Eco-Friendly Carboxymethyl Cellulose/Pullulan Blend Films: Preparation, Characterization and Proposed Applications

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Abstract

Background and Objectives: In recent years, the heavy reliance on petroleum-based synthetic plastics particularly for single-use products has sparked significant environmental concern because of their long-lasting nature and resistance to breaking down naturally. Despite the functional advantages and convenience these materials offer, their accumulation in natural ecosystems has emerged as a critical ecological issue. Consequently, research efforts have increasingly shifted toward the development of sustainable, bio-based alternatives aimed at mitigating the environmental impact associated with conventional plastic use. Carboxymethyl cellulose (CMC) is a biodegradable polymer derived from naturally abundant cellulose, renowned for its excellent film-forming properties. However, its high-water solubility and inherent brittleness limit its practical utility in various applications. This study aims to enhance the water resistance and physical strength of CMC films through the citric acid crosslinking and the incorporation of pullulan. The modified films were subsequently evaluated for their structural integrity and biocompatibility to assess their potential applications.

Methodology: The modification of CMC-based films to enhance both water resistance and mechanical strength was accomplished through an approach involving the transformation of the linear structure of CMC into a three-dimensional network. This transformation employed citric acid as a non-toxic crosslinking agent, selected for its effectiveness in promoting crosslinking without compromising the biocompatibility of the resulting materials. Additionally, CMC/pullulan blending was performed to achieve uniform dispersion and to improve the overall properties of the films. Film formation was conducted using the solution casting method. The resultant crosslinked films combining CMC with pullulan, a polysaccharide known for its film-forming capabilities, were characterized to evaluate their properties. Characterization techniques included ATR-FTIR spectroscopy to assess the functional groups present in the films. Water solubility and swelling tests were conducted to evaluate the films' resistance to water, a critical property for many applications. Tensile strength analysis was used to determine the mechanical performance of the films, indicating their ability to withstand stress without failure. Furthermore, SEM provided detailed insights into the films' surface morphology. To ensure the biosafety of these innovative films, cell viability assays were conducted using African green monkey fibroblast cells (Vero) and immortalized human keratinocytes (HaCaT). These assays were crucial for evaluating potential cytotoxic effects, thereby confirming the films' suitability for biomedical applications and other uses where biocompatibility is essential, such as drug delivery matrices.

Main Results: Citric acid crosslinking significantly improved the overall properties of CMC films, particularly enhancing their water resistance and mechanical strength. The study identified that incorporation of 15% citric acid yielded the most advantageous performance characteristics among the variations tested. This level of citric acid not only provided a network structure to the films but also contributed to their durability when exposed to moisture. In addition to citric acid, the incorporation of pullulan, a natural polysaccharide, further enhanced the flexibility of the CMC-based films. This addition was particularly beneficial as it did not compromise the optical transparency of the films, ensuring that they maintained clarity even with the modifications. The ability to retain transparency while improving flexibility is crucial for a range of applications, especially in fields such as packaging and biomedical materials. To confirm the successful formation of crosslinks and the compatibility of the polymeric components, characterization techniques such as ATR-FTIR and SEM were employed. The ATR-FTIR analysis demonstrated a new band associated with the covalent crosslinking through esterification reactions between the carboxylic acid groups of citric acid and the hydroxyl groups on the polymer chains. Meanwhile, the SEM images revealed a uniform and homogenous surface structure, providing further evidence of the effective interaction between the components. Importantly, the optimized film exhibited high cell viability for both Vero (95%) and HaCaT (90%) cell lines, highlighting its excellent biocompatibility. The positive results in these cell lines suggest that the modified CMC films are safe for biological applications. The combination of mechanical strength, flexibility, transparency, and biocompatibility makes the CMC based films, produced through citric acid crosslinking and pullulan incorporation, promising candidates for a wide range of future biomedical and packaging applications.

