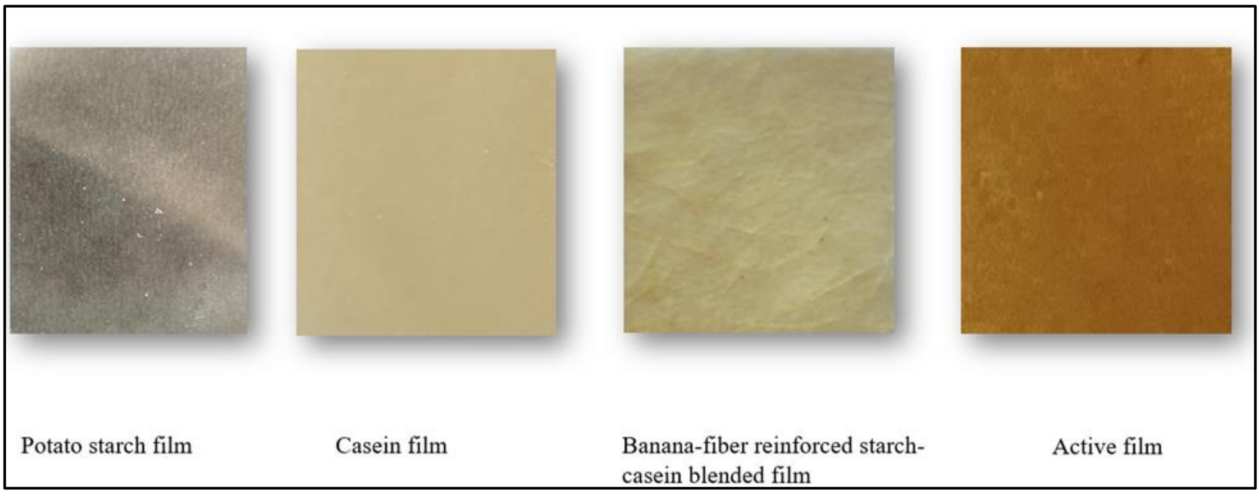

Fig. 6.3 Appearance of developed films

6.3.4.3. Film solubility

Fig. 6.4 illustrates the solubility behavior of banana fiber-reinforced potato starch blended films incorporated with different concentrations of CNO and CLO at 0.5, 1.0, and 1.5%. The results show that the film with 0.5% CNO exhibited the highest solubility (approximately 16.5%), which was significantly different ($p<0.05$) from the other concentrations. Films containing 1.0% and 1.5% CNO showed slightly lower solubility values (around 16.3% and 16.2% respectively. The decreased solubility with increasing CNO concentration can be attributed to the hydrophobic nature of essential oils and their interaction with starch molecules (Khan et al., 2024). This finding aligns with research by Acevedo-Fani et al. (2015) and Ghasemlou et al. (2013), who showed that EOs can form cross-links with starch molecules and create a more hydrophobic network, thereby reducing the film's water solubility. The slight decrease in solubility with increasing CNO concentration (from 16.5% to 16.2%) follows a similar trend reported by Hafsa et al. (2016) in chitosan films containing citrus EOs, where they observed reduced solubility due to the formation of a more compact and hydrophobic matrix. Lower solubility often correlates with improved barrier properties against gases and water vapor which can lead to better preservation of food quality and extended shelf life (Dutta and Sit, 2024). Similarly, Pelissari et al. (2009) demonstrated that incorporating oregano EO into starch-based films decreased water solubility due to the enhancement of hydrophobic interactions within the polymer network. The relatively high overall solubility values (>16%) observed here are comparable to those noticed by Travalini et al. (2019) for natural fibers reinforced cassava starch films, suggesting that the banana fiber reinforcement maintains film biodegradability while providing structural support indicating a saturation point in the polymer-oil interactions. Moreover, Kuorwel et al. (2013) reported similar findings with starch-based films containing thymol, where the solubility reduction reached a plateau after certain essential oil concentrations, suggesting an optimal loading capacity for maintaining film functionality.

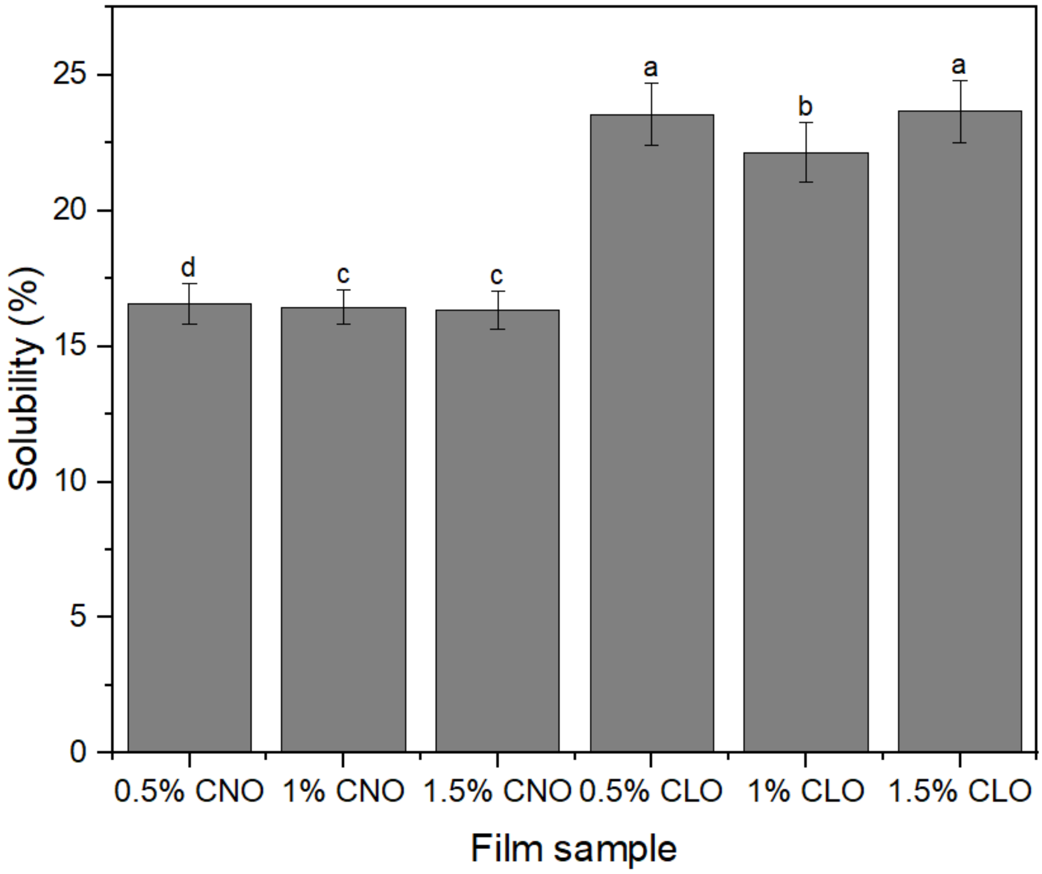

Figure 6.4. Film solubility (%) of active PS-CS blended film reinforced with BPF (CNO-cinnamon EO, CLO-clove EO)

6.3.4.4.Optical properties

Table 6.3 appears to show opacity measurements for active banana fiber-reinforced PS-casein blended films with varying concentrations of cinnamon and CLO

(EO). The opacity values decrease from 0.88 to 0.469 as the concentration of CNO is presumably increased, suggesting that the incorporation of CNO makes the films more transparent. This reduction in opacity can be attributed to the plasticizing effect of essential oils, which can modify the polymer matrix structure and improve light transmission through the film. This finding aligns with research by Souza et al. (2013) who observed that essential oils can alter the internal structure of starch-based films, leading to better light transmission. Additionally, the interaction between the hydrophobic essential oil compounds and the hydrophilic starch-casein matrix may create a more homogeneous structure that allows better light penetration, as demonstrated in similar work by Atarés and Chiralt (2016) with essential oil-incorporated biopolymer films. Lower opacity values facilitate better light transmission and can help in UV-based sterilization processes. It also enables the use of active packaging technologies that require visibility. Studies by Singh et al. (2021) have shown that transparent packaging can increase product sales by up to 40% compared to opaque alternatives.

6.3.4.5.Fourier-transform infrared spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (FTIR) analysis is a crucial characterization method used to identify surface functional groups and confirm the formation of covalent bonds, providing concrete evidence of successful reinforcement and modification (Dutta and Sit, 2024). From **Fig. 6.5**, the FTIR analysis revealed characteristic peaks in the CNO and CLO spectrum, including -OH (3517 cm^{-1}), C=O (1228 cm^{-1}), and C=C bonds (1619, 1523, and 1438 cm^{-1}) in the phenyl ring. The developed active film exhibited distinctive stretching vibrations for hydroxyl groups (3278 cm^{-1}), aliphatic CH_2 and CH_3 groups (2985, 2961, and 2895 cm^{-1}), amide-I C=O bonds (1654 cm^{-1}), amide-II CN and N-H groups (1543 cm^{-1}), and amide-III C-N bonds (1237 cm^{-1}). The active film with incorporation of CNO and CLO FTIR spectrum demonstrated stretching vibrations corresponding to both PS, casein, and banana-fiber incorporation and pure EO, encompassing amide I, II, and III groups along with phenyl ring C=C bonds and C=O bonds, confirming successful EO incorporation in the starch-casein matrix (Bertolo et al., 2022). The pure PS film displayed distinctive peaks at 3462, 2928, and 1614 cm^{-1}, representing -OH, CH, and H-O-H groups respectively, while peaks at 905, 986, and 1032 cm^{-1} indicated C-O-C bonds in the starch chain. The addition of CLO and CNO into the PS-casein matrix developed alterations to the location and strength of peaks of pure starch, indicating the successful integration of these nanoparticles into the bio-nanocomposite films' structure. As illustrated in **Fig. 6.5**, the hydroxyl group peak at 3462 cm^{-1} in pure starch broadened and shifted to 3449 cm^{-1} upon addition of EO-containing banana fiber particles, suggesting hydrogen bond formation between the film matrix and banana fiber particles (Chen et al., 2018). These findings align with previous research regarding FTIR spectral changes. Alinaqi et al. (2021) reported the disappearance of the amide-II peak when incorporating zein nanoparticles into starch-based films, attributing this to new bond formation, while also noting hydroxyl group bond position changes due to new hydrogen bonds. Moreover, Bertolo et al. (2022) observed increased hydroxyl group peak intensity when studying FTIR spectra of poly (ethylene oxide)-based composite films containing zein nanoparticles, confirming interaction and compatibility between zein and the PEO matrix. The enhanced properties of the EOs incorporated PS-casein blended banana pseudostem fiber-reinforced film can be attributed primarily to the interplay between added EO-loaded fiber particles and PS and casein. This interaction strengthens the film's structural network, as evidenced by both the film's morphological features shown in **Fig. 10** along with the results obtained from FTIR analysis (Zhang et al., 2022).

