

Research Article

Enhanced sodium alginate-Based active packaging films via sonication, CaCl_2 crosslinking, and patchouli essential oil incorporation

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ABSTRACT

This study explored the development of enhanced sodium alginate (SA)-based active packaging films using a synergistic approach that combines sonication, calcium chloride (CaCl_2) crosslinking, and incorporation of patchouli essential oil (PEO). The fabrication of the films involved optimized sonication (5 min at 50 % amplitude), internal crosslinking with 2 % CaCl_2 , and 1 % (w/v) PEO. The resulting film (S5/C2/PEO) exhibited significant ($p < 0.05$) improvements in tensile strength (31.8 MPa), reduced water vapor permeability ($2.11 \times 10^{-11} \text{ g m}^{-1} \text{ h}^{-1} \cdot \text{Pa}^{-1}$), and enhanced thermal stability. FTIR analysis revealed intensified hydrogen bonding, indicating the successful integration of PEO and structural reinforcement via Ca^{2+} crosslinking. A qualitative *in vivo* preservation test on cherry tomatoes demonstrated mold inhibition by the S5/C2/PEO film over four days of storage, in contrast to rapid spoilage in the control. Although these antimicrobial results are preliminary and based on visual assessment, they underscore the potential of the film as a biodegradable active food packaging solution. Future studies should incorporate quantitative microbiological and sensory analyses to validate its efficacy and address the potential aroma transfer.

1. Introduction

Food packaging plays a critical role in preserving food quality, extending shelf life, and preventing microbial contamination. However, the widespread use of synthetic polymers such as polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET) in packaging has raised significant environmental concerns. These non-biodegradable materials contribute to the accumulation of millions of tons of plastic waste globally. By 2020, global plastic production reached approximately 367 million tons, with PET being the third most widely used polymer after PE and PP [1,2]. An estimated 79 % of all plastics produced end up in landfills or as unmanaged waste, exacerbating pollution and ecological degradation [3]. This growing crisis underscores the urgent need for sustainable packaging alternatives, particularly those derived from renewable biopolymers such as sodium alginate (SA).

Sodium alginate (SA) is a naturally occurring polysaccharide extracted from brown algae, recognized for its biocompatibility, biodegradability, and non-toxicity traits aligned with sustainability directives [4]. Structurally, SA consists of alternating mannuronate (M) and guluronate (G) residues, enabling the formation of films with desirable mechanical and barrier properties. However, native SA films exhibit poor tensile strength and high water vapor permeability (WVP), which limit their effectiveness in food packaging applications [5].

To overcome these limitations, researchers have employed various modification strategies. Physical treatments such as ultrasonication enhance film homogeneity by facilitating polymer chain rearrangement, thereby improving mechanical and barrier performance [6,7]. Additionally, ionic crosslinking with divalent cations especially calcium ions (Ca^{2+}), induces the formation of an "egg-box" structure within the SA matrix, improving tensile strength and water resistance. Beyond

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structural modification, the incorporation of bioactive agents such as essential oils (EOs) offers antimicrobial functionality. Zhou et al. [8] reported that integrating EOs into biopolymer films enhances their ability to inhibit microbial growth and prolong food shelf life.

Patchouli essential oil (PEO), derived from *Pogostemon cablin*, contains patchouli alcohol and sesquiterpenes with well-documented antimicrobial activity against foodborne pathogens and spoilage organisms [9,10]. PEO disrupts microbial membranes, increases cell permeability, and induces lysis, making it a promising additive for preserving perishable products [11]. Incorporating PEO into SA films may therefore enhance their antimicrobial efficacy and extend product shelf life.

Recent studies have focused on integrating EOs such as oregano [12], thyme [13], and clove [14] into various biopolymer matrices, including chitosan, whey protein, gelatin, and starch. However, limited research has examined the incorporation of PEO into SA films, particularly in combination with other film-enhancing treatments. Furthermore, the synergistic effects of ultrasonication, CaCl₂ crosslinking, and PEO incorporation on film microstructure and functional performance remain underexplored.

This study aims to address these gaps by developing an SA-based film modified through ultrasonic treatment, CaCl₂ crosslinking, and PEO integration. We hypothesized that the combined application of these treatments would significantly improve the physicochemical, mechanical, thermal, and antimicrobial properties of the films, thereby enhancing their potential as biodegradable active packaging for perishable food products.

2. Materials and methods

2.1. Materials

Low-viscosity sodium alginate (SA) (MWt.: 30,000–100,000 g/mol; M/G ratio: 1.56) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Glycerol and calcium chloride (CaCl₂) were sourced from R&M Chemicals, and PEO was sourced from Phatol. All chemicals were of analytical grade and were not purified before use in any film-forming process for consistency purposes.

2.2. Preparation of SA films

The films were prepared using the solution casting method. Sodium alginate (2 % w/v) was dissolved in 200 mL of deionized water with continuous magnetic stirring at 300 rpm and heated to 90 °C until fully solubilized. Glycerol (25 % w/w on SA) was then added as a plasticizer. After complete mixing, 50 g of the solution was cast into 150 × 25 mm Petri dishes and dried at 50 °C for 24 h. The dried films were stored at 25 ± 2 °C and 51 ± 2 % RH in a desiccator prior to analysis. The untreated film was considered the control film (SA-Control).

To prepare the sonicated films, SA solutions were subjected to ultrasonication using a VCX800 ultrasonic processor (Sonics & Materials Inc., USA; 13 mm probe) at 50 % amplitude for 0, 5, 10, 20, or 30 min. Each 50 g aliquot of the treated solution was cast and dried under the same conditions as the control.

For the crosslinked films, 10 mL of CaCl₂ solution (1–4 % w/v) was added dropwise to 200 mL of SA solution, followed by magnetic stirring at 1500 rpm for uniform dispersion. The resulting mixtures were cast into Petri dishes and dried at 50 °C for 24 h. To prepare the combined-treatment films, 10 mL of 2 % CaCl₂ solution was added to the SA solution before sonication at 50 % amplitude for 5 min. The final solutions were cast and dried using the same procedures. The films were designated based on sonication time and CaCl₂ content (e.g., S5/C2 indicates 5 min sonication and 2 % CaCl₂).

For the antimicrobial formulation, PEO was incorporated at a fixed concentration of 1 % (w/v) relative to the total film-forming solution volume. PEO was first mixed with Tween 80 at a 2:1 vol ratio to improve its dispersion. The emulsion was then added dropwise to the SA/CaCl₂

solution and sonicated at 50 % amplitude for 5 min. The resulting solution (50 g) was cast into Petri dishes and dried at 50 °C for 24 h. The films were stored at 25 °C and 51 % RH for 48 h before use. The PEO-incorporated films were designated as S5/C2/PEO. Table 1 summarizes the composition and treatment conditions of all film formulations used in this study.

2.3. Film thickness

The thickness of each film sample was measured with a precision of 0.001 mm, using a digital micrometer (Mitutoyo, Japan). To obtain an accurate average, measurements were performed at five randomly selected points on each film, and the final thickness was recorded in millimeters (mm).

2.4. Color and opacity analysis

The color of the SA films was assessed in triplicate (n = 3) using a Hunter Lab Colorimeter (USP 1431 Ultrascan Pro, USA) according to the CIE L*a*b* color system. To ensure consistency, each sample was calibrated using a standard white plate (L* = 97.39, a* = 0.03, b* = 1.77). The lightness (L*), greenness/redness (a*), and blueness/yellowness (b*) values of the films were measured. The color difference (ΔE) and whiteness index were determined using Eq. (1).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Film opacity was determined by measuring light transmittance using an exact color spectrophotometer across wavelengths ranging from 200 to 700 nm. Percent transmittance at 600 nm was calculated using Eq. (2).

$$\text{Opacity, \%} = \frac{T_{600}}{100} \times L \quad (2)$$

where T₆₀₀ is the transmittance at 600 nm and L is the film thickness (mm).