Conclusions: The incorporation of pullulan into crosslinked CMC matrices has proven to be a significant advancement in the field of biodegradable materials. This integration not only maintained the visual appeal and structural integrity of the films but also led to enhancements in their overall functional performance. The synergistic effects of combining CMC with pullulan resulted in the creation of innovative biodegradable films that possess improved characteristics. An optimal citric acid concentration of 15% was identified, as higher concentrations resulted in decreased mechanical performance. Incorporating 15% and 30% pullulan into the CMC matrix further enhanced the films' flexibility and tensile properties. These enhanced properties make the films well-suited for a broad range of applications. Their appealing visual appearance, combined with

mechanical strength and flexibility, suggests that the crosslinked CMC/pullulan films can effectively meet consumer expectations while supporting environmental sustainability and reducing plastic waste.
Keywords: pullulan ; carboxymethyl cellulose ; biodegradable film ; citric acid ; packaging film

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Introduction

In recent decades, there has been a heavy reliance on petroleum-derived synthetic plastics, particularly for single-use applications. While these materials offer convenience and functionality, their persistence and non-biodegradability pose significant environmental challenges (Kumari *et al*., 2023). In response to growing ecological concerns, there has been a marked shift in research toward sustainable, bio-based alternatives. Among these, natural polysaccharides, especially cellulose and its derivatives, have garnered considerable attention due to their abundance, renewability, biodegradability, and environmental compatibility (Aleksanyan, 2023; Cheng *et al*., 2024). Cellulose, the most abundant biopolymer, is a fundamental component of plant cell walls. However, its extensive intra- and intermolecular hydrogen bonding driven by numerous hydroxyl groups limits its solubility and practical utility in its native form (Yildirim-Yalcin *et al*., 2022). To overcome these limitations, cellulose is frequently converted into carboxymethyl cellulose (CMC), a water-soluble derivative widely recognized for its non-toxicity, biocompatibility, and excellent film-forming ability (Feng *et al*., 2022). Despite these favorable properties, CMC-based films frequently exhibit mechanical limitations, including brittleness and poor flexibility (An *et al*., 2021). To address these limitations, various studies have investigated the incorporation of complementary biopolymers to improve the structural and mechanical integrity of CMC films (Zhao *et al*., 2022). Among these, pullulan, a linear, water-soluble polysaccharide composed of maltotriose units linked by α-(1,6)-glycosidic bonds, has attracted considerable attention due to its excellent film-forming ability, biodegradability, and proven biosafety (Zhang *et al*., 2021). Blending pullulan with other polysaccharides has been shown to markedly improve film flexibility and strength, highlighting its potential for the development of biodegradable packaging and other sustainable functional materials (Priyadarshi *et al*., 2021). In addition to polymer blending, chemical crosslinking has been widely adopted to further enhance film stability and mechanical performance. Conventional crosslinkers such as glutaraldehyde, epichlorohydrin, and sodium trimetaphosphate have been employed for this purpose (Khabibi *et al*., 2021; Han *et al*., 2023); however, their use is often accompanied by concerns regarding residual toxicity and environmental sustainability.

The novelty of the present study lies in the strategic development of crosslinked CMC/pullulan blend films using citric acid as a multifunctional crosslinker and bio-based crosslinker, an approach that had not been extensively explored within this polymer system. Citric acid is a naturally occurring, non-toxic, and environmentally organic acid containing three carboxyl (–COOH) groups and one hydroxyl (–OH) group which confer high reactivity and enable multiple crosslinking sites. These functional groups allow citric acid to effectively form covalent bonds with the abundant hydroxyl groups of CMC and pullulan via esterification reactions under suitable thermal conditions. This process generates a stable three-dimensional network within the polymer matrix, thereby enhancing structural integrity, water resistance, and mechanical durability. Consequently, citric acid crosslinking reinforces the blend films, resulting in improved functional properties suitable for sustainable packaging applications (de Lima *et al.,* 2020). A comprehensive characterization of the films' structural, mechanical, and optical properties was conducted to evaluate their functional performance. Additionally, a cytotoxicity assessment using African green monkey fibroblast (Vero) and immortalized human keratinocytes (HaCaT) cells confirmed the films' biocompatibility, supporting their suitability for contact-sensitive applications. The synergistic combination of CMC and pullulan, crosslinked with citric acid, presented a novel material with strong potential to biodegradable applications, particularly in packaging and biomedical fields.