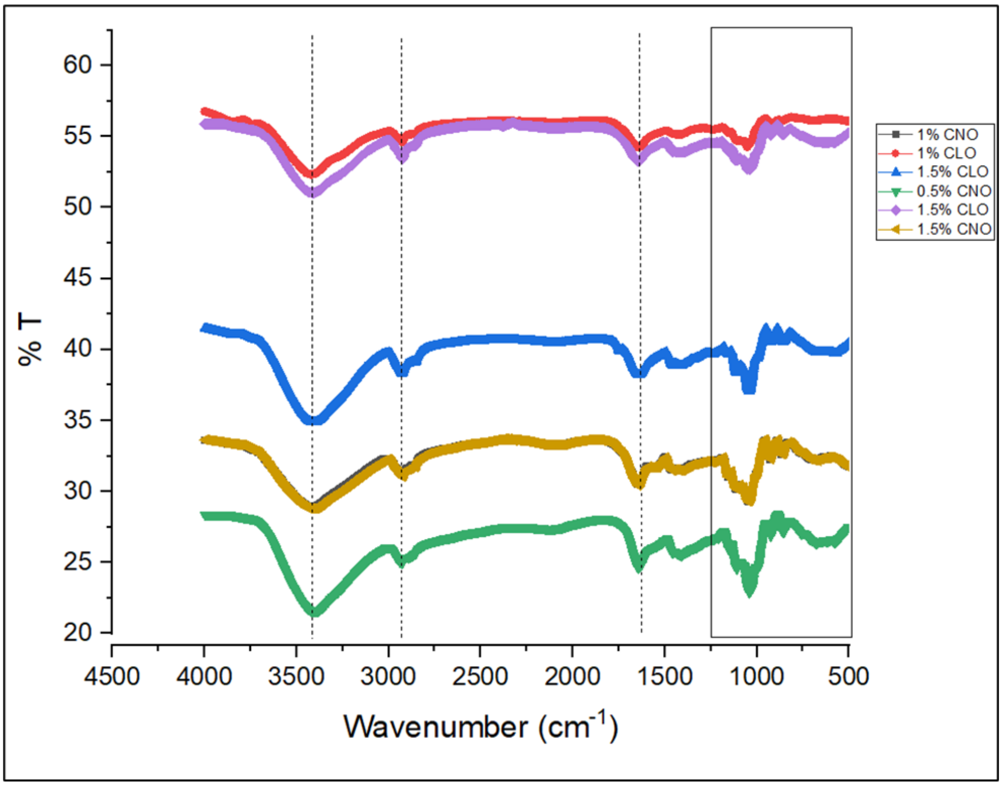

Fig 6.5. FT-IR spectra of active PS-CS blended film reinforced with BPF

(CNO-Cinnamon EO, CLO-clove EO)

6.3.4.6.Water vapor permeability (WVP)

Fig. 6.6. displays the Water Vapor Permeability (WVP) values in g.mm/m2/h/kPa for active banana fiber-reinforced PS and casein blended films with different concentrations of cinnamon and clove (CNO/CLO) at 0.5, 1.0, and 1.5%. The WVP values show slight variations across different CNO concentrations, ranging from approximately 0.15 to 0.288 g.mm/m^2/h/kPa. The relatively stable WVP values despite increasing CNO concentration suggest that the EO's hydrophobic nature may be effectively counter balanced by the hydrophilic properties of starch and casein in the film matrix. This observation aligns with research by Atarés and Chiralt (2016), who found that EOs can create a tortuous path for water molecules without significantly altering the overall water barrier properties of biopolymer films. Additionally, studies by Hosseini et al. (2015) have shown that protein-polysaccharide matrices can maintain relatively stable water barrier properties even with the addition of hydrophobic compounds due to their strong internal network structure and the formation of stable emulsions with the EOs. The water vapor transmission rate (WVTR) measures how quickly water in gas form passes through a film or membrane. The interaction between lipid materials and the biopolymer matrix hinders water molecule interactions, leading to lower WVTR (Xue et al., 2023). Films with minimum WVTR are preferred for food packaging as they help prevent moisture migration and reduce spoilage. The WVP values observed in the current study align well with findings by Souza et al. (2013) for cassava starch films containing CNO (0.13-0.17 g.mm/m^2/h/kPa) and de Moraes Crizel et al. (2018) for fiber-reinforced starch-based films (0.14-0.19 g.mm/m^2/h/kPa). Interestingly, while the current study shows relatively stable WVP across different CNO concentrations, contrasting trends were observed by Dong et al. (2019) and López-Cano et al. (2023), who reported significant decreases in WVP with increasing essential oil concentration in gelatin and starch films respectively. These variations can be attributed to different matrix compositions, relative humidity conditions during testing, film preparation methods, and complex interactions between multiple components (starch, casein, pseudostem fiber, and CNO) that influence the barrier properties. The relatively higher WVP values in the current study may be primarily because of the dominant hydrophilic behaviour of both starch and casein in the film matrix, which persists despite CNO addition.

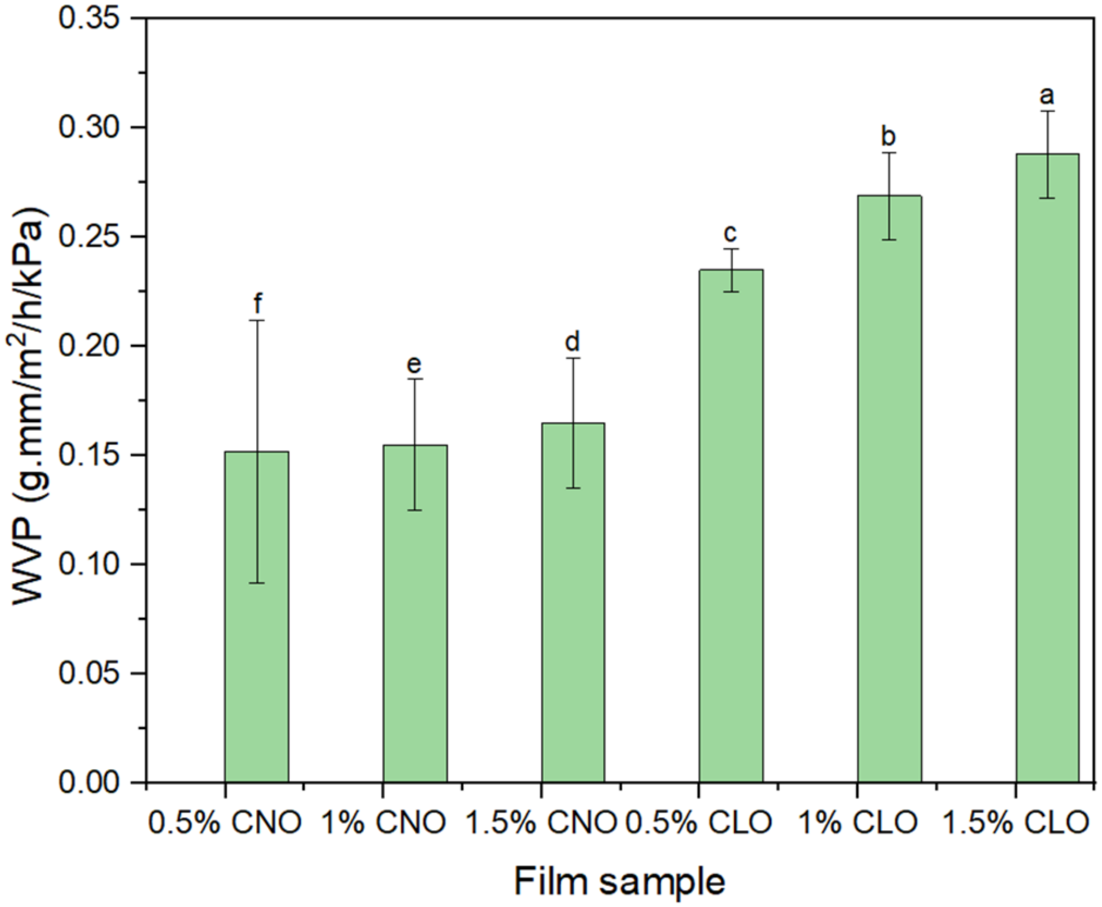

Fig. 6.6. Water vapor permeability (WVP) of developed active active PS-CS blended film reinforced with BPF

(Cinnamon EO-CNO, Clove EO-CLO)

6.3.4.7. Mechanical properties

Incorporating EOs like cinnamon and clove in biopolymer films is a known approach to enhance their properties**. Fig. 6.7. (a and b)** show the tensile strength (TS) and EAB (%) of banana pseudostem fiber-reinforced potato starch-casein blended films, which have been included with varied amount of CLO/CNO: 0.5%, 1%, and 1.5% presenting significant differences ($p<0.05$). 1% CNO shows the highest tensile strength among the samples, suggesting that this concentration may optimize the mechanical reinforcement effect of CNO in the film. 1.5% CNO demonstrates a lower tensile strength than the 1% CNO, implying that an increased concentration of CNO may not linearly improve the tensile strength and may even have an adverse effect at higher concentrations.