2.5. Determination of moisture content

To determine the moisture content (MC) of each film, small samples (1 cm²) were carefully weighed, dried at 105 °C for 24 h, and weighed again. The difference between the initial (M₁) and final (M₂) weights was used to calculate the MC, which provided insights into the water retention properties of the film. All measurements were performed in triplicate (n = 3).

$$\text{MC, \%} = \frac{M_1 - M_2}{M_1} \times 100 \quad (3)$$

Table 1

Composition of untreated (control) and treated SA-based film samples with varying concentrations of CaCl₂ crosslinking, sonication time, and the addition of patchouli essential oil (PEO) addition.

Samples	SA (%) w/v	Glycerol (%) w/w	Sonication time (min)	CaCl ₂ (%)	PEO (%) w/v
Control Film	2	25	–	–	–
S5	2	25	5	–	–
S10	2	25	10	–	–
S20	2	25	20	–	–
S30	2	25	30	–	–
C1	2	25	–	1	–
C1.5	2	25	–	1.5	–
C2	2	25	–	2	–
C2.5	2	25	–	2.5	–
S5/C2	2	25	5	2	–
S5/C2/ PEO	2	25	5	2	1

2.6. Water vapor permeability

WVP was measured in triplicate and gravimetrically using a water vapor permeability cup (Model No. 318, Yasuda Seiki Seisakusho Ltd., Nishinomiya, Japan) according to the JIS Z 0208 standard. A wax mixture of paraffin and beeswax in an 8:2 ratio was melted using a magnetic stirring hotplate. Each permeability cup filled with 10 g of anhydrous CaCl_2 to maintain 0 % RH. Circular film samples (7 cm in diameter) were cut and sealed in a cup using wax solution. The cups were placed in a desiccator containing saturated magnesium nitrate solution at 25 °C to ensure a stable RH of 51 %. The temperature and humidity within the desiccator were continuously monitored using a digital temperature-humidity meter (Proskit NT-312, Techno Tools & Equipment Sdn. Bhd., Ampang Jaya, Malaysia). The weight change in each cup was recorded hourly over 24 h, and a graph of weight loss versus time was plotted. Duplicate WVP measurements were performed for each film sample.

The water vapor transmission rate (WVTR) was determined using Eq. (4) as follows:

$$\text{WVTR} = \frac{w/t}{A} \quad (4)$$

where w/t represents the weight change slope (g) over time (h) and A is the transmission area of the film (28 cm^2). WVP was calculated using Eq. (5).

$$\text{WVP} = \frac{(\text{WVTR} \times L)}{\Delta P} \quad (5)$$

where L is the average film thickness (mm), ΔP is the water vapor partial pressure inside and outside the desiccator at 51 % RH ($21.64 \times 10^5 \text{ Pa}$).

2.7. Thermogravimetric analysis

TGA was performed to evaluate the thermal profiles of the films. Approximately 10 mg of each sample was heated from 25 °C to 600 °C at a rate of 10 °C/min in a nitrogen atmosphere. Thermogravimetric (TG) and derivative thermogravimetric (DTG) curves were used to record the temperatures at which the degradation occurred, providing insights into the heat resistance of the films.

2.8. Morphology

The surface and cross-sectional structural features of the films were examined using a scanning electron microscope (SEM; JSM 6400, JEOL, Japan) operated at an accelerating voltage of 2 kV. To examine the impact of sonication, CaCl_2 crosslinking, and PEO integration on the morphology of the films, surface and cross-sectional images were obtained at 1000 $\times$ magnification. This investigation focused on surface roughness, porosity, and cross-sectional density to assess the influence of various treatments on the microstructure of the films.

2.9. In vivo antimicrobial activity of films using tomato cherry

An *in vivo* study was conducted to evaluate the antimicrobial activity of the films on the cherry tomatoes. The procedure involved affixing small film pieces ($50 \times 50 \text{ mm}$) to the inner surfaces of Petri dish lids, followed by placing tomatoes at the base of each dish. The dishes were sealed and stored at 25 °C. This setup facilitated indirect contact, and visible signs of spoilage or microbial growth were monitored daily over four days.

2.10. Statistical analysis

All experimental measurements were conducted in triplicate ($n = 3$), and the results are expressed as mean $\pm$ standard deviation. Statistical

analysis was performed using one-way analysis of variance, followed by Tukey's post hoc test, to determine significant differences among the treatment groups at a confidence level of $p < 0.05$, using the SPSS software (version 29, IBM Corp., Armonk, NY, USA). Significant differences are indicated in the corresponding figures and tables using different superscript letters.

3. Results and discussion

3.1. FT-IR spectroscopy

Fig. 1 presents the FT-IR spectra of untreated (control) and treated sodium alginate (SA) films subjected to sonication, CaCl_2 crosslinking, and PEO incorporation. Table 2 summarizes the corresponding wave-number shifts of key functional groups and the related molecular interactions.

The control film (Fig. 1(a)) exhibited characteristic peaks of the SA spectrum, including a prominent O–H stretching vibration at 3302 cm^{-1} , indicating the presence of hydroxyl groups. A peak at 2932 cm^{-1} corresponded to C–H stretching, typical of polysaccharides. Peaks at 1604 cm^{-1} and 1416 cm^{-1} reflected the asymmetric and symmetric stretching vibrations of carboxylate (COO^-) groups, respectively, which play a critical role in the gel-forming properties of SA [15]. Additional bands at 1082 and 1026 cm^{-1} were attributed to C–O–C antisymmetric stretching, with the latter showing greater intensity, suggesting strong O–H bonding within the matrix. Peaks at 942 , 892 , and 814 cm^{-1} were associated with vibrations of guluronic and mannuronic acid units. The treated films showed similar spectral patterns but exhibited notable peak shifts, indicating structural modifications.

In the S5 film (Fig. 1(b)), the O–H stretching band shifted from 3302 cm^{-1} to 3260 cm^{-1} , indicating enhanced intermolecular hydrogen bonding and polymer chain rearrangement induced by sonication. These changes reflect stronger internal interactions, contributing to improved film structure. In the combined treatment (S5/C2) and PEO-incorporated film (S5/C2/PEO), the C–H stretching band shifted to 2928 cm^{-1} , compared to 2934 cm^{-1} in other samples. This shift was most pronounced in the PEO-containing film, reflecting its high hydrocarbon content. The asymmetric COO^- stretch shifted from 1604 cm^{-1} in the control to 1588 cm^{-1} in the S5 film, suggesting stronger ionic interactions.

A minor shift in the symmetric COO^- stretching vibration to 1412 cm^{-1} further indicated structural reorganization of the SA matrix following sonication, likely due to energy-driven molecular rearrangements. Additional shifts to 1590 , 1606 , and 1612 cm^{-1} in treated

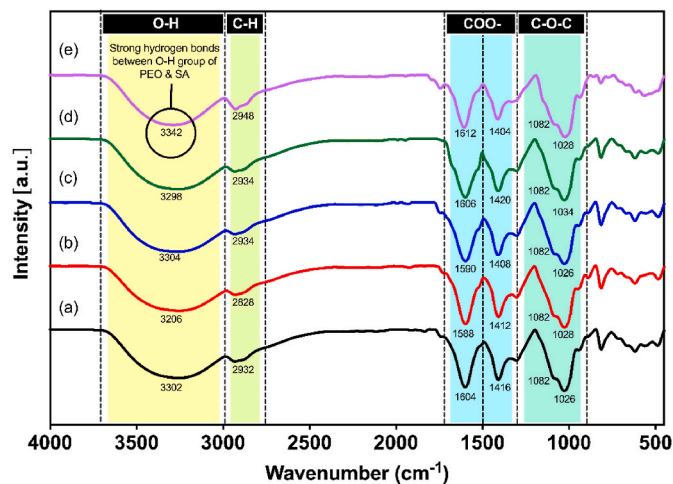


Fig. 1. FTIR spectra for the following SA-based films: (a) control, (b) S5, (c) C2, (d) S5/C2, and (e) S5/C2/PEO.