Methodology

1. Materials

Carboxymethyl cellulose, sodium salt (CMC; average MW 90,000 g/mol) was purchased from Thermo Fisher Scientific (USA). Pullulan (CAS No.9057-02-7; viscosity: 160.0 mPa·s at 10% in H_2O) was obtained from Tokyo Chemical Industry Co., Ltd. (Japan). Citric acid monohydrate was sourced from Honeywell International Inc. (USA). Glycerol (AR Grade) was purchased from QRec (Malaysia).

2. Preparation of Films

Blend films were prepared using the solution casting technique. 2% (w/v) aqueous solutions of carboxymethyl cellulose (CMC) and pullulan were prepared by dissolving the polymers in distilled water at 80 °C. Citric acid, employed as a crosslinking agent, was added at concentrations of 15% and 30% (w/w, relative to the total dry weight of the polymers), followed by continuous stirring at 80 °C for 3 h. Subsequently, glycerol (10% w/w, based on the dry polymer weight) was incorporated as a plasticizer, and the mixture was stirred for an additional 30 min at 80 °C. The resulting solution was then subjected to sonication for 5 min to remove air bubbles. The homogeneous polymer solution was poured into Petri dishes and dried at 60 °C for 20 h. The dried films were carefully peeled off and stored until further characterization. The prepared films were designated as 100C, 100C-15A, 100C-30A, 15P, 15P-15A, 15P-30A, 30P, 30P-15A, and 30P-30A, reflecting the specific polymer blend ratios and citric acid (A) concentrations (Table 1).

Table 1 Chemical compositions of the blend films

Samples*	2% CMC (mL)	2% Pullulan (mL)	Citric acid (g)	10% Glycerol (μL)
100C	40.0	-	-	800
100C-15A	40.0	-	0.12	800
100C-30A	40.0	-	0.24	800
15P	34.0	6.0	-	800
15P-15A	34.0	6.0	0.12	800
15P-30A	34.0	6.0	0.24	800
30P	28.0	12.0	-	800
30P-15A	28.0	12.0	0.12	800
30P-30A	28.0	12.0	0.24	800

*C = Carboxymethyl cellulose, P = Pullulan and A = Citric acid

3. Characterization of Films

3.1 Fourier Transform Infrared Spectroscopy (FTIR)

Chemical functionalities and potential interactions within the film matrix were analyzed using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy (PerkinElmer System 2000). Spectra were recorded over the range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1}, averaging four scans per sample.

3.2 Optical Properties

Colorimetric analysis of the films was performed using a WR-10 colorimeter, following the Hunter Lab color space system. The parameters measured included L* (lightness), a* (red-green scale), and b* (yellow-blue scale). The total color difference (ΔE) between control and modified films was calculated using the following equation (Roy & Rhim, 2021):

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

where ΔL, Δa, and Δb represents the differences between each color value of the control and the test films.

3.3 Surface Morphology

The morphology of the blend films was characterized using a scanning electron microscope (SEM, LEO1450VP). The films were mounted on carbon tapes attached to aluminum stubs and then vacuum sputter-coated with gold prior to analysis. SEM images of the blend films were captured at a magnification of 3.5KX.

3.4 Swelling Behavior and Water Solubility

Swelling and solubility of the films were evaluated using a gravimetric method. Pre-dried film samples (2 cm × 2 cm) were immersed in 30 mL of distilled water for 1 h, taken out and weighed. After swelling, samples were re-dried to determine residual mass. %Swelling and %water solubility were calculated based on the differences between initial, swollen, and final dry weights (Kanatt & Makwana, 2020).

$$\%\text{Swelling} = [(W_{swell} - W_{dry}) / W_{dry}] \times 100 \quad (2)$$

$$\%\text{Water solubility} = [(W_{dry} - W_{sol}) / W_{dry}] \times 100 \quad (3)$$

Here, W_{dry} represents the initial weight of the dried film before immersion, W_{swell} denotes the weight after swelling in distilled water, and W_{sol} corresponds to the remaining weight of the dried film after testing.

3.5 Tensile Properties

The mechanical properties, including tensile strength (TS) and percent elongation at break (%EB), were evaluated using a texture analyzer (TA.XTplus, Stable Micro Systems). The fabricated films were cut into specimens measuring 10 mm × 50 mm. The specimens were mounted with a grip distance of 30 mm and stretched at a rate of 2.0 mm/s. The TS and %EB values were determined from stress-strain curves, and Young's modulus was calculated from the slope of the initial linear region.