Ghasemlou et al. (2013) examined the effect of CNO on starch-based films, finding that optimal concentrations improved mechanical properties due to enhanced intermolecular interactions. Similarly, Alizadeh-Sani et al. (2018) explored the use of EOs in protein-based films and observed improved tensile strength at certain oil concentrations due to the plasticizing effect and hydrophobic interactions. The tensile strength values obtained in this study are comparable to other biopolymer films, such as those noticed by Pelissari et al. (2009) for banana starch-chitosan films (4-8 MPa), indicating that these films have promising mechanical properties for potential packaging applications. The figure demonstrates the elongation at break (EAB%) behavior of banana fiber-reinforced potato starch-casein blended films with varying concentrations of CNO (CNO). The results reveal that 1% CNO exhibits the highest elongation at break of approximately 3.5%, followed by 1.5% CNO at 3.1% and 0.5% CNO at 2.7%. This trend can be defined by the action of plasticization of CNO at an optimal concentration (1% CNO), which enhances the flexibility of the polymer matrix by reducing intermolecular forces and increasing the mobility of polymer chains. However, the decrease in EAB% at higher CNO concentration (1.5% CNO) could be attributed to the phase separation and structural discontinuities in the polymer matrix, as observed in similar research by Atarés et al. (2010) in their study on cinnamon-fortified sodium caseinate films. The EAB% values obtained in this study align with those reported by Martelo-Guzmán et al. (2022) for cassava starch films with essential oils (2-4%), where they also found that excessive essential oil content led to decreased film flexibility. The moderate elongation values suggest that while these films maintain some flexibility, they are relatively rigid compared to conventional synthetic packaging materials, which typically show EAB% values above 10%. This characteristic rigidity can be attributed to the strong bond between banana fibers and the starch-casein matrix, including the crystalline nature of the reinforcing fibers (Das and Kalyani, 2023). Also, the results reveal that 1% CNO exhibits the highest elongation at break (EAB) of approximately 3.5%, followed by 1.5% CNO at 3.1% and 0.5% CNO at 2.7%. This trend can be explained by the action of plasticization of CNO at an optimal concentration (1% CNO), which enhances the flexibility of the polymer matrix by enhancing polymer chain mobility and decreasing intermolecular pressures (Xue et al., 2024). However, the decrease in EAB% at higher CNO concentration (1.5 CNO) could be attributed to the phase separation and structural discontinuities in the polymer matrix, as observed in similar research by Atarés et al. (2010) in their study on cinnamon-fortified sodium caseinate films. The EAB% values noticed in this study align with those reported by Valencia-Sullca et al. (2018) for cassava starch films with essential oils (2-4%), where they also found that excessive essential oil content led to decreased film flexibility. The moderate elongation values suggest that while these films maintain some flexibility, they are relatively rigid compared to conventional synthetic packaging materials, which typically show EAB% values above 10%. Furthermore, CNO was incorporated which changed the shape of oil droplets, making them easily distorted, which increased the film's elongation (Mittal et al., 2023). Improved mechanical properties could be also due to the even distribution of a huge surface area of the matrix incorporated with EOs by hydrogen bonding improving the interfacial adhesion and crystallinity between the polymer phases (Xue et al., 2024) also discussed in FTIR and X-RD sections. Thus, it demonstrates that the reinforcement and incorporation raise the ability of potato starch and casein matrix for absorption of energy with applied force. Therefore, the bio-composites utilized in the study incorporated with 1% CNO are suitable for usage as functional packaging materials.

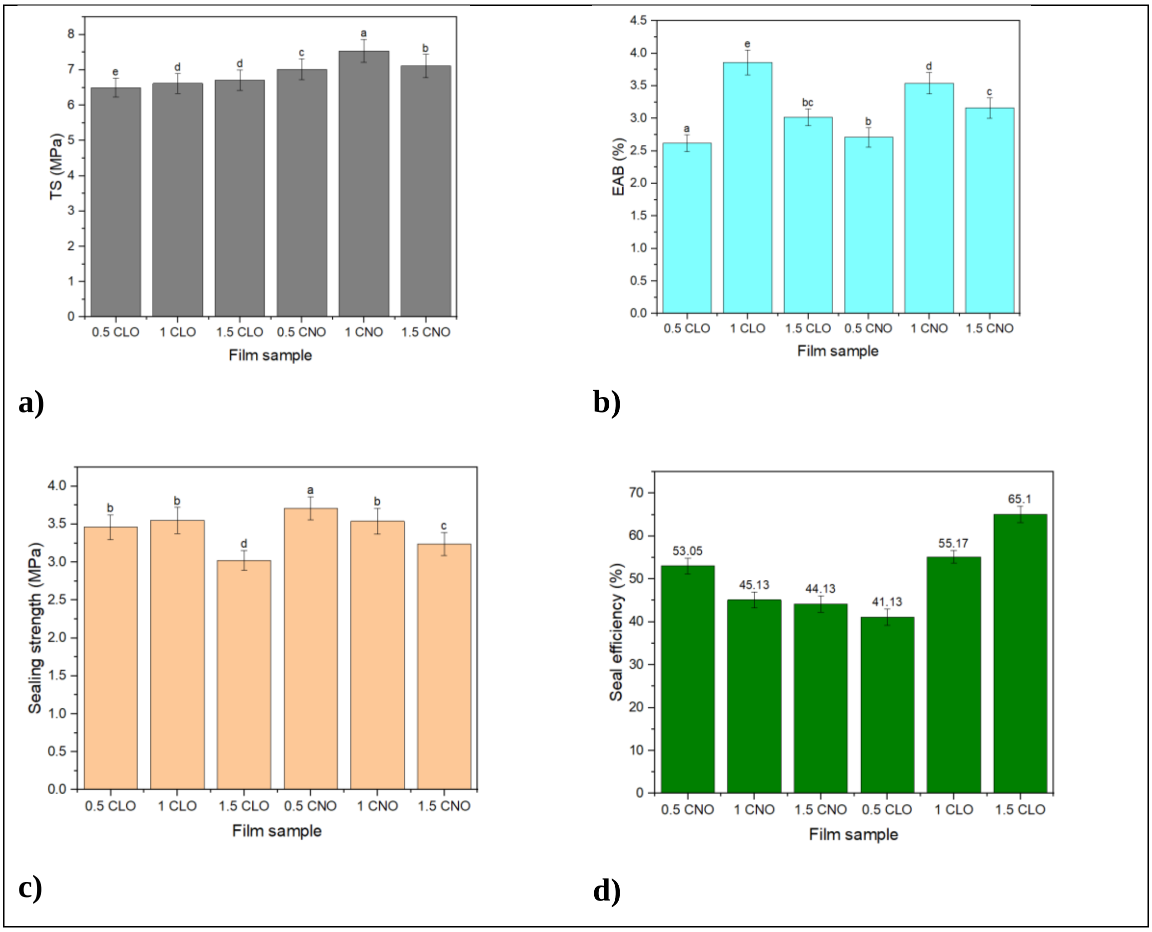

Fig. 6.7. a) and b) Tensile strength (TS) and Elongation at break (EAB%) c) Sealing strength d) Sealing efficiency of developed active PS-CS blended film reinforced with BPF, (Cinnamon EO-CNO, Clove EO-CLO)

6.3.4.8.Sealing properties

The **Figs. 6.7 c)** and **d)** have presented the seal strength (MPa) and sealing efficiency (%) of banana fiber-reinforced potato starch-casein blended films with varying concentrations of CNO and CLO varying significantly. The sealing efficiency shows a decreasing trend with increasing CNO concentration, with the highest value of approximately 52% at 0.5 CNO, followed by 45% at 1 CNO, and 42% at 1.5 CNO. Similarly, the seal strength demonstrates a declining pattern, with values of about 3.7 MPa, 3.4 MPa, and 3.2 MPa for 0.5, 1, and 1.5 CNO concentrations, respectively. This decrease in both sealing properties with increasing CNO could be ascribed to the hydrophobic nature of EOs, which may interfere with polymer chain interactions at the sealing interface (Mittal et al., 2023). Also, decrease in sealing efficiency can be attributed to possible migration of CNO to film surface affecting seal integrity. This phenomenon is consistent with research by Siripatrawan and Harte (2010) on chitosan films containing green tea extract, where higher concentrations of hydrophobic components decreased film sealability. The seal strength values obtained are in agreement to those reported by Shankar et al. (2019) for alginate-based films (2.5-4.0 MPa), though lower than conventional synthetic packaging materials. The statistical differences ($p<0.05$) suggest that CNO concentration significantly affects the sealing properties, likely due to the oil's impact on surface properties and polymer chain mobility during heat sealing. The moderate sealing properties indicate that while these films can be heat-sealed, their performance might be limited for high-barrier packaging applications requiring very strong seals. The sealing properties obtained were similar to results by Roy and Rhim et al. (2019) for agar-based films (3.0-3.8 MPa) and better than protein-based films by González et al. (2019) (2.5-3.0 MPa). The results demonstrate promising sealing properties, particularly in applications for dry food packaging where moderate seal strength is adequate. Also, the results provide valuable insights for developing improved bio-based packaging materials with enhanced sealing characteristics.