Table 2

Comparative FTIR peaks of untreated and treated SA-based films, both with and without PEO incorporation.

Functional Group	Samples' wavenumber (cm ⁻¹)					Interpretation
	Control	S5	C2	S5/C2	S5/C2/ PEO	
O–H stretching (hydrogen bonding)	3302	3260	3298	3294	3342	Stronger H-bonding (sonication); and new bonding sites (Patchoulol)
C–H stretching (aliphatic CH)	2932	2928	2934	2926	2928	Slight shift reflects the hydrocarbon content of PEO
COO ⁻ asymmetric stretching	1604	1588	1590	1606	1612	Ionic crosslinking with Ca ²⁺ ; structural rearrangement
COO ⁻ symmetric stretching	1416	1412	1408	1420	1404	Reinforced COO ⁻ –Ca ²⁺ interaction and minor matrix compaction
C–O–C stretching (polysaccharides)	1082, 1026	1082, 1028	1084, 1028	1084, 1028	1082, 1034	Retained backbone; some matrix reorganization
PEO O–H (patchoulol)	–	–	–	–	3342	Presence of PEO, confirms hydrogen bonding with the SA matrix

samples (Fig. 1(c)–(e)) confirmed successful Ca²⁺ crosslinking and enhanced ionic interactions. These COO⁻–Ca²⁺ associations correlate with improved TS and reduced WVP, in agreement with previous studies on crosslinked SA films [16]. In Fig. 1(d), the appearance of a peak at 1034 cm⁻¹ reflects structural modification due to crosslinking, further amplified by sonication.

Incorporation of PEO intensified the O–H stretching peak, which shifted to 3342 cm⁻¹ (Fig. 1(e)), consistent with the typical O–H vibration of patchoulol, a major component of PEO. This shift suggests the formation of strong hydrogen bonds between PEO and the SA matrix, indicating enhanced interactions between the hydrophilic regions of SA and the polar functional groups of PEO [17].

Despite PEO incorporation, the C–H stretching band at 2928 cm⁻¹ remained unchanged, implying that the interaction mechanism was primarily hydrogen bonding rather than hydrocarbon chain alteration. The asymmetric COO⁻ peak at 1612 cm⁻¹ also remained stable, indicating that the crosslinked network was not disrupted. Similarly, the slight shift in the symmetric COO⁻ band to 1404 cm⁻¹ confirmed preservation of the carboxylate framework. All samples consistently maintained the C–O–C stretching band near 1082 cm⁻¹, proving that the treatments preserved the polysaccharide backbone. Overall, the main structural modifications involved hydrogen bonding and ionic interactions, with the core SA structure remaining intact.

3.2. Mechanical properties

The TS and EaB are key indicators of a film's mechanical suitability for flexible packaging. Fig. 2 shows the effects of sonication, CaCl₂

crosslinking, and PEO incorporation on the TS and EaB of SA films.

The control SA film exhibited a TS of 5.5 MPa and EaB of 25.0 %, indicating high flexibility but limited strength. Sonication for 5 min (S5) significantly increased TS to 31.8 MPa, while reducing EaB to 12.2 %. This enhancement can be attributed to improved polymer chain dispersion and the reduction of matrix defects caused by ultrasonic cavitation [18]. However, extending sonication to 30 min further decreased EaB to 8.1–12.2 %, likely due to partial degradation and chain scission induced by high-energy cavitation. Ultrasonic waves generate localized cavitation bubbles and intense shear forces, which can cleave polymer chains, reduce molecular weight, and compromise flexibility. Abrial et al. [19] reported similar findings in PVA films, where TS increased with sonication, but EaB declined by approximately 30 % after 7.5 min of exposure.

Crosslinking the SA film with 2 % CaCl₂ (C2) increased TS to 7.8 MPa and EaB to 21.8 %, suggesting that the formation of an ionic “egg-box” structure enhances structural cohesion and elasticity [20]. However, increasing CaCl₂ to 2.5 % led to a TS decline to 6.2 MPa, likely due to excessive crosslinking. This may cause elevated charge density in the SA matrix, resulting in electrostatic repulsion, increased intermolecular spacing, and weakened bonding forces [21].

The combination of sonication and CaCl₂ crosslinking (S5/C2) yielded a TS of 12.4 MPa, surpassing the C2 film but slightly lower than the S5 film. This synergistic effect reflects the dual contribution of sonication, which aligns polymer chains, and crosslinking, which reinforces the network via ionic bonding. The treatment reduced EaB compared to the control, indicating increased rigidity. While studies on the combined use of sonication and crosslinking in SA-based films are limited, most

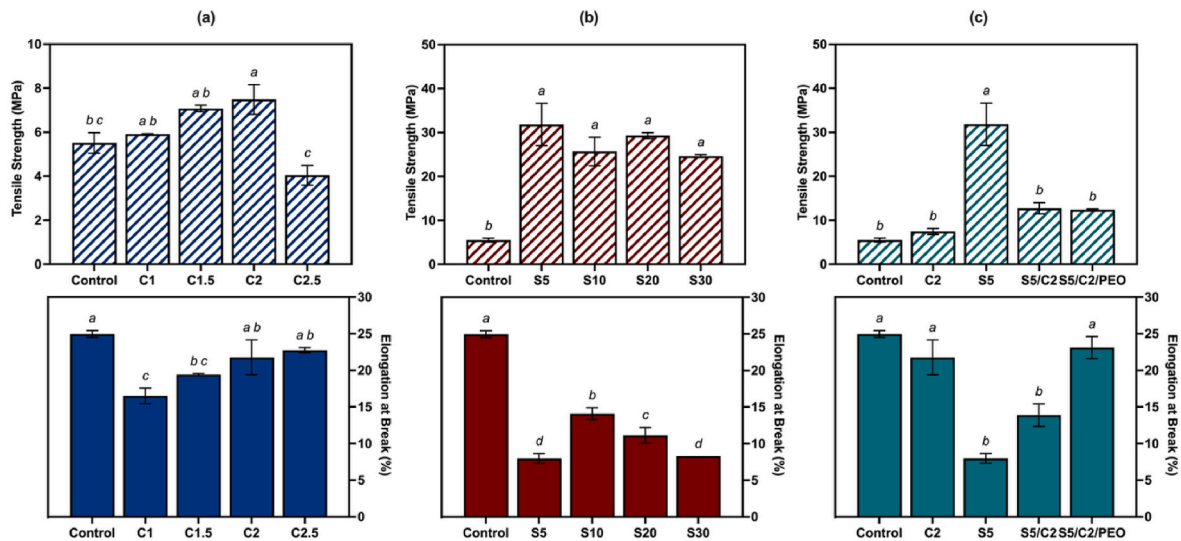


Fig. 2. Tensile strength (TS) and elongation at break (EaB) of SA films (a) sonicated at different times; (b) crosslinked with CaCl₂ at various concentrations, and (c) untreated (control) and treated SA-based films with or without PEO incorporation. Different superscript letters indicate significant differences between the samples ($p < 0.05$).

existing research has addressed these treatments separately or in alternate pairings.