3.6 Cytotoxicity

The cytotoxicity was evaluated using an MTT assay using African green monkey fibroblast cells (Vero) and human keratinocyte immortalized cells (HaCaT). The sample films were immersed in culture medium (DMEM + 5% FBS) for 24 h. The obtained medium was then added to cells (1×10^5 cells/mL, in a 96-well plate) and incubated at 37 $^{\circ}$C under a 5% CO_2 atmosphere for 24 h. MTT solution (10 µL/well) was added, and incubation continued for an additional 4 h. Subsequently, the MTT solution was removed, and 100 µL/well of a dissolving solution for the formazan crystals (100% DMSO:10% SDS, 1:9) was added. Absorbance was measured at 570 nm after swirling the plate for 5 minutes. The percentage of cytotoxicity was calculated using the following equation:

$$\%Cytotoxicity = [(A-B) / A] \times 100 \quad (4)$$

where A represents the absorbance of the well containing cells in culture medium, and B represents the absorbance of the well containing cells with the sample solution.

Results

1. Properties of the Crosslinked CMC Films

In this study, citric acid was employed as a non-toxic crosslinking agent to enhance the functional properties of CMC films. The effects of crosslinking on the physical and chemical characteristics of the modified 100C-15A and 100C-30A films, in comparison with the uncrosslinked 100C films, are summarized in Figure 1. These include differences in visual appearance, Fourier-transform infrared (FTIR) spectra, water swelling behavior, solubility, and mechanical performance. Digital photographs in Figure 1(a) show that all films were transparent with smooth, uniform surfaces. Colorimetric analysis (Table 2) revealed that the incorporation of citric acid did not significantly affect the visual appearance of the films. The ΔE values for 100C-15A and 100C-30A were minimal, measuring 0.33 and 0.36, respectively, indicating a negligible perceptible color change compared to the unmodified 100C film. ATR-FTIR spectroscopy was conducted to confirm successful crosslinking with citric acid (Figure 1(b)). The spectrum of unmodified 100C exhibited characteristic absorption bands around 3300 cm^{-1} and 2900 cm^{-1}, corresponding to O–H and C–H stretching vibrations, respectively. A prominent band at 1590 cm^{-1} was attributed to carboxylate ($–COO^-$) groups. Additional peaks at 1470 cm^{-1} and 1325 cm^{-1} were assigned to CH_2 scissoring and O–H bending, respectively, while the peak near 970 cm^{-1} was associated with C–O–C stretching of the cellulose ether backbone (Reshma *et al.,* 2020). Upon crosslinking, new absorption bands emerged near 1720 cm^{-1} in the spectra of 100C-15A and 100C-30A, indicative of C=O stretching from ester bonds, confirming successful esterification and covalent crosslink formation within the CMC matrix (Ghorpade *et al.,* 2019).

Water solubility testing demonstrated a pronounced difference in water resistance between the unmodified and crosslinked films. The pristine 100C film completely dissolved in water within 2 minutes at room temperature, highlighting its poor aqueous stability. In contrast, the crosslinked films (100C-15A and 100C-30A) exhibited markedly enhanced water resistance, with swelling values reduced to 1195% and 915%, respectively, and corresponding solubility values of 60% and 37% (Figure 1(c)). These findings suggest that crosslinking effectively improved water resistance, likely through the formation of a stable three-dimensional polymer network (Pratinthong *et al.,* 2024).