6.3.4.9.Thermal properties

Table 6.4 and **Fig. 6.8** illustrate the thermal degradation stages of PS-casein banana fiber-reinforced films incorporating 0.5%, 1%, and 1.5% CNO and CLO. The thermal degradation data is organized into three stages with respective mass loss percentages and peak temperatures. The thermal degradation behavior of banana fiber-reinforced PS-casein films with varying CNO concentrations (0.5%, 1%, and 1.5%) shows distinct three-stage degradation patterns. The first stage (around 111-118°C) represents moisture evaporation and volatile compounds loss, with 1.5% CNO showing the highest mass loss (-96.62%) due to greater CNO content. The second stage (239-251°C) corresponds to the decomposition of starch and protein components, showing mass losses of -44.98%, -37.80%, and -42.34% for 0.5%, 1%, and 1.5% CNO respectively. The third stage (506-599°C) represents the degradation of more thermally stable components and char formation, with mass losses ranging from -27.22% to -34%. The residual mass varies significantly, with 1.5% CNO showing negative residual mass (-88.05%) indicating complete degradation, while 0.5% and 1% CNO retain about 13% mass. For the 0.5% CNO concentration, the peak degradation temperatures are observed at 111.1°C, 251.0°C, and 599.1°C, with a residual mass of 13.28%. At the 1% CNO concentration, the peak degradation temperatures occur at 118.5°C, 239.7°C, and 507.2°C, and the residual mass is similar to that of the 0.5% CNO sample at 13.03%. For the 1.5% CNO concentration, the second and third stages show mass losses of 42.34% and 27.22%, with peak degradation temperatures at 115.7°C, 250.8°C, and 506.8°C. The residual mass for this concentration is -88.05%, indicating almost complete decomposition. This thermal behavior aligns with research by Ghasemlou et al. (2013) who reported similar three-stage degradation in essential oil-incorporated starch films. The degradation temperatures are comparable to findings by Liu et al. (2017) for protein-based films, though showing higher thermal stability in the second stage. The impact of CNO concentration on thermal stability corresponds with the work by Zhang et al. (2015), who observed decreased thermal stability with increasing essential oil content in composite films. The residual mass trends can be compared to research by Wang et al. (2018) on fiber-reinforced biopolymer films, though showing better thermal resistance at lower EO concentrations. From the results, each sample exhibits distinct degradation patterns that correlate with the mass loss percentages and peak temperatures in the table. The 0.5% and 1% CNO samples show stable decomposition phases with clear degradation steps, while the 1.5% CNO sample has a more unstable profile with rapid mass loss in the initial stage. After the incorporation of CNO, an improvement in thermal stability was observed. The presence of CNO generally enhances thermal stability due to the EO's hydrophobic nature and its potential interactions with the polymer matrix. However, excessive EO concentration (as in 1.5% CNO) can lead to structural instability and rapid mass loss at lower temperatures, which agrees with similar findings in biopolymer films containing volatile compounds (Xu et al., 2018; Hosseini et al., 2022). Studies by Arfat et al. (2014) and Liu et al. (2020) report that moderate essential oil levels improve stability, but higher concentrations can disrupt film integrity, leading to premature degradation. The enhanced thermal properties of the CNO banana-reinforced starch-casein films were also could be attributed to the homogeneous dispersion of microfibers and increased interfacial hydrogen bonding, which restricted the mobility of starch polymer chains at higher temperatures (Dutta and Sit, 2024).

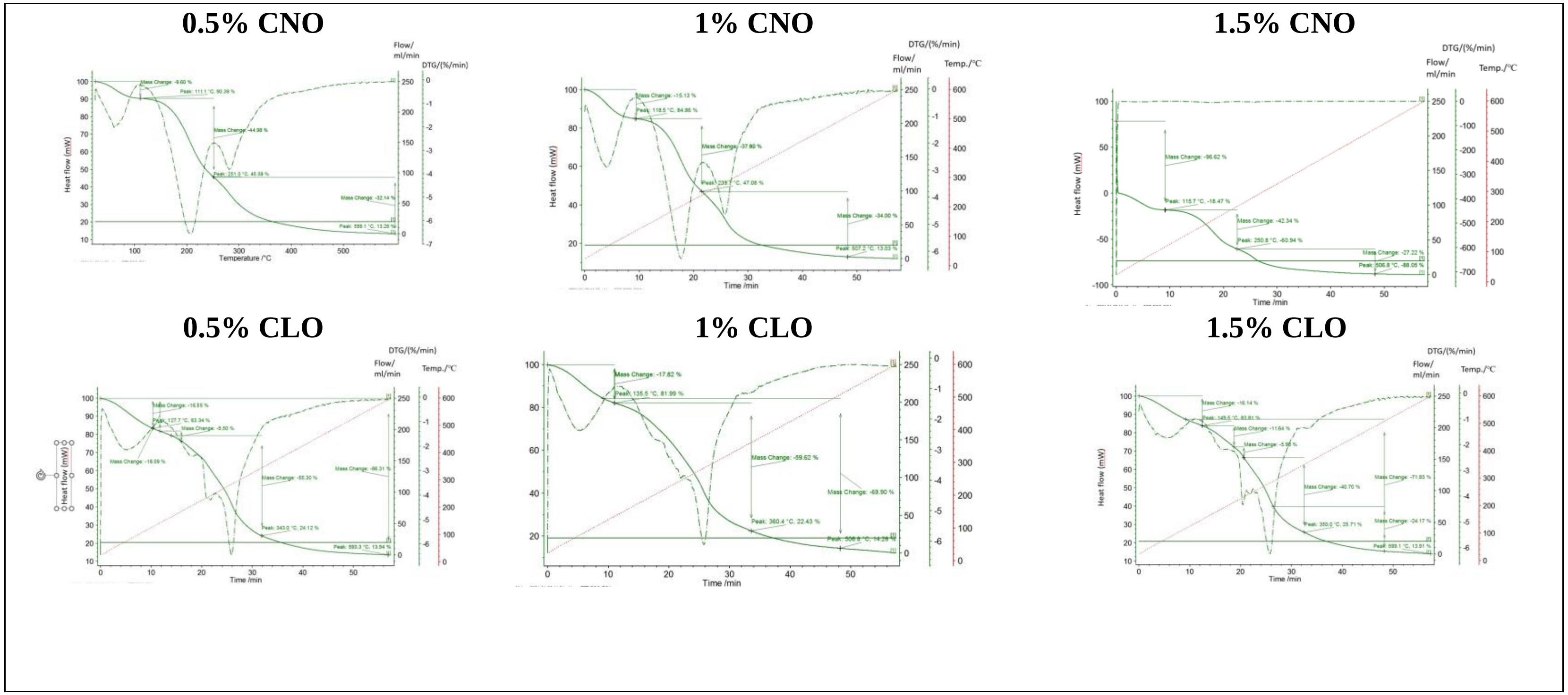

Fig. 6.8 Thermo-gravimetric analysis of developed PS-casein blended BPF incorporated with CNO and CLO, (Cinnamon EO-CNO, Clove EO-CLO)

Table 6.4 Thermal degradation stages of PS-casein BPF-reinforced film incorporated with EOs

Film Sample	**Mass changes (stage wise)**			**Peak**			**Residual mass (%)**
	1ST (%)	**2nd (%)**	**3rd (%)**	**1 (°C)**	**2 (°C)**	**3 (°C)**	
0.5 CNO	9.60	-44.98	-32.14	111.1	251.0	599.1	13.28
1 CNO	-15.13	-37.80	-34	118.5	239.7	507.2	13.03
1.5 CNO	-96.62	-42.34	-27.22	115.7	250.8	506.8	-88.05
0.5 CLO	-18.02	-55.30	-86.31	127.7	343.0	593.3	13.54
1 CLO	-17.82	-59.62	-69.90	135.5	360.4	508.8	14.26
1.5 CLO	-16.14	-40.70	-71.93	149.5	350.0	599.1	13.91

(CNO- Cinnamon EO, CLO- Clove EO)

6.3.4.10. Antioxidant properties

DPPH radical scavenging activity data for different concentrations of CNO (CNO) in banana fiber-reinforced potato starch-casein films, showed a clear trend presented in **Fig. 6.9**. The film with 1% CNO showed the highest DPPH radical scavenging activity at approximately 330%, followed by 1.5% CNO at around 240%, while 0.5% CNO exhibited the lowest activity at about 200% indicating statistically significant differences between all concentrations ($p>0.05$). This trend in antioxidant activity can be explained by the bioactive compounds present in CNO, particularly cinnamaldehyde and cinnamic acid, which collectively contribute to its strong antioxidant properties known for their free radical scavenging properties. The optimal activity at 1% CNO suggests there's an ideal concentration for maximum antioxidant effectiveness. The lower activity at 1.5% CNO might be due to the aggregation of essential oil components at higher concentrations, potentially reducing their availability for free radical scavenging. Wang et al. (2024) also reported optimal antioxidant activity in starch-based films at moderate essential oil concentrations. Similarly, MO Syafiq et al. (2020) found that incorporating CNO in biopolymer films enhanced their antioxidant properties, with peak activity observed at intermediate concentrations. Yang et al. (2024) also documented comparable DPPH radical scavenging patterns in similar composite films. The composite films produced exhibit strong antioxidant properties, making them promising candidates for usage in active packaging of foods. They could offer protection for foods prone to oxidation, aiding in quality preservation and shelf-life extension (Zhang et al., 2022).