Incorporating PEO into the dual-treated film (S5/C2/PEO) slightly reduced TS to 11.7 MPa but significantly increased EaB to 23.1 %. This outcome suggests that PEO functioned as a plasticizer, softening the matrix and enhancing flexibility. The hydrophobic nature of PEO may disrupt polymer uniformity and weaken interchain bonding, thereby permitting greater elongation under stress. SEM micrographs support this interpretation, revealing a rougher surface and porous internal structure in the S5/C2/PEO film—features consistent with increased chain mobility and higher EaB. In contrast, the denser, compact morphologies of S5 and S5/C2 films correlate with their higher TS and lower EaB values.

These observations align with previous studies, such as that by Ardjoum et al. [22], where the incorporation of *Thymus vulgaris* (thyme) EO reduced TS while increasing EaB from 5.8 % to 13.5 %. Other EOs, including ylang-ylang, have also been shown to enhance film flexibility without significantly compromising strength [23]. Despite the slight reduction in TS caused by PEO, the value of 11.7 MPa still reflects a substantial improvement over unmodified SA films, which typically exhibit inferior mechanical strength [24].

3.3. Thickness

Table 3 presents the thickness of SA films subjected to various treatments, along with corresponding changes in moisture content (MC) and water vapor permeability (WVP). These results demonstrate how sonication, CaCl₂ crosslinking, and PEO incorporation influence key physical properties.

Table 3 presents the thickness of SA films subjected to various treatments, along with corresponding changes in moisture content (MC) and water vapor permeability (WVP). These results demonstrate how sonication, CaCl₂ crosslinking, and PEO incorporation influence key physical properties.

Sonicated films were thinner than those treated with CaCl₂, combined treatments, or PEO incorporation. This trend persisted across increasing sonication durations (5–30 min), suggesting that ultrasonic treatment induces microstructural changes via cavitation. Such changes—particularly enhanced polymer chain dispersion and the

removal of entrapped air—produce denser, thinner films that improve packaging efficiency. This observation aligns with Fang et al. [25], who reported consistent film thickness across sonication times in flaxseed gum films, and with Keawpeng et al. [26], who found that sonication reduced the thickness of mung bean phyto-films.

In contrast, the addition of CaCl₂ to the SA matrix increased film thickness, ranging from 0.062 mm at 1.0 % concentration to 0.070 mm at 2.5 %. This increase is attributed to the formation of ionic bridges between carboxylate groups in the SA chains, which enhance structural cohesion and matrix density. Similar effects have been reported by Rahman et al. [27] and Giz et al. [28], who noted that optimal cross-linking concentrations improve film robustness without significantly reducing flexibility.

Incorporating PEO further increased thickness, reaching 0.086 mm. This expansion likely results from the intercalation of oil molecules between SA chains, which reduces intermolecular bonding and promotes a more open matrix structure. Prior studies have shown that EOs can improve film uniformity, reinforce the biopolymer network, and contribute to increased thickness [24,29]. Ren et al. [29] also observed that oil incorporation facilitates moisture retention and cohesive matrix formation.

The observed increase in thickness due to PEO incorporation has implications for both mechanical and barrier performance. Thicker films typically retain more moisture and may exhibit enhanced mechanical strength due to a reinforced network structure. However, flexibility may be compromised depending on the oil concentration and its interactions within the polymer matrix.

3.4. Moisture Content (MC)

Table 3 presents the moisture content (MC) of SA films subjected to various treatments. Sonicated films showed MC values ranging from 21.4 % to 22.6 %, slightly lower than the control (23.7 %), likely due to localized heat generated during sonication that promoted partial water evaporation. Zou et al. [30] reported that ultrasonic treatment can induce mild thermal effects that facilitate water loss without compromising film integrity. Notably, extending the sonication time did not significantly alter the MC, indicating a plateau in water retention behavior beyond a certain treatment duration.

CaCl₂ crosslinking significantly ($p < 0.05$) reduced MC from 20.2 % at 1 % concentration to 15.4 % at 2.5 %. This reduction is attributed to the formation of the "egg-box" structure, in which Ca²⁺ ions interact with carboxylate (COO[−]) groups in the SA chains and tighten the polymer network. Ionic crosslinking reduces hydrophilicity by limiting water molecule mobility within the matrix through enhanced interchain bonding [31].

The combined treatment incorporating PEO (S5/C2/PEO) resulted in the lowest MC value of 14.1 %. This decrease is primarily due to the hydrophobic nature of PEO, which lowers overall film hydrophilicity and water-holding capacity. Sonication likely facilitated the uniform incorporation of PEO into the crosslinked matrix, enhancing film cohesion and reducing moisture uptake. These findings are consistent with Asghar et al. [32], who observed improved moisture barrier properties in EO-enriched chitosan and gelatin films, supporting their suitability for food packaging applications.

3.5. Color, opacity, and total color difference

The optical properties of SA films, including color and opacity, are critical indicators of their suitability for food packaging, as they influence both consumer perception and product protection. Table 4 and Fig. 3 present the colorimetric parameters, opacity values, and visual characteristics of the control, treated, combined, and PEO-incorporated SA films.

The optical properties of SA films, including color and opacity, are critical indicators of their suitability for food packaging, as they

Table 3

Thickness, moisture content (MC), and water vapor permeability (WVP) of untreated (control) and treated SA-based films with varying concentrations of CaCl₂ crosslinking, sonication time, and patchouli essential oil (PEO) addition. Values represent mean $\pm$ standard deviation ($n = 3$). Different superscript letters indicate significant differences among samples ($p < 0.05$).

Sample	Thickness (mm)	MC (%)	WVP ($\times 10^{-11}$ g cm ^{−2} h ^{−1} Pa ^{−1})
Control Film	0.051 $\pm$ 0.001 ^a	23.72 $\pm$ 0.42 ^e	3.24 $\pm$ 0.09 ^a
S5	0.051 $\pm$ 0.004 ^{ab}	22.63 $\pm$ 0.25 ^{de}	2.82 $\pm$ 0.13 ^b
S10	0.048 $\pm$ 0.001 ^a	22.13 $\pm$ 0.31 ^{de}	3.42 $\pm$ 0.19 ^a
S20	0.047 $\pm$ 0.001 ^a	21.88 $\pm$ 0.28 ^{de}	3.50 $\pm$ 0.86 ^a
S30	0.046 $\pm$ 0.001 ^a	21.35 $\pm$ 0.19 ^{cde}	3.92 $\pm$ 0.18 ^a
C1	0.062 $\pm$ 0.002 ^c	20.22 $\pm$ 0.34 ^{cde}	3.16 $\pm$ 0.35 ^a
C1.5	0.064 $\pm$ 0.004 ^c	19.86 $\pm$ 0.41 ^{cd}	2.95 $\pm$ 0.17 ^b
C2	0.067 $\pm$ 0.001 ^c	19.15 $\pm$ 0.29 ^{cd}	2.20 $\pm$ 0.19 ^b
C2.5	0.070 $\pm$ 0.010 ^d	15.39 $\pm$ 0.26 ^{bc}	2.25 $\pm$ 0.09 ^b
S5/C2	0.076 $\pm$ 0.002 ^d	17.79 $\pm$ 0.33 ^{ab}	2.38 $\pm$ 0.86 ^b
S5/C2/PEO	0.086 $\pm$ 0.004 ^e	14.06 $\pm$ 0.31 ^a	2.11 $\pm$ 0.18 ^b

Table 4

Color parameters (L^* , a^* , b^*), total color difference (ΔE^*), and opacity values of untreated (control) and treated SA-based films with varying concentrations of CaCl_2 crosslinking, sonication time, and PEO addition. Values represent mean $\pm$ standard deviation ($n = 3$). Different superscript letters indicate significant differences among samples ($p < 0.05$).