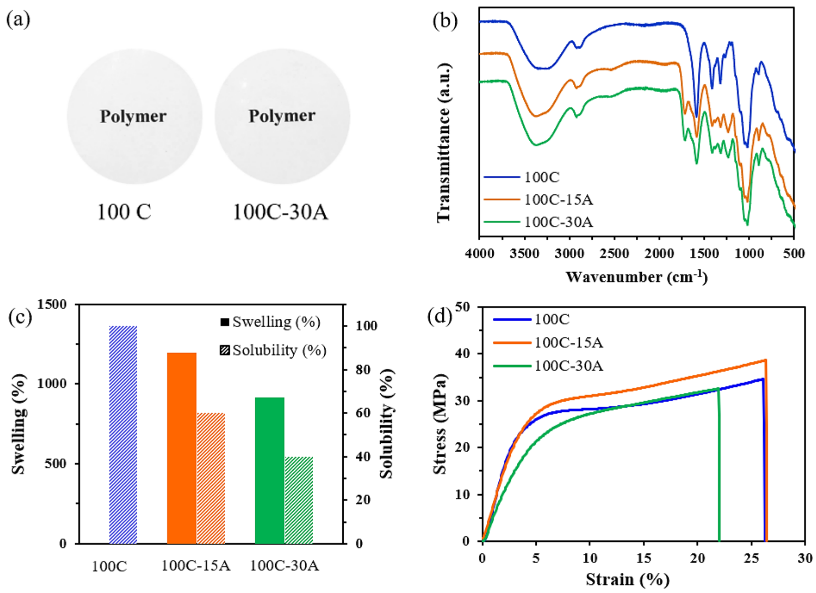


Figure 1 Characteristics of pristine CMC and citric acid-crosslinked CMC films: (a) digital photographs, (b) ATR-FTIR spectra, (c) swelling behavior and solubility in distilled water and (d) stress-strain curves

Mechanical testing was conducted to assess the influence of citric acid crosslinking on tensile strength and elongation at break (Figure 1(d)). Crosslinking slightly enhanced the mechanical strength of CMC films, with 100C-15A achieving optimal values of 39.69 MPa in tensile strength and 26.91% elongation at break. However, a further increase in citric acid concentration (100C-30A) resulted in a modest decline in properties, with a tensile strength of 33.01 MPa and an elongation at break of 22.48%. Despite the inclusion of glycerol as a plasticizer, all CMC-based films exhibited relatively limited flexibility and remained brittle. To further enhance mechanical properties, additional studies were undertaken involving the incorporation of pullulan, a natural polysaccharide known for its film-forming and plasticizing properties (Zhang *et al.,* 2023), into the CMC matrix.

Table 2 Color parameters and tensile properties of pristine CMC and citric acid-crosslinked CMC films.

Films	Film Thickness (mm)	Color parameters				Tensile properties		
		L	a	b	ΔE	TS (MPa)	EB (%)	EM* (MPa)
100C	0.087 ±0.001	92.49 ±0.24	-0.79 ±0.03	2.05 ±0.01	-	34.18 ±0.67	26.23 ±0.14	5.29 ±0.61
100C-15A	0.090 ±0.011	92.79 ±0.38	-0.70 ±0.05	2.07 ±0.09	0.33 ±0.17	39.69 ±0.89	26.91 ±2.05	5.97 ±2.37
100C-30A	0.092 ±0.009	92.76 ±0.11	-0.73 ±0.03	2.24 ±0.09	0.36 ±0.07	33.01 ±1.01	22.48 ±2.81	4.03 ±1.21