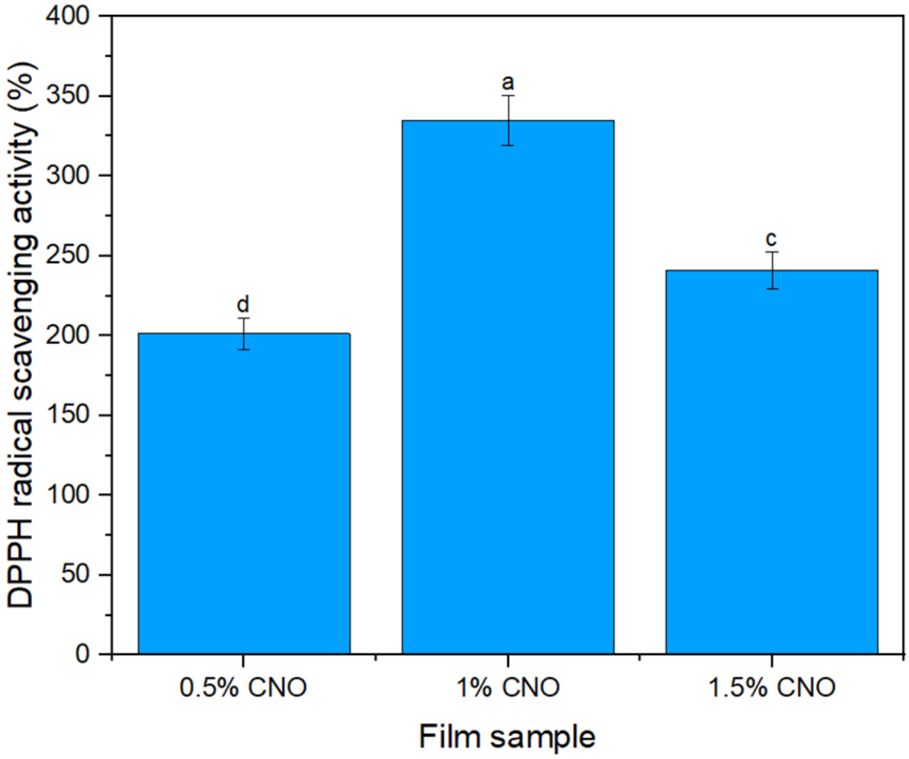

Fig. 6.9 DPPH radical scavenging activity of developed active PS-CS blended film reinforced with BPF (CNO-Films incorporated with cinnamon essential oil)

6.3.4.11. Antimicrobial activity

Table 6.5 shows the antimicrobial activity against three pathogenic bacteria (*S. aureus*, and *E. coli*) for different concentrations of cinnamon EO (CNO) in banana pseudostem fiber-reinforced potato starch-casein films. For *S. aureus*, the inhibition zone increased significantly with increasing CNO concentration, showing the highest activity (75 mm) at 1.5% CNO compared to 33 mm at 0.5% CNO. However, for *E. coli*, the trend was the opposite - the inhibition zones decreased with increasing CNO concentration. *E. coli* exhibited zones of 45 mm, 38 mm, and 32 mm. This differential antimicrobial effectiveness can be attributed to the complex interaction between CNO's active compounds (primarily cinnamaldehyde) and the different cell wall structures of gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria. The improved activity towards *S. aureus* at higher concentrations aligns with previous research findings in antimicrobial packaging materials. Sharma et al. (2020) highlighted the antimicrobial properties of CNO (EO) in a PLA/PBAT blend for active food packaging. Brnawi et al. (2019) showed its strong effectiveness against *Salmonella typhimurium* and *Listeria monocytogenes*. Zhang et al. (2016) explored CNO's antibacterial mechanism in case of *E. coli* and *S. aureus*, revealing that it disrupts cell membranes at the minimum inhibitory concentration (MIC) and kills cells at the minimum bactericidal concentration (MBC). Additionally, CNO caused an early increase in electrical conductivity due to electrolyte leakage, raised protein and nucleic acid levels in suspensions, and reduced bacterial metabolic activity by 3-5-fold. The result found that films containing the incorporation of active ingredient demonstrated strong antibacterial effects, though an excessively high concentration of CNO reduced this inhibitory ability. Micropores in the films may enhance CNO diffusion. Therefore, an optimal CNO (1%) is needed to improve the films' antimicrobial effectiveness (Sun et al., 2020).

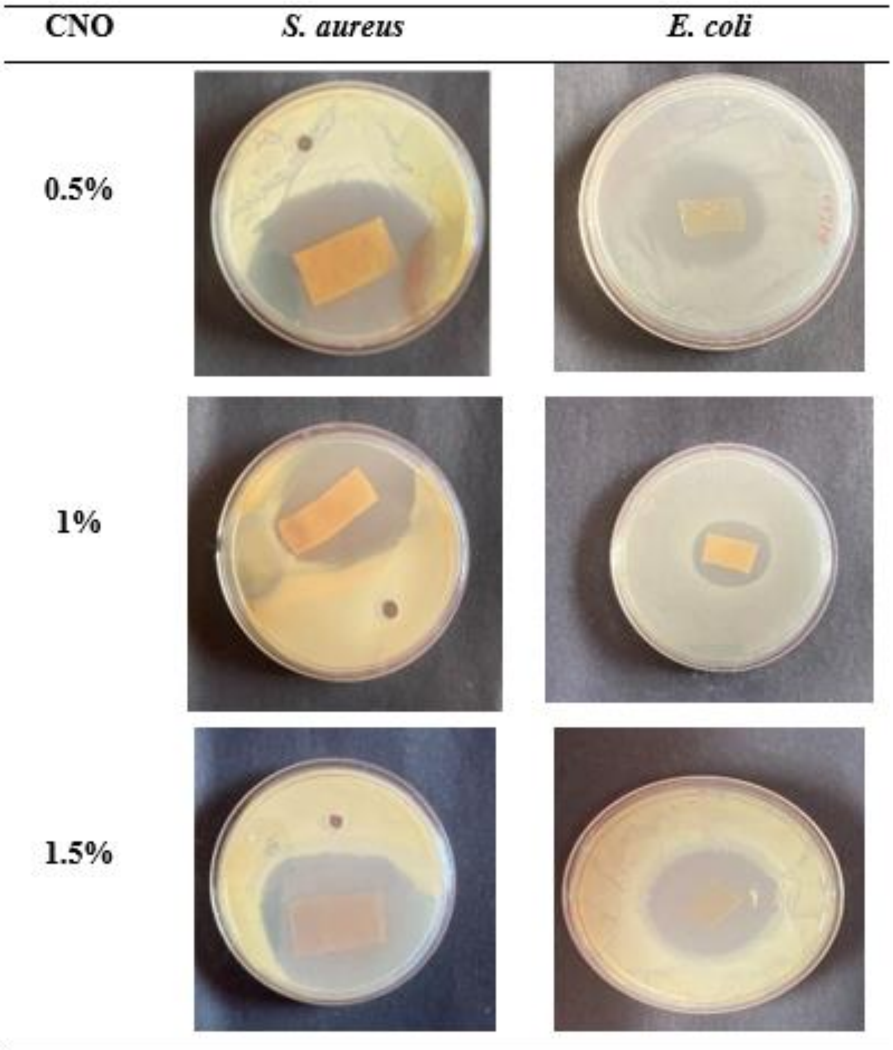

Fig. 6.10 Antimicrobial activity of developed active PS-CS blended film reinforced with BPF film against *S. aureus* and *E. coli* (CNO-Films incorporated with cinnamon essential oil)

Table 6.5 Inhibition zone of cinnamon EO-incorporated potato starch-casein banana fiber-reinforced films against *S. aureus* and *E. coli*

Film sample	*S. aureus*	*E. coli*
0.5% CNO	37	28
1% CNO	35	15
1.5% CNO	40	25

(CNO-Films incorporated with cinnamon essential oil)

6.3.4.12. Release behavior

Fig. 6.11 a), b), and c) demonstrates the release behavior of 0.5%, 1%, and 1.5% CNO incorporated banana fiber-reinforced PS-casein blended films in different solvents (ethanol, acetic acid, and distilled water) over 4 days with significant differences. In the 0.5% CNO concentration graph, ethanol shows the highest release percentage starting at around 38% and gradually increasing to approximately 40% over the 4 days. Acetic acid demonstrates an intermediate release profile, maintaining a relatively stable release percentage of around 36%. The distilled water condition shows the lowest initial release (around 35%) with a slight increase over time. For the 1% CNO concentration, the release profiles appear more stable across all three conditions. Ethanol maintains the highest release percentage at approximately 37%, while both acetic acid and distilled water show similar release patterns around 35-36%. The 1.5% CNO concentration demonstrates the most distinct separation between the three conditions. The release behavior observed are affected by a number of variables, including as the structure of the polymer matrix, EO concentration, and the nature of the release medium. Similar release patterns have been observed in previous studies of EO-incorporated starch-based films (Alvarez-Pérez et al., 2021). The incorporation of banana fiber likely affects the film's microstructure and consequently the release behavior, as natural fibers can create a more indirect path for compound diffusion (Pinto et al., 2020). The increasing CNO concentration enhances the differentiation between release media, suggesting that higher EO content may affect the film's interaction with different solvents. This could be attributed to changes in the film's hydrophobic-hydrophilic balance and matrix organization (Wang et al., 2019). The stable release behavior throughout simulant conditions over time suggests good structural integrity maintained by the crosslinking between PS, casein, banana pseudostem fiber, and, CNO, making these films suitable for food packaging applications requiring moisture resistance.

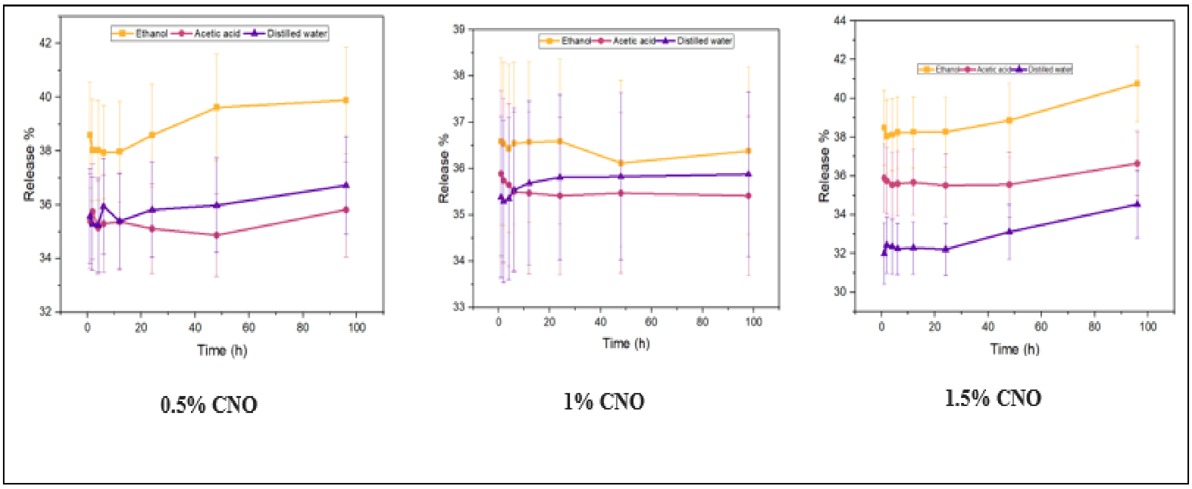

Fig. 6.11 Release behavior of Cinnamon EO (CNO) incorporated PS-casein blended film reinforced by BPF