Sample	L^*	a^*	b^*	ΔE^*	Opacity
Control Film	93.08 ± 0.04^a	-1.41 ± 0.01^a	4.37 ± 0.08^a	7.79 ± 0.04^a	0.035 ± 0.006^a
S5	93.08 ± 0.02^a	-1.41 ± 0.01^a	4.42 ± 0.09^a	7.81 ± 0.06^a	0.037 ± 0.000^a
S10	93.05 ± 0.03^a	-1.41 ± 0.03^a	4.39 ± 0.12^a	7.83 ± 0.06^a	0.037 ± 0.000^a
S20	93.11 ± 0.02^a	-1.41 ± 0.04^a	4.42 ± 0.03^a	7.80 ± 0.01^a	0.038 ± 0.000^a
S30	92.85 ± 0.08^b	-1.41 ± 0.05^a	4.39 ± 0.12^a	8.00 ± 0.13^b	0.037 ± 0.001^a
C1	92.89 ± 0.05^b	-1.41 ± 0.01^a	4.41 ± 0.05^a	7.97 ± 0.06^a	0.037 ± 0.000^a
C1.5	92.85 ± 0.03^b	-1.41 ± 0.01^a	4.38 ± 0.09^a	7.98 ± 0.04^a	0.037 ± 0.000^a
C2	92.93 ± 0.04^b	-1.42 ± 0.01^a	4.47 ± 0.06^a	7.97 ± 0.05^a	0.038 ± 0.000^a
C2.5	92.75 ± 0.01^b	-1.42 ± 0.02^a	4.66 ± 0.30^a	8.21 ± 0.16^b	0.037 ± 0.001^a
S5/C2	92.85 ± 0.08^b	-1.42 ± 0.01^a	4.42 ± 0.01^a	8.01 ± 0.07^b	0.037 ± 0.001^a
S5C2/PEO	91.08 ± 1.47^c	-1.90 ± 0.15^b	6.52 ± 2.43^b	10.72 ± 2.63^c	0.106 ± 0.003^b

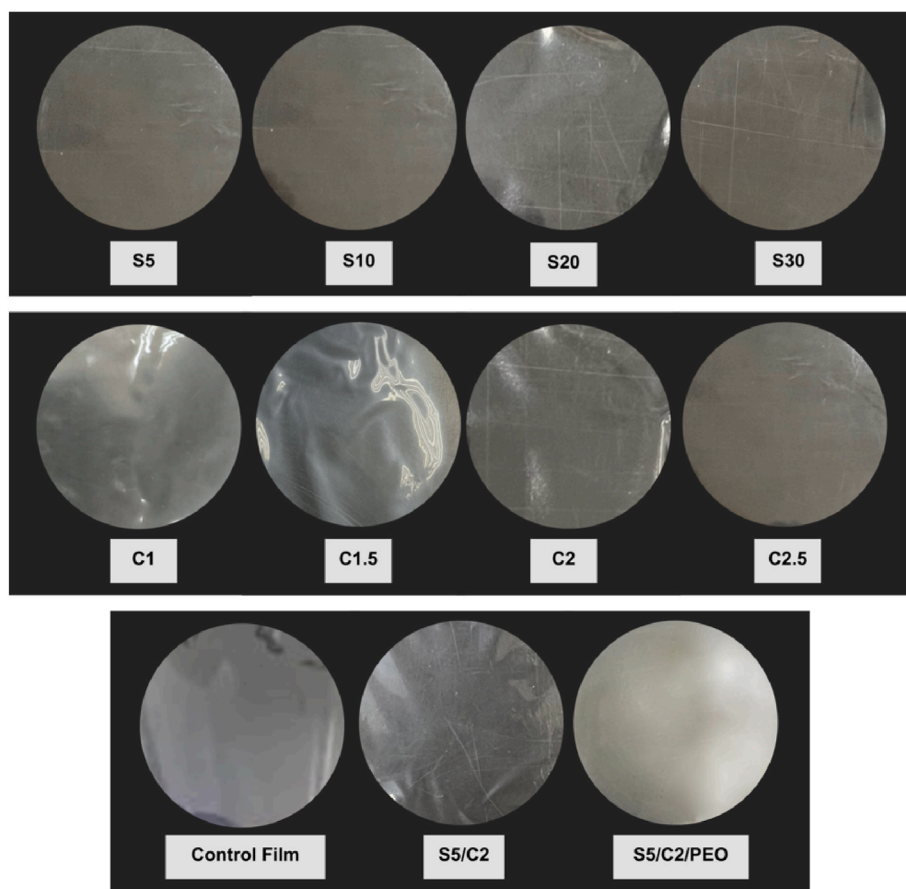


Fig. 3. Visual appearance of untreated (control) and treated SA-based films, with varying concentrations of CaCl_2 crosslinking, sonication time, and patchouli essential oil (PEO) addition.

influence both consumer perception and product protection. Table 4 and Fig. 3 present the colorimetric parameters, opacity values, and visual characteristics of the control, treated, combined, and PEO-incorporated SA films.

The control film exhibited a lightness (L^*) value of 93.08, indicating a translucent appearance. Sonicated films showed comparable L^* values, suggesting that sonication had no significant impact on lightness. A slight reduction in L^* was observed in films treated with higher concentrations of CaCl_2 and in combination treatments, likely due to increased matrix compactness, which reduced light transmission. This darkening effect aligns with previous findings showing that CaCl_2 crosslinking produces denser films with reduced transparency [33].

The S5/C2/PEO film exhibited a significant decrease in L^* ($p <$

0.05), with a value of 91.08 (Table 4), consistent with the visual darkening observed in Fig. 3. The natural yellow-brown hue of PEO, combined with its emulsified dispersion in the film matrix, increased light absorption and scattering. These changes resulted in a total color difference (ΔE^*) of 10.72, confirming the strong visual impact of PEO incorporation. Socaciu et al. [34] reported similar trends, where the addition of tarragon essential oil to whey protein isolate films significantly altered color metrics, depending on oil type and concentration.

All samples exhibited negative a^* values, indicating a greenish tint, likely due to residual chlorophyll in the SA [35]. This parameter remained relatively unchanged with sonication and CaCl_2 crosslinking. However, PEO incorporation led to more noticeable color variation. The b^* values (yellowness) significantly increased ($p < 0.05$) in the

S5/C2/PEO film, attributable to the intrinsic color of PEO [36]. Although b^* remained relatively stable in other treated films, PEO markedly increased the opacity to 0.106. This rise in opacity reflects a more heterogeneous matrix capable of scattering light more effectively [37]. As depicted in Fig. 3, the S5/C2/PEO film appears more opaque and exhibits a matte surface compared to other samples.

The altered color and opacity observed in the treated films may offer functional advantages for specific packaging applications. Higher opacity enhances light protection, which is essential for preserving light-sensitive products prone to lipid oxidation or nutrient degradation. Prior studies have demonstrated that high-opacity packaging effectively shields perishable items such as meat, dairy, and nuts from photo-degradation [38–40]. However, for products that benefit from consumer visibility such as fresh produce, transparent packaging with low opacity may be preferred.

In this study, PEO incorporation enhanced the film's protective qualities by increasing opacity and reducing lightness, which could be advantageous for shielding packaged foods from light-induced deterioration. In contrast, the higher L^* values and lower opacity of the S5 and C2 films may be more suitable for applications requiring product visibility and visual quality monitoring during storage.

3.6. Water vapor permeability

Table 3 presents the water vapor permeability (WVP) of SA-based films subjected to various treatments. Among the sonicated films, S5 exhibited the lowest WVP at 2.81. However, prolonged sonication (30 min) increased the WVP to 3.92, suggesting that excessive exposure to ultrasonic energy may compromise the film structure. This increase is likely due to enhanced chain mobility and the formation of microvoids that facilitate water vapor diffusion. Hafizulhaq et al. [41] reported that while sonication can promote structural homogeneity, extended treatment may disrupt the polymer matrix and create pathways for vapor permeation.