*Elastic modulus at 0.5%elongation

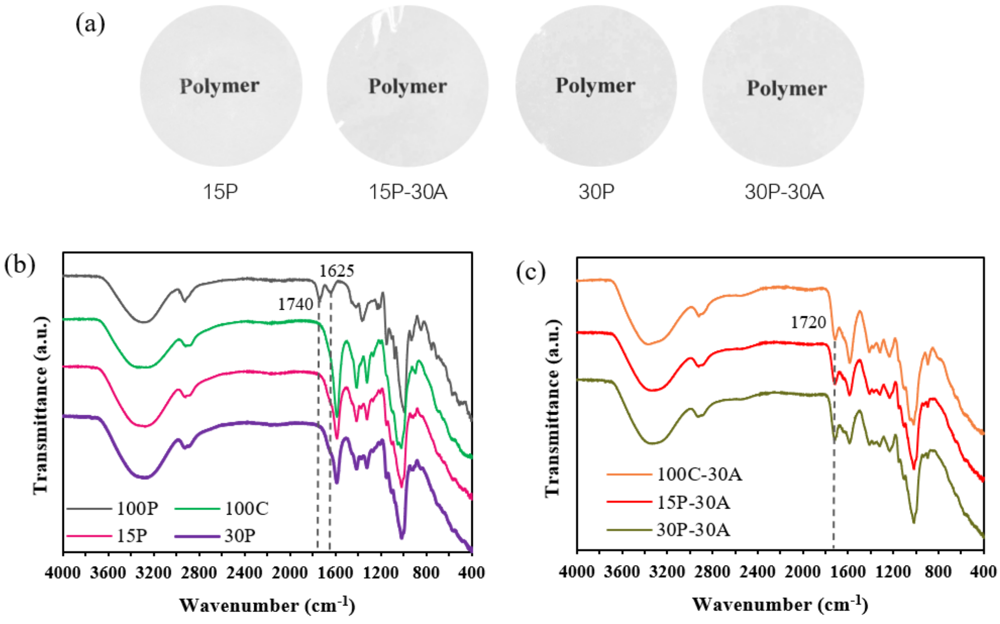


Figure 2 Characteristics of CMC/pullulan films: (a) Digital photographs of the films, (b) ATR-FTIR spectra of the linear blend films, (c) ATR-FTIR spectra of the crosslinked films

2. Properties of the Crosslinked CMC/Pullulan Films

To enhance the flexibility of crosslinked carboxymethyl cellulose, pullulan was incorporated into the blend solution prior to the crosslinking and solution casting processes. The resulting films exhibited a smooth and transparent surface, indicating successful interaction between the two polymers and the absence of any visible phase separation (Figure 2(a)). The homogeneous nature of the films is crucial, especially when considering their applications, as uniformity often correlates with enhanced performance characteristics.

The color parameters of the films and ΔE calculated from the differences in parameter between 100C and the modified films, as summarized in Table 3, demonstrate that both the crosslinking process and the incorporation of pullulan into CMC matrix did not significantly alter the transparency or visual appeal of the films. This finding is particularly relevant for packaging materials, where clarity can impact consumer perception. The transparent nature of films suggests that they are suitable for applications where visibility of the product is essential. ATR-FTIR spectra of the linear blend films are depicted in Figure 2(b). A noticeable shift of the bands at 1740 cm^{-1} and 1625 cm^{-1} in pristine pullulan towards lower wavenumbers was recorded. This spectral change indicates a significant interaction between the pullulan and CMC, as these bands eventually merged with the main characteristic carboxylate group of CMC. Such intermolecular interactions are indicative of the formation of a complex network, suggesting enhanced compatibility and potential synergistic effects between the two polysaccharides. Furthermore, the successful crosslinking of the blend films containing 15% and 30% pullulan was confirmed by the emergence of a carboxylic ester band at 1720 cm^{-1}, as shown in Figure 2(c). This spectral feature implies a covalent bond formation through the esterification process, which may lead to an increase in the integrity and stability of the films (Yildirim-Yalcin *et al*., 2022).

Table 3 Color parameters of the citric acid crosslinked CMC/pullulan films

Films	L	a	b	**Δ**E*
15P	92.31 $\pm$ 0.08	0.07 $\pm$ 0.02	1.44 $\pm$ 0.02	0.36 $\pm$ 0.07
15P-15A	92.32 $\pm$ 0.04	0.04 $\pm$ 0.01	1.42 $\pm$ 0.13	0.37 $\pm$ 0.03
15P-30A	92.19 $\pm$ 0.18	0.12 $\pm$ 0.04	1.46 $\pm$ 0.14	0.31 $\pm$ 0.14
30P	92.31 $\pm$ 0.03	0.13 $\pm$ 0.02	1.49 $\pm$ 0.14	0.40 $\pm$ 0.04
30P-15A	92.42 $\pm$ 0.14	0.12 $\pm$ 0.02	1.35 $\pm$ 0.11	0.49 $\pm$ 0.13
30P-30A	92.36 $\pm$ 0.02	0.13 $\pm$ 0.01	1.31 $\pm$ 0.17	0.46 $\pm$ 0.07

*ΔE calculated from the differences in parameter between 100C and modified films

The equilibrium water absorption and solubility of CMC/pullulan blend films, key parameters for evaluating their suitability as practical materials, were assessed, with results presented in Figure 3. In distilled water, the linear blends (15P and 30P) exhibited rapid water uptake and complete solubility (100%). In contrast, all crosslinked films showed markedly reduced water absorption and solubility. Notably, the 15P-30A and 30P-30A films demonstrated the lowest solubility, approximately 34%.