6.3.4.13. Biodegradability

The biodegradation of developed active films over 3 weeks period in soil is illustrated in **Fig. 6.12**. The statistical differences (*p<0.05*) indicate significant variations in degradation rates among different CNO concentrations, suggesting that CNO content can effectively modulate the biodegradation rate for specific applications. Initially (day 0), films with higher CNO concentrations showed greater weight loss (1.5% CNO ~0.64%, 1% CNO ~0.61%, 0.5% CNO ~0.54%), likely due to the volatile nature of EOs. However, as degradation progressed, an interesting trend emerged: films with lower CNO concentration (0.5 CNO) consistently showed higher weight loss percentages across weeks 1-3. By week 3, the weight loss stabilized at approximately 0.11%, 0.05%, and 0.07% for 0.5, 1, and 1.5% CNO respectively. The incorporation of EO accelerated the rate of degradation, likely due to the formation of void spaces. These gaps increased the films' brittleness, making them more susceptible to microbial attack and consequently facilitating faster degradation (Mittal et al., 2023). This behavior can be compared to research by Mei et al. (2013) on starch-based films with EOs, where similar degradation patterns were observed. The slower degradation rate in films with higher CNO concentrations can be attributed to the antimicrobial properties of CNO and its hydrophobic nature, which may inhibit microbial activity and moisture absorption necessary for biodegradation. This aligns with the research by Priyadarshi et al. (2018), who observed that incorporating EOs in chitosan-based films reduced their biodegradation rate from 85% to 65% over 28 days.

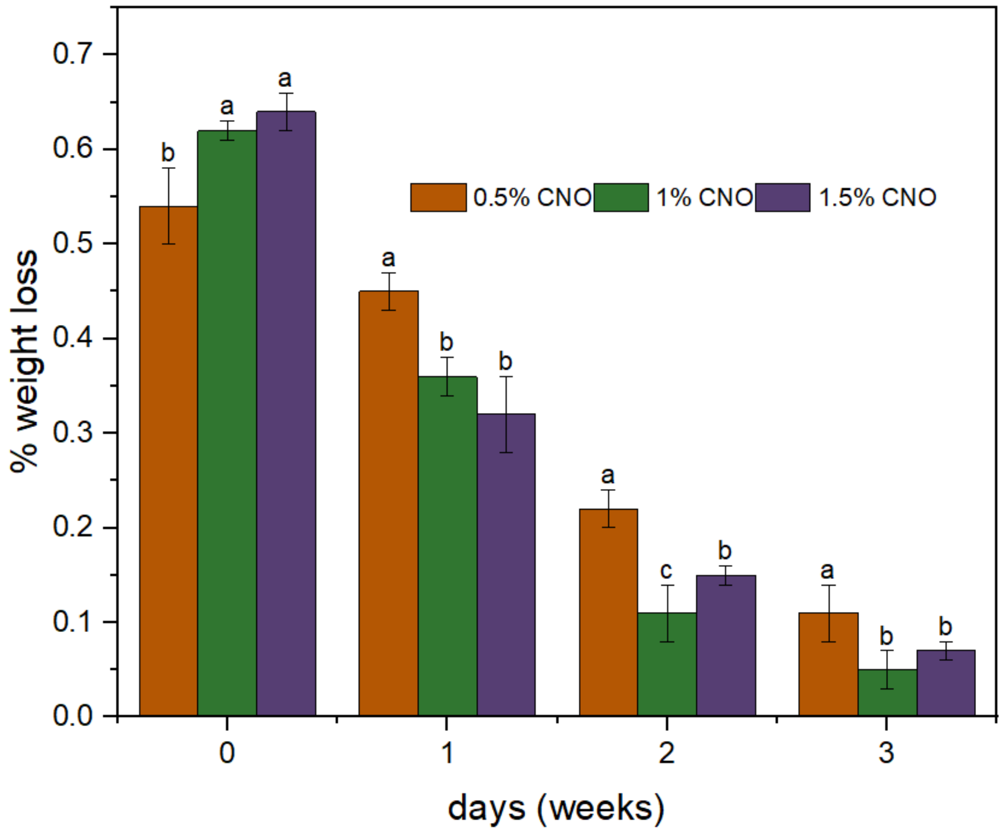

Fig. 6.12 Degradation of developed active PS-CS blended film reinforced with BPF (CNO-Films incorporated with cinnamon essential oil)

6.4. Conclusions

The developed active films, made from modified potato starch/casein/banana-fiber blends and active ingredients such as cinnamon and clove essential oils, exhibit enhanced properties which qualifies them for use in food packaging. These properties include improved optical clarity, mechanical strength, structural composition, crystallinity, and thermal stability. The chapter finds that CNO particularly at a 1% concentration, enhances the film’s antioxidant, antimicrobial, and mechanical properties. However, higher concentrations (1.5%) of CNO negatively impact sealing efficiency and thermal stability, indicating that optimal EO concentration is critical for performance. The addition of CNO increases the film's transparency and reduces water vapor permeability, contributing to its potential as a packaging material. Adding CNO, the thickness of the blended films was considerably increased including active properties while reducing water solubility and vapor permeation. SEM and FT-IR analyses confirmed the films' good compatibility and strong internal interactions. The study emphasizes the need to optimize EO concentrations to balance mechanical, barrier, and sealing properties effectively. Films containing CNO were also shown to be biodegradable, non-toxic, and eco-friendly, making them viable alternatives to conventional synthetic packaging materials. The films' moderate mechanical and barrier features support their usage in dry food packaging, with moderate seal strength. Overall, the result identifies cinnamon essential oil (CNO) at a 1% concentration as the most effective active ingredient, significantly enhancing the film’s mechanical, antioxidant, and antimicrobial properties. Among the developed films, the potato starch-casein-banana fiber blend incorporated with CNO demonstrated the best overall performance in terms of structural integrity, barrier properties, and biodegradability. These bio-composite films present a promising sustainable alternative to conventional plastic packaging, with strong potential for extending food shelf life while reducing environmental impact. Further research is recommended to explore the scalability, cost-effectiveness, and long-term stability of these films in real-world food packaging applications, as well as to evaluate their impact on shelf life and food safety.

References to Chapter 6

Acevedo-Fani, A., Salvia-Trujillo, L., Rojas-Graü, M. A., & Martín-Belloso, O. (2015). Edible films from essential-oil-loaded nanoemulsions: Physicochemical characterization and antimicrobial properties. *Food hydrocolloids*, *47*, 168-177.

Adilah, Z. M., Jamilah, B., & Hanani, Z. N. (2018). Functional and antioxidant properties of protein-based films incorporated with mango kernel extract for active packaging. *Food Hydrocolloids*, *74*, 207-218.

Ahmad, M., Benjakul, S., Prodpran, T., & Agustini, T. W. (2012). Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. *Food hydrocolloids*, *28*(1), 189-199.

Alinaqi, Z., Khezri, A., & Rezaeinia, H. (2021). Sustained release modeling of CLO from the structure of starch-based bio-nanocomposite film reinforced by electrosprayed zein nanoparticles. *International Journal of Biological Macromolecules*, *173*, 193-202.

Alizadeh-Sani, M., Khezerlou, A., & Ehsani, A. (2018). Fabrication and characterization of the bionanocomposite film based on whey protein biopolymer loaded with TiO2 nanoparticles, cellulose nanofibers and rosemary essential oil. *Industrial crops and products*, *124*, 300-315.

Alvarez-Pérez, O. B., Chávez-González, M. L., Iliná, A., Martínez-Hernández, J. L., Segura-Ceniceros, E. P., Ramos-González, R., & Aguilar, C. N. (2021). Essential oils in active food packaging. In *Handbook of Research on Food Science and Technology* (pp. Vol1-131). Apple Academic Press.

Anuar, H., Nur Fatin Izzati, A. B., Sharifah Nurul Inani, S. M., Siti Nur E'zzati, M. A., Siti Munirah Salimah, A. B., Ali, F. B., & Manshor, M. R. (2017). Impregnation of CNO into plasticised polylactic acid biocomposite film for active food packaging. *Journal of Packaging Technology and Research*, *1*, 149-156.

Arfat, Y. A., Benjakul, S., Prodpran, T., Sumpavapol, P., & Songtipya, P. (2014). Properties and antimicrobial activity of fish protein isolate/fish skin gelatin film containing basil leaf essential oil and zinc oxide nanoparticles. *Food Hydrocolloids*, *41*, 265-273.

Atarés, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends in food science & technology*, *48*, 51-62.

Atarés, L., Bonilla, J., & Chiralt, A. (2010). Characterization of sodium caseinate-based edible films incorporated with cinnamon or ginger essential oils. *Journal of Food Engineering*, *100*(4), 678-687.

Azizkhani, M., Jafari Kiasari, F., Tooryan, F., Shahavi, M. H., & Partovi, R. (2021). Preparation and evaluation of food-grade nanoemulsion of tarragon (Artemisia dracunculus L.) essential oil: antioxidant and antibacterial properties. *Journal of Food Science and Technology*, *58*, 1341-1348.

Bertolo, M. R. V., Dias, L. D., de Oliveira Filho, J. G., Alves, F., Marangon, C. A., Martins, V. D. C. A., & Junior, S. B. (2022). Central composite design optimization of active and physical properties of food packaging films based on chitosan/gelatin/pomegranate peel extract. *Food Packaging and Shelf Life*, *34*, 100986.

Bhatia, S., Al-Harrasi, A., Ullah, S., Shah, Y. A., Al-Azri, M. S., Jawad, M., & Aydemir, L. Y. (2024). Fabrication, characterization and antioxidant activities of pectin and gelatin based edible film loaded with Citrus reticulata L. essential oil. *Journal of Food Process Engineering*, *47*(4), e14583.