SA, being a hydrophilic polymer, tends to absorb moisture under high relative humidity (RH), which can further increase WVP. The absorbed water acts as a plasticizer, weakening interchain interactions and increasing chain flexibility and permeability [42,43]. Although sonication improves dispersion and film uniformity, the resulting increase in film flexibility may inadvertently raise WVP when applied excessively.

Crosslinking with CaCl_2 significantly improved the moisture barrier properties. The C1 and C1.5 films showed moderate WVP reductions to 3.16 and 2.95, respectively. The C2 film recorded the lowest WVP at 2.20, indicating that optimal CaCl_2 concentration enhances matrix cohesion and restricts water vapor diffusion. In contrast, the C2.5 film showed a slight increase in WVP, likely due to excessive crosslinking, which can produce overly dense calcium-alginate domains. These rigid structures may reduce network compactness, hindering efficient film packing and permitting greater vapor penetration [31,44].

Farias et al. [45] described Ca^{2+} -induced crosslinking as forming a reticulated “egg-box” network that reduces polymer chain mobility and impedes water diffusion through the matrix. Consistent with this, the film subjected to both sonication and 2 % CaCl_2 crosslinking (S5/C2) showed a WVP of 2.38, which was lower than that of the control, indicating improved barrier performance. This enhancement is attributed to the dual effect of polymer alignment via sonication and crosslinking-induced structural reinforcement.

These findings are consistent with Khadsai et al. [46], who demonstrated that Ca^{2+} crosslinking significantly reduces WVP by creating a compact polymer network. Such networks limit molecular diffusion, thereby improving moisture resistance and extending the functional lifespan of the packaging material.

3.7. Thermal properties

The thermogravimetric (TGA) and derivative thermogravimetric (DTG) curves of the films are shown in Fig. 4(a) and (b), revealing distinct multistep degradation profiles. The control and S5 films exhibited the typical three-step degradation behavior, while the C2, S5/C2, and S5/C2/PEO films displayed more complex four-step patterns due to the presence of CaCl_2 and PEO.

The first degradation stage occurred below 120 °C and corresponded to the evaporation of free and bound water, resulting in an average mass loss of approximately 16 % across all samples [47,48]. A second degradation stage, observed exclusively in the S5/C2/PEO film between 120 and 200 °C, involved a ~6 % mass loss, likely due to the volatilization of PEO constituents such as patchoulol. This step was absent or negligible in other samples.

The primary degradation stage between 200 and 300 °C was attributed to the cleavage of glycosidic bonds in the sodium alginate backbone. The control film experienced a 43 % mass loss during this stage. As shown in Fig. 4(b), the maximum degradation temperature (T_{max}) of this step increased slightly with treatment: 227.67 °C (Control), 232.67 °C (S5), 233.00 °C (C2), and 232.17 °C (S5/C2). The onset degradation temperature also increased, from 200.67 °C in the control to 221.83 °C in the S5/C2/PEO film (Table 5).

The increase in both onset and T_{max} values in the S5 film suggests that ultrasonication enhanced thermal resistance by promoting molecular alignment and reducing structural irregularities. Similarly, the C2 and S5/C2 films exhibited delayed degradation onset, attributed to the formation of ionic crosslinks between the carboxylate groups of SA and Ca^{2+} ions. These interactions formed a more rigid polymer matrix, consistent with the “egg-box” model, which enhances thermal stability by restricting molecular mobility and requiring greater energy for degradation.

Above 300 °C, all films entered the final degradation stage, corresponding to the breakdown of residual components such as Tween-80, EO residues, and chitosan nanoparticles. The S5/C2/PEO film exhibited the highest residual mass, indicating superior thermal stability. This enhancement reflects a dual effect of PEO, while its volatile components contribute to early-stage mass loss, its hydroxyl-rich fractions promote hydrogen bonding with the SA matrix, reinforcing the network against thermal degradation.

The broader and less intense DTG peak of the S5/C2/PEO film (Fig. 4 (b)) supports this mechanism, indicating a more gradual decomposition process. These results are in agreement with previous findings by Almasi et al. [49] and Kowalonek et al. [24], who reported similar thermal behaviors in SA films incorporated with essential oils. Overall, the combined application of sonication, CaCl_2 crosslinking, and PEO incorporation enhanced the thermal resistance of the SA-based films, reinforcing their potential for active food-packaging applications.

3.8. Surface and cross-sectional morphology

Fig. 5 presents SEM micrographs of the surface and cross-sectional morphology of untreated and treated SA-based films. The control film displayed a smooth and uniform surface without pores or cracks, indicating a consistent and homogenous microstructure. Although the cross-section appeared compact, minor structural roughness was observed, possibly due to shrinkage or internal stress during the drying process.

The S5 film showed linear protuberances on the surface; however, the structure remained dense and compact, lacking visible cracks or pores. Sonication introduced uneven cross-sectional morphology and increased porosity, likely due to partial SA chain degradation and the formation of microchannels. Despite the increased porosity, the film retained a compact architecture, consistent with previous findings that report improved mechanical strength and reduced WVP following controlled sonication [50–53]. The observed aligned layers and reduced surface irregularities are likely a result of ultrasonic cavitation-induced

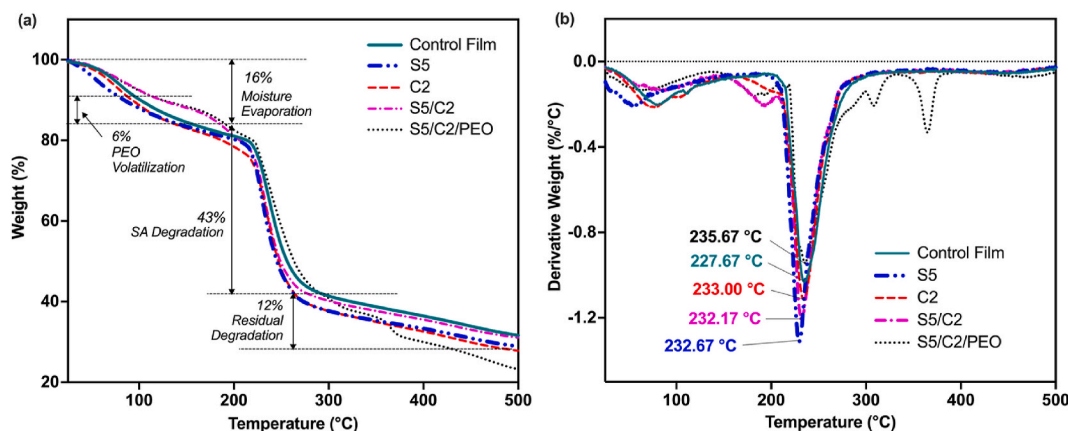


Fig. 4. Thermal curves of untreated (control) and treated SA-based films with or without PEO incorporation: (a) TG and (b) DTG.

Table 5

Onset and thermal degradation temperature (T_{max}) of untreated (control) and treated SA-based films with or without PEO incorporation.

Samples	Onset temperature (°C)	Degradation temperature, T_{max} (°C)
Control Film	200.67	227.67
S5	202.33	232.67
C2	215.33	233.00
S5/C2	214.00	232.17
S5/C2/PEO	221.83	235.67

depolymerization and molecular rearrangement. This denser structure corresponds to enhanced TS and moisture barrier properties, as supported by FTIR spectra (Fig. 1), which revealed intensified COO^- peaks and shifts in O–H stretching indicative of stronger intermolecular hydrogen bonding.

Crosslinked films (C2) exhibited rougher surfaces with small protrusions, attributed to rapid gelation and heterogeneous CaCl_2 diffusion during ionic crosslinking [54]. FTIR analysis confirmed the formation of ionic bonds between Ca^{2+} and COO^- groups, forming the characteristic “egg-box” structure [55]. Crosslinking resulted in a denser and more compact internal matrix, evident in the cross-section, which enhanced barrier performance and rigidity.