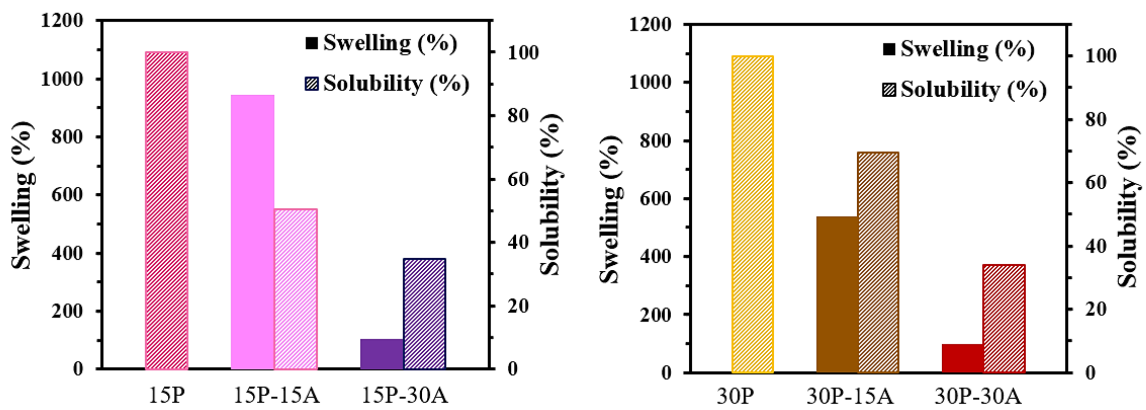


Figure 3 Swelling and solubility of the films containing different contents of pullulan and citric acid

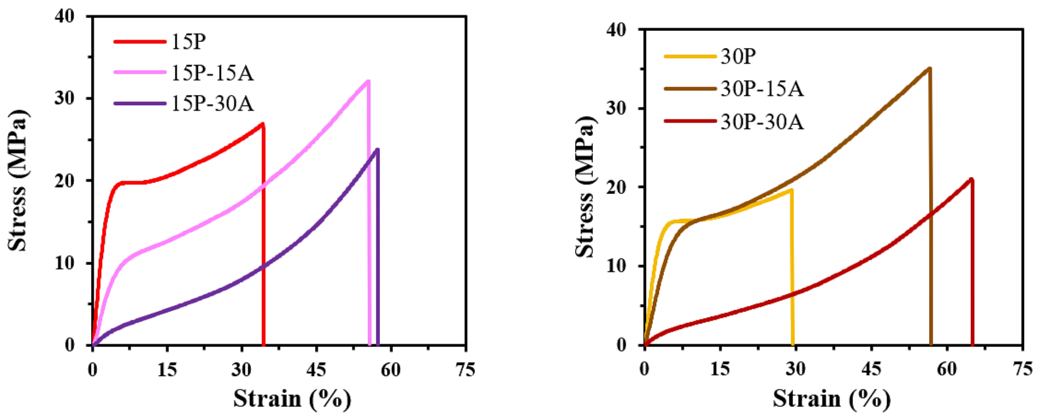


Figure 4 Tensile properties of films containing varying contents of pullulan and citric acid

Table 4 The tensile properties of crosslinked CMC/pullulan blend films

Films	Thickness (mm)	TS (MPa)	EB (%)	EM* (MPa)
15P	0.089 ± 0.009	27.90 ± 0.93	34.46 ± 2.11	5.33 ± 0.25
15P-15A	0.092 ± 0.007	33.39 ± 1.80	56.39 ± 1.23	1.51 ± 0.54
15P-30A	0.093 ± 0.015	27.27 ± 1.68	58.09 ± 6.17	0.57 ± 0.11
30P	0.084 ± 0.015	19.49 ± 0.19	28.63 ± 0.80	6.45 ± 3.35
30P-15A	0.091 ± 0.008	35.00 ± 0.14	56.62 ± 0.05	2.30 ± 0.43
30P-30A	0.095 ± 0.013	21.19 ± 0.59	64.65 ± 2.94	0.40 ± 0.43