Brnawi, W. I., Hettiarachchy, N. S., Horax, R., Kumar-Phillips, G., & Ricke, S. (2019). Antimicrobial activity of leaf and bark CNOs against Listeria monocytogenes and Salmonella typhimurium in broth system and on celery. *Journal of Food Processing and Preservation*, *43*(3), e13888.

Brodowska, K. M., Brodowska, A. J., Śmigielski, K., & Łodyga-Chruścińska, E. (2016). Antioxidant profile of essential oils and extracts of cinnamon bark (Cinnamomum cassia). *European Journal of Biological Research*, *6*(4), 310-316.

Chen, S., Han, Y., Sun, C., Dai, L., Yang, S., Wei, Y., & Gao, Y. (2018). Effect of molecular weight of hyaluronan on zein-based nanoparticles: Fabrication, structural characterization and delivery of curcumin. *Carbohydrate Polymers*, *201*, 599-607.

Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial activity of some essential oils—present status and future perspectives. *Medicines*, *4*(3), 58.

Chutia, H., & Mahanta, C. L. (2021). Properties of starch nanoparticle obtained by ultrasonication and high-pressure homogenization for developing carotenoids-enriched powder and Pickering nanoemulsion. *Innovative Food Science & Emerging Technologies*, *74*, 102822.

Dalli, M., Azizi, S. E., Kandsi, F., & Gseyra, N. (2021). Evaluation of the in vitro antioxidant activity of different extracts of Nigella sativa L. seeds, and the quantification of their bioactive compounds. *Materials Today: Proceedings*, *45*, 7259-7263.

de Moraes Crizel, T., de Oliveira Rios, A., Alves, V. D., Bandarra, N., Moldão-Martins, M., & Flôres, S. H. (2018). Active food packaging prepared with chitosan and olive pomace. *Food Hydrocolloids*, *74*, 139-150.

Das, S., & Kalyani, M. I. (2023). From trash to treasure: Review on upcycling of fruit and vegetable wastes into starch-based bioplastics. *Preparative Biochemistry & Biotechnology*, *53*(7), 713-727.

Eldahshan, O. A., Zakaria, G., Mostafa, N. M., & El-Ezz, A. (2023). Evaluation of Antioxidant Activity of *Cinnamomum glanduliferum* Leaf Oil Using Several In-vitro Assays. *Archives of Pharmaceutical Sciences Ain Shams University*, *7*(1), 200-207.

Fahma, F., Fauzan, R. M., Sunarti, T., Sugiarto, S., Halim, A., Lin, K. H., ... & Enomae, T. (2020). Production of silica-alginate-nanocellulose composite beads with CNO for antimicrobial sachet. *Journal of Nanostructures*, *10*(4), 779-792.

Fan, S., Wang, D., Wen, X., Li, X., Fang, F., Richel, A., & Zhang, D. (2023). Incorporation of CNO-loaded Pickering emulsion for improving antimicrobial properties and control release of chitosan/gelatin films. *Food Hydrocolloids*, *138*, 108438.

Ghasemlou, M., Aliheidari, N., Fahmi, R., Shojaee-Aliabadi, S., Keshavarz, B., Cran, M. J., & Khaksar, R. (2013). Physical, mechanical and barrier properties of corn starch films incorporated with plant essential oils. *Carbohydrate polymers*, *98*(1), 1117-1126.

González, A., Gastelú, G., Barrera, G. N., Ribotta, P. D., & Igarzabal, C. I. Á. (2019). Preparation and characterization of soy protein films reinforced with cellulose nanofibers obtained from soybean by-products. *Food Hydrocolloids*, *89*, 758-764.

Guimarães, A. C., Meireles, L. M., Lemos, M. F., Guimarães, M. C. C., Endringer, D. C., Fronza, M., & Scherer, R. (2019). Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules*, *24*(13), 2471.v

Hafsa, J., ali Smach, M., Khedher, M. R. B., Charfeddine, B., Limem, K., Majdoub, H., & Rouatbi, S. (2016). Physical, antioxidant and antimicrobial properties of chitosan films containing Eucalyptus globulus essential oil. *LWT-food Science and Technology*, *68*, 356-364.

He, X., Li, M., Gong, X., Niu, B., & Li, W. (2021). Biodegradable and antimicrobial CSC films containing CNO for preservation applications. *Food Packaging and Shelf Life*, *29*, 100697.

Hosseini, M. H., Razavi, S. H., & Mousavi, M. A. (2009). Antimicrobial, physical and mechanical properties of chitosan-based films incorporated with thyme, clove and CNOs. *Journal of food processing and preservation*, *33*(6), 727-743.

Hosseini, S. F., Ghaderi, J., & Gómez-Guillén, M. C. (2022). Tailoring physico-mechanical and antimicrobial/antioxidant properties of biopolymeric films by cinnamaldehyde-loaded chitosan nanoparticles and their application in packaging of fresh rainbow trout fillets. *Food Hydrocolloids*, *124*, 107249.

Hosseini, S. F., Rezaei, M., Zandi, M., & Farahmandghavi, F. (2015). Bio-based composite edible films containing Origanum vulgare L. essential oil. *Industrial Crops and products*, *67*, 403-413.

Huang, X., Li, J., He, J., Luo, J., Cai, J., Wei, J., & Zhong, H. (2024). Preparation of curcumin-loaded chitosan/polyvinyl alcohol intelligent active films for food packaging and freshness monitoring. *International Journal of Biological Macromolecules*, 133807.

Johari, A., & Khan, M. H. (2022). Evaluation of antioxidant activity of essential oils of some Indian medicinal plants by DPPH, FRAP and ABTS assay. *Journal of Pharmaceutical Negative Results*, 2602-2607.

Khajeh, N., Mohammadi Nafchi, A., & Nouri, L. (2024). Antioxidant and antimicrobial activities of tarragon and Zataria multiflora Boiss essential oils and their applications as active agents to improve the shelf life of freshly cut potato strips. *Food Science & Nutrition*.

Khan, A., Riahi, Z., Kim, J. T., & Rhim, J. W. (2024). Chitosan/gelatin-based multifunctional films integrated with sulfur-functionalized chitin for active packaging applications. *Food Hydrocolloids*, *149*, 109537.

Kumar, P., Sandeep, K. P., Alavi, S., Truong, V. D., & Gorga, R. E. (2010). Effect of type and content of modified montmorillonite on the structure and properties of bio-nanocomposite films based on soy protein isolate and montmorillonite. *Journal of food science*, *75*(5), N46-N56.

Kuorwel, K. K., Cran, M. J., Sonneveld, K., Miltz, J., & Bigger, S. W. (2013). Migration of antimicrobial agents from starch-based films into a food simulant. *LWT-Food Science and Technology*, *50*(2), 432-438.

Liu, F., Chiou, B. S., Avena-Bustillos, R. J., Zhang, Y., Li, Y., McHugh, T. H., & Zhong, F. (2017). Study of combined effects of glycerol and transglutaminase on properties of gelatin films. *Food hydrocolloids*, *65*, 1-9.

Liu, S., Zhao, C., Cao, Y., Li, Y., Zhang, Z., Nie, D., ... & Li, Y. (2023). Comparison of chemical compositions and antioxidant activity of essential oils from Litsea Cubeba, Cinnamon, Anise, and Eucalyptus. *Molecules*, *28*(13), 5051.

Liu, X., Xie, Y., Li, C., & Xue, F. (2022). Comparative studies on physicochemical properties of gluten-and glutenin-based films functionalized by polyphenols. *Cereal Chemistry*, *99*(3), 640-651.

López-Mata, M. A., Ruiz-Cruz, S., Silva-Beltrán, N. P., Ornelas-Paz, J. D. J., Ocaño-Higuera, V. M., Rodríguez-Félix, F., & Shirai, K. (2015). Physicochemical and antioxidant properties of chitosan films incorporated with cinnamon oil. *International Journal of Polymer Science*, *2015*(1), 974506.

López-Cano, A. A., Martínez-Aguilar, V., Peña-Juárez, M. G., López-Esparza, R., Delgado-Alvarado, E., Gutiérrez-Castañeda, E. J., & Gonzalez-Calderon, J. A. (2023). Chemically Modified Nanoparticles for Enhanced Antioxidant and Antimicrobial Properties with CNO. Antioxidants, 12(12), 2057.

Lv, F., Liang, H., Yuan, Q., & Li, C. (2011). In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International*, *44*(9), 3057-3064.

Martelo-Guzmán, J., Collazo-Bigliardi, S., & Ortega-Toro, R. (2020). Comparative study of active edible coatings based on Yam Starch and Cassava Starch. Revista EIA, 17(34), 312-325.

Mei, J., Yuan, Y., Guo, Q., Wu, Y., Li, Y., & Yu, H. (2013). Characterization and antimicrobial properties of water chestnut starch-chitosan edible films. *International Journal of Biological Macromolecules*, *61*, 169-174.

Mittal, M., Ahuja, S., Yadav, A., & Aggarwal, N. K. (2023). Development of poly (hydroxybutyrate) film incorporated with nano silica and CLO intended for active packaging of brown bread. *International Journal of Biological Macromolecules*, *233*, 123512.

MO Syafiq, R., Sapuan, S. M., & Zuhri, M. R. (2020). Effect of CNO on morphological, flammability and thermal properties of nanocellulose fibre–reinforced starch biopolymer composites. *Nanotechnology Reviews*, *9*(1), 1147-1159.

Pandita, G., de Souza, C. K., Gonçalves, M. J., Jasińska, J. M., Jamróz, E., & Roy, S. (2024). Recent progress on Pickering emulsion stabilized essential oil added biopolymer-based film for food packaging applications: A review. *International Journal of Biological Macromolecules*, 132067.