The S5/C2 film showed a more stratified and compact morphology than the C2 film alone. Sonication appeared to refine the crosslinked structure by minimizing surface irregularities, producing a film that balanced mechanical strength and flexibility. This structural optimization is consistent with the film’s elevated TS and moderate EaB.

In contrast, the S5/C2/PEO film exhibited a coarse, heterogeneous surface with visible oil droplet inclusions. These disruptions are likely due to hydrophobic interactions and reduced hydrogen bonding in the presence of PEO, which interfere with matrix uniformity [56]. The cross-section revealed a sponge-like, porous architecture that correlates with increased opacity and reduced EaB. However, this porous network may facilitate the gradual release of antimicrobial agents. Phal et al. [57] reported similar morphological changes in SA films incorporated with turmeric essential oil, noting increased roughness and porosity. The inclusion of PEO in this study yielded comparable structural alterations, influencing WVP, opacity, and EaB, despite a reduction in TS compared to untreated films [58].

3.9. Antimicrobial activity in vivo

SA-based films, particularly those incorporated with essential oils (EOs), have attracted significant attention as active packaging materials for prolonging the shelf life of perishable foods such as cherry tomatoes (Fig. 6). Fig. 7 presents the visual appearance of cherry tomatoes

packaged with different SA-based films over a four-day storage period, highlighting qualitative differences in spoilage and preservation across treatments.

The S5/C2/PEO film exhibited notable antimicrobial activity, effectively preserving the quality of cherry tomatoes with minimal shrinkage and no visible mold growth throughout the four-day storage period. In contrast, the control SA film showed rapid spoilage, discoloration, and fungal development by day four (Fig. 7). Previous studies have extensively explored the incorporation of essential oils (EOs) into SA-based films, consistently reporting enhanced antimicrobial effects. Tian et al. [59], for example, showed that SA films containing emulsified peppermint EO completely inhibited microbial infection in cherry tomatoes over six days at room temperature. Although the present study relies on qualitative observations, the suppression of fungal growth in the S5/C2/PEO film parallels the quantitative findings of Tian et al. [59], reinforcing the efficacy of EO-enriched SA films in microbial control.

In addition to their bioactivity, film structure plays a key role in sustained release. Mahcene et al. [60] showed that crosslinking polymer matrices with Ca^{2+} ions effectively trap EO molecules and facilitates controlled release, thereby maintaining antimicrobial efficacy. Despite the proven benefits of EOs such as cinnamon and clove, their intense aromas may limit their use in specific food applications. In contrast, PEO provides a milder, woody aroma and offers distinct mechanical characteristics, making it a promising candidate for active packaging systems where sensory neutrality is essential [61].

EOs typically disrupt bacterial membranes, increase permeability, and induce cell death [62,63]. The antimicrobial activity observed in the S5/C2/PEO film likely resulted from the intrinsic bioactivity of PEO combined with structural enhancements in the sodium alginate matrix. In vitro studies have reported broad-spectrum efficacy of patchouli alcohol, a major component of PEO, with minimum inhibitory concentrations ranging from 1.5 to 200 $\mu\text{g}/\text{mL}$ against gram-positive bacteria and 25–768 $\mu\text{g}/\text{mL}$ against gram-negative strains [64]. PEO also demonstrated a notable 6.80 mm inhibition zone against *Shigella* sp., outperforming citronella and nutmeg oils, which showed no effect on this resistant gram-negative pathogen [65]. Rajan et al. [66] further validated PEO’s antimicrobial efficacy in PLA/PBS-based active packaging, which suppressed *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa* in fresh chicken meat.

In addition to its bioactivity, PEO’s sesquiterpene-rich composition imparts a neutral, woody aroma, enhancing its suitability for odor-sensitive food products. In this study, no perceptible off-odors were observed in PEO-treated cherry tomatoes, suggesting favorable sensory compatibility. These findings provide preliminary evidence for the antimicrobial potential of the S5/C2/PEO film; however, confirmation through quantitative assays such as colony-forming unit counts or

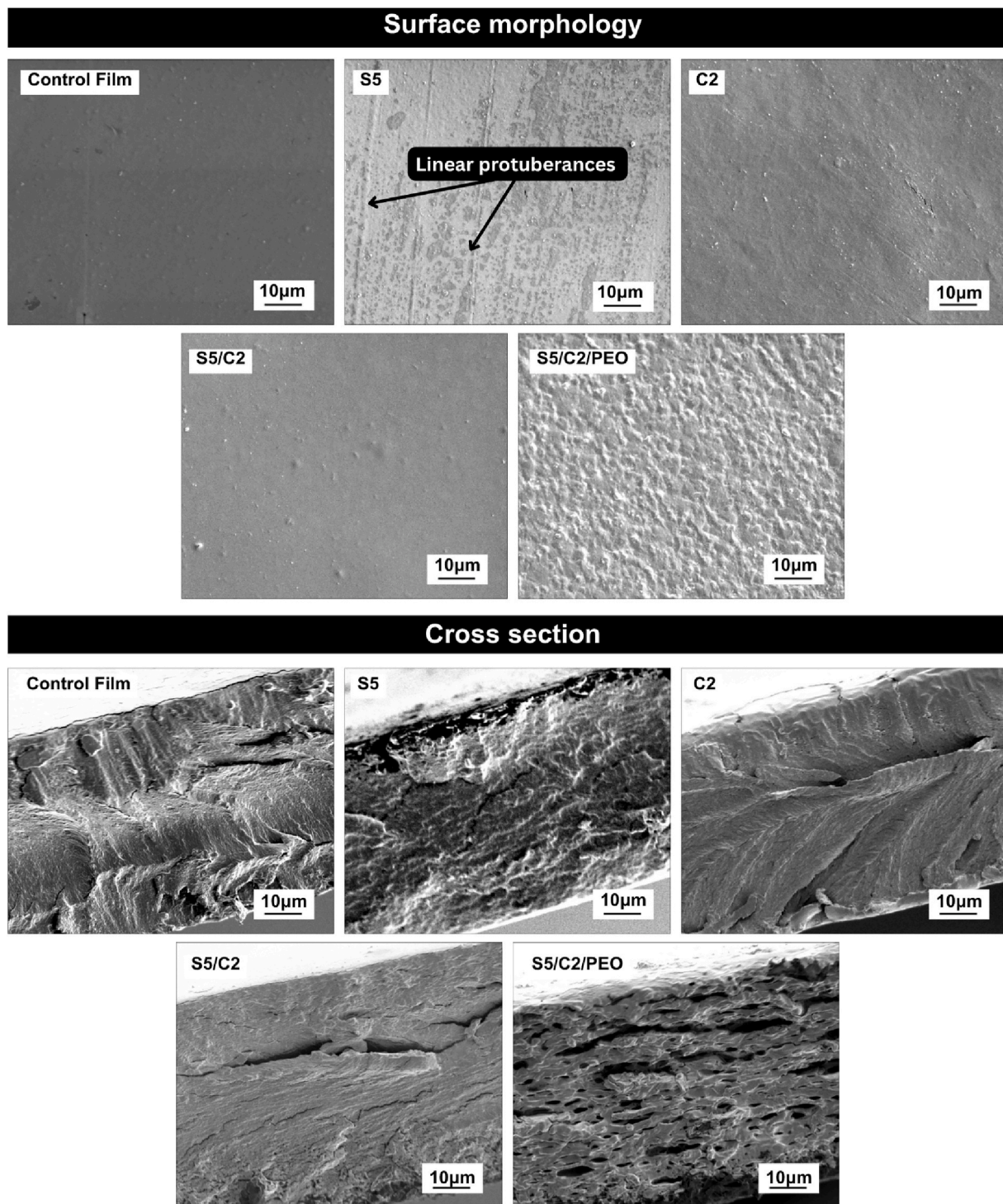


Fig. 5. Micrographs of the surfaces and cross-sections of the untreated (control) and treated SA-based films, with or without PEO incorporation at x1000 magnification: control film, S2, C2, S5/C2, and S5/C2/PEO.

inhibition zone analysis is necessary.

From a regulatory standpoint, the U.S. Food and Drug Administration (FDA) classifies PEO as Generally Recognized as Safe (GRAS) under 21 CFR 182.20, authorizing its use in food and food-contact materials. Similarly, the European Union permits its use under Framework Regulation (EC) No. 1935/2004, provided it does not compromise food safety or alter sensory attributes [67,68]. These regulatory endorsements support the safe and practical application of PEO in commercial food-packaging systems.

4. Conclusion

The combination of sonication, CaCl_2 crosslinking, and PEO incorporation significantly improved the physicochemical, mechanical, and barrier properties of the SA film. The optimized film (S5/C2/PEO) exhibited enhanced TS, reduced WVP, and improved thermal stability. Qualitative visual assessments also indicated preliminary antimicrobial activity, as evidenced by the delayed spoilage and mold inhibition in cherry tomatoes.

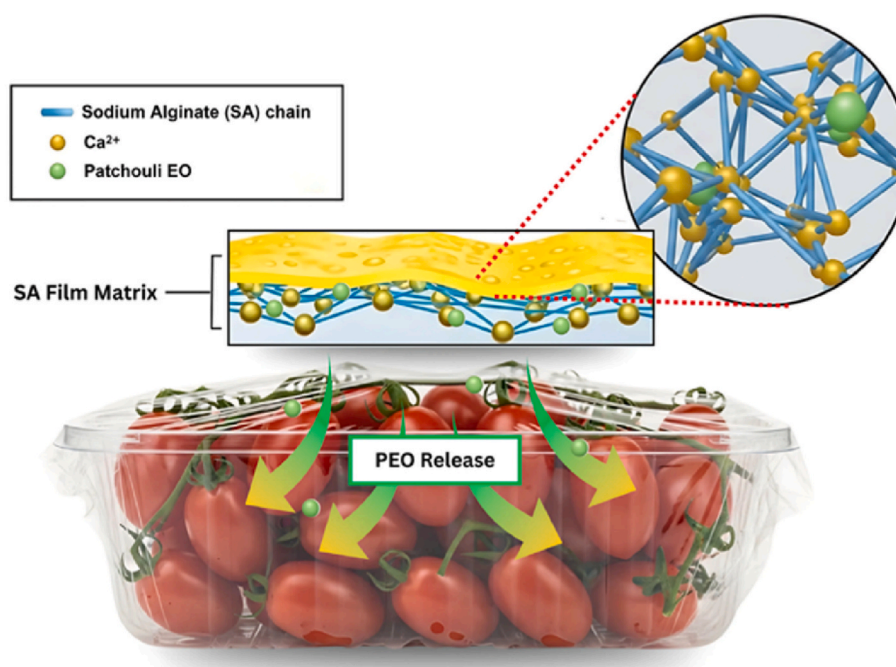


Fig. 6. Schematic representation of the preservation mechanism of SA films for cherry tomatoes. The SA film matrix was crosslinked with calcium ions (Ca^{2+}) and infused with patchouli essential oil (PEO). Ca^{2+} ions form ionic bridges among alginate chains, enhancing film strength, while PEO is released slowly from the matrix, exhibiting antimicrobial activity and extending shelf life.

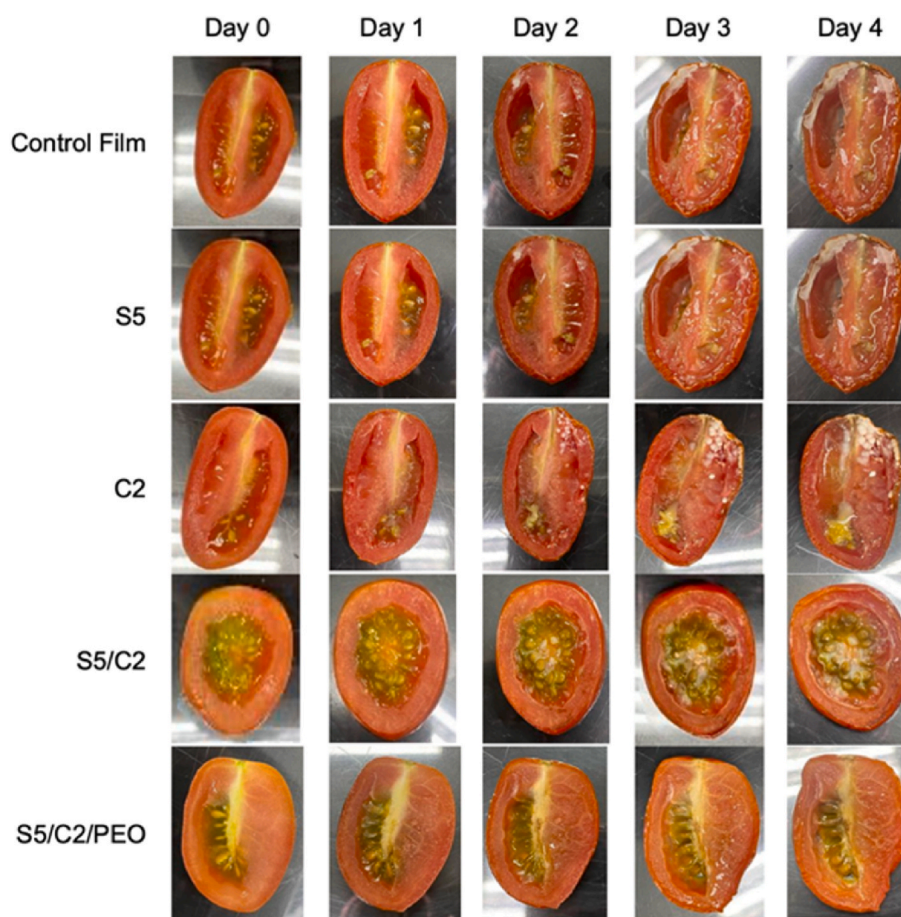


Fig. 7. Visual appearance of microbial growth on cherry tomatoes with untreated (control) and treated SA-based films, with or without PEO incorporation, up to 4 days of storage at 25 °C.

These findings support the potential of PEO-loaded SA films as biodegradable alternatives to the conventional synthetic packaging materials. Current antimicrobial assessments remain qualitative and require validation through quantitative microbiological assays. Researchers should also evaluate the potential for aroma transfer from PEO components using sensory and migration analyses. Future research should investigate encapsulation techniques, such as chitosan-based nanoparticles, to control release kinetics, enhance film compatibility, and prolong shelf-life performance. Researchers must also address regulatory compliance, assess consumer acceptance, and conduct long-term storage trials to advance the practical application of these films in food packaging.

CRediT authorship contribution statement

Farhana Azmira Asmadi: Writing – review & editing, Visualization, Methodology, Formal analysis, Conceptualization. **Nurul Afwani Khalid:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Mohd Salahuddin Mohd Basri:** Supervision. **Siti Hajar Othman:** Supervision. **Rosnita A. Talib:** Supervision. **Thinesh Sharma Balakrishnan:** Writing – review & editing. **Intan Syafinaz Mohamed Amin Tawakkal:** Writing – review & editing, Funding acquisition, Conceptualization.

Data availability statement

This study's supporting data can be obtained from the corresponding author upon reasonable request

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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