Pelissari, F. M., Grossmann, M. V., Yamashita, F., & Pineda, E. A. G. (2009). Antimicrobial, mechanical, and barrier properties of cassava starch− chitosan films incorporated with oregano essential oil. *Journal of agricultural and food chemistry*, *57*(16), 7499-7504.

Perdones, Á., Chiralt, A., & Vargas, M. (2016). Properties of film-forming dispersions and films based on chitosan containing basil or thyme essential oil. *Food Hydrocolloids*, *57*, 271-279.

Pranoto, Y., Rakshit, S. K., & Salokhe, V. M. (2005). Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *LWT-Food Science and Technology*, *38*(8), 859-865.

Priyadarshi, R., Kumar, B., Deeba, F., Kulshreshtha, A., & Negi, Y. S. (2018). Chitosan films incorporated with Apricot (Prunus armeniaca) kernel essential oil as active food packaging material. *Food Hydrocolloids*, *85*, 158-166.

Ratanasongtham, P., Sukplee, P., & Wongnongwa, Y. (2024). Development of antioxidant film based on gelatin and carboxymethylcellulose incorporated with Tecoma stans (L.) Juss. ex Kunth Petals extract for biodegradable food packaging. *Journal of Metals, Materials and Minerals*, *34*(2), 1781-1781.

Rawat, R., & Saini, C. S. (2024). A novel biopolymeric composite edible film based on sunnhemp protein isolate and potato starch incorporated with clove oil: Fabrication, characterization, and amino acid composition. *International Journal of Biological Macromolecules*, *268*, 131940.

Roy, S., & Rhim, J. W. (2019). Agar-based antioxidant composite films incorporated with melanin nanoparticles. *Food Hydrocolloids*, *94*, 391-398.

Roy, S., & Rhim, J. W. (2021). Carrageenan/agar-based functional film integrated with zinc sulfide nanoparticles and Pickering emulsion of tea tree essential oil for active packaging applications. *International Journal of Biological Macromolecules*, *193*, 2038-2046.

Roy, S., Priyadarshi, R., & Rhim, J. W. (2022). Gelatin/agar-based multifunctional film integrated with copper-doped zinc oxide nanoparticles and CLO Pickering emulsion for enhancing the shelf life of pork meat. *Food Research International*, *160*, 111690.

Sánchez-González, L., Cháfer, M., González-Martínez, C., Chiralt, A., & Desobry, S. (2011). Study of the release of limonene present in chitosan films enriched with bergamot oil in food simulants. *Journal of Food Engineering*, *105*(1), 138-143.

Sarengaowa, Feng, K., Li, Y., Long, Y., & Hu, W. (2023). Effect of alginate-based edible coating containing thyme essential oil on quality and microbial safety of fresh-cut potatoes. *Horticulturae*, *9*(5), 543.

Shah, A. Y., Bhatia, S., Al-Harrasi, A., Naseer, A., Jawad, M., Khan, T. S., ... & Aydemir, L. Y. (2024). The Applications of Lime (Citrus aurantifolia) Essential Oil as a Functional Ingredient in Gelatin/Kappa-Carrageenan Composite Films for Active Packaging. *ACS Food Science & Technology*, *4*(5), 1199-1208.

Shahid, M. Z., Saima, H., Yasmin, A., Nadeem, M. T., Imran, M., & Afzaal, M. (2018). Antioxidant capacity of cinnamon extract for palm oil stability. *Lipids in health and disease*, *17*, 1-8.

Shankar, S., Danneels, F., & Lacroix, M. (2019). Coating with alginate containing a mixture of essential oils and citrus extract in combination with ozonation or gamma irradiation increased the shelf life of Merluccius sp. fillets. *Food Packaging and Shelf Life*, *22*, 100434.

Sharma, S., Barkauskaite, S., Jaiswal, S., Duffy, B., & Jaiswal, A. K. (2020). Development of essential oil incorporated active film based on biodegradable blends of poly (lactide)/poly (butylene adipate-co-terephthalate) for food packaging application. *Journal of Packaging Technology and Research*, *4*(3), 235-245.

Singh, A. K., Ramakanth, D., Kumar, A., Lee, Y. S., & Gaikwad, K. K. (2021). Active packaging technologies for clean label food products: a review. *Journal of Food Measurement and Characterization*, *15*(5), 4314-4324.

Singla, M., Singh, A., & Sit, N. (2023). Effect of microwave and enzymatic pretreatment and type of solvent on kinetics of ultrasound assisted extraction of bioactive compounds from ripe papaya peel. *Journal of Food Process Engineering*, *46*(6), e14119.

Siripatrawan, U., & Harte, B. R. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food hydrocolloids*, *24*(8), 770-775.

Souza, A. C. D., Goto, G. E. O., Mainardi, J. A., Coelho, A. C. V., & Tadini, C. C. (2013). Cassava starch composite films incorporated with CNO: Antimicrobial activity, microstructure, mechanical and barrier properties. *LWT-Food Science and Technology*, *54*(2), 346-352.

Srivastava, K. R., Dixit, S., Pal, D. B., Mishra, P. K., Srivastava, P., Srivastava, N., & Abd_Allah, E. F. (2021). Effect of nanocellulose on mechanical and barrier properties of PVA–banana pseudostem fiber composite films. *Environmental Technology & Innovation*, *21*, 101312.

Sun, H., Li, S., Chen, S., Wang, C., Liu, D., & Li, X. (2020). Antibacterial and antioxidant activities of sodium starch octenylsuccinate-based Pickering emulsion films incorporated with CNO. *International journal of biological macromolecules*, *159*, 696-703.

Sun, H., Li, S., Chen, S., Wang, C., Liu, D., & Li, X. (2020). Antibacterial and antioxidant activities of sodium starch octenylsuccinate-based Pickering emulsion films incorporated with CNO. *International journal of biological macromolecules*, *159*, 696-703.

Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V., De Pasquale, A., & Saija, A. (2005). Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. *Food chemistry*, *89*(4), 549-554.

Travalini, A. P., Lamsal, B., Magalhães, W. L. E., & Demiate, I. M. (2019). Cassava starch films reinforced with lignocellulose nanofibers from cassava bagasse. *International journal of biological macromolecules*, *139*, 1151-1161.

Valencia-Sullca, C., Vargas, M., Atarés, L., & Chiralt, A. (2018). Thermoplastic cassava starch-chitosan bilayer films containing essential oils. Food hydrocolloids, 75, 107-115.

Wahab, D. N. A., Siddique, M. B. M., Chew, J. J., Su, H. T., Khairuddin, N., Khaerudini, D. S., & Sunarso, J. (2023). Characterization of starch biofilm reinforced with cellulose microfibers isolated from Musa Saba'midrib residue and its application as an active packaging film. *Journal of Applied Polymer Science*, *140*(48), e54720.

Wang, D., Dong, Y., Chen, X., Liu, Y., Wang, J., Wang, X., ... & Song, H. (2020). Incorporation of apricot (Prunus armeniaca) kernel essential oil into chitosan films displaying antimicrobial effect against Listeria monocytogenes and improving quality indices of spiced beef. *International journal of biological macromolecules*, *162*, 838-844.

Wang, W., Zhang, H., Jia, R., Dai, Y., Dong, H., Hou, H., & Guo, Q. (2018). High performance extrusion blown starch/polyvinyl alcohol/clay nanocomposite films. *Food Hydrocolloids*, *79*, 534-543.

Wang, Y., Ju, J., Diao, Y., Zhao, F., & Yang, Q. (2024). The application of starch-based edible film in food preservation: a comprehensive review. *Critical Reviews in Food Science and Nutrition*, 1-34.

Wu, J., Sun, X., Guo, X., Ge, S., & Zhang, Q. (2017). Physicochemical properties, antimicrobial activity and oil release of fish gelatin films incorporated with CNO. *Aquaculture and Fisheries*, *2*(4), 185-192.

Xu, T., Gao, C., Yang, Y., Shen, X., Huang, M., Liu, S., & Tang, X. (2018). Retention and release properties of CNO in antimicrobial films based on chitosan and gum arabic. *Food Hydrocolloids, 84*, 84-92.

Xu, Y., Liu, X., Jiang, Q., Yu, D., Xu, Y., Wang, B., & Xia, W. (2021). Development and properties of bacterial cellulose, curcumin, and chitosan composite biodegradable films for active packaging materials. *Carbohydrate polymers*, *260*, 117778.

Xue, F., Li, C., & Adhikari, B. (2024). Physicochemical properties of active films of rose essential oil produced using soy protein isolate-polyphenol conjugates for cherry tomato preservation. *Food Chemistry, 452*, 139614.

Yang, Z., Zhang, D., & Cai, J. (2024). Robust design of starch composite nanofibrous films for active food packaging: Towards improved mechanical, antioxidant, and antibacterial properties. *International Journal of Biological Macromolecules*, *260*, 129329.

Zhang, K., Huang, T. S., Yan, H., Hu, X., & Ren, T. (2020). Novel pH-sensitive films based on starch/polyvinyl alcohol and food anthocyanins as a visual indicator of shrimp deterioration. *International journal of biological macromolecules*, *145*, 768-776.

Zhang, W., Jiang, H., Rhim, J. W., Cao, J., & Jiang, W. (2022). Effective strategies of sustained release and retention enhancement of essential oils in active food packaging films/coatings. *Food Chemistry*, *367*, 130671.

Zhang, Y., Liu, X., Wang, Y., Jiang, P., & Quek, S. (2016). Antibacterial activity and mechanism of CNO against Escherichia coli and Staphylococcus aureus. *Food control*, *59*, 282-289.

Zhang, Y., Ma, Q., Critzer, F., Davidson, P. M., & Zhong, Q. (2015). Physical and antibacterial properties of alginate films containing cinnamon bark oil and soybean oil. *LWT-Food Science and Technology*, *64*(1), 423-430.

Zhou, Y., Wu, X., Chen, J., & He, J. (2021). Effects of CNO on the physical, mechanical, structural and thermal properties of cassava starch-based edible films. *International Journal of Biological Macromolecules*, *184*, 574-583.