*Elastic modulus at 0.5 % elongation

The tensile properties of the blends were evaluated to assess the influence of pullulan content and the degree of crosslinking on their mechanical behavior. As illustrated in Figure 4 and Table 4, an optimal degree of crosslinking significantly improved both tensile strength and elongation at break, effectively overcoming the primary mechanical limitations of CMC-based films. In the series of linear blends containing 15% pullulan, the tensile strengths at break were 27.90, 33.39, and 27.27 MPa, while the elongation at break values were 34.46, 56.39, and 58.09% for the 15P, 15P-15A, and 15P-30A blends, respectively. Similar effects were observed in the films containing 30%pullulan. Notably, the optimal citric acid content was determined to be 15% by weight of the polymer; exceeding this concentration led to a decline in mechanical performance. Previous research has shown that an excess of citric acid acts as a plasticizer in the polymer network, enhancing elongation and flexibility but compromising strength (Sharmin *et al.,* 2022). This increase in flexibility is further demonstrated in Figure 5, where the 15P-15A and 30P-15A blends endure deformation forces without breaking.

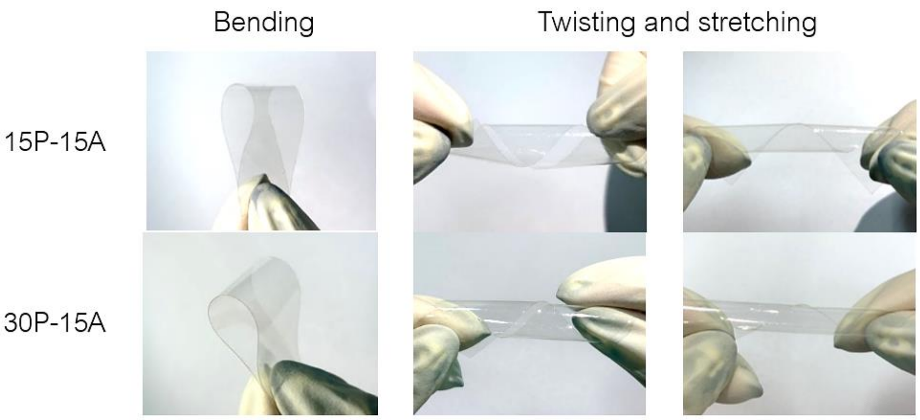


Figure 5 Digital photographs of the crosslinked 15P-15A and 15P-30A films under different deformations

High-magnification SEM micrographs of the crosslinked 15P-15A and 15P-30A films are presented in Figure 6. These films showed no signs of phase separation and exhibited smooth surface morphologies, indicating good compatibility among all components within the matrices.

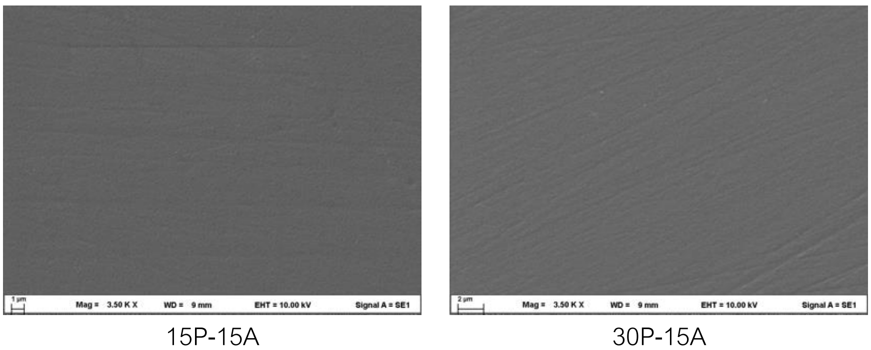
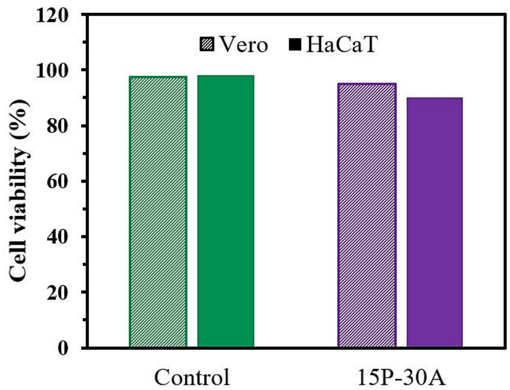


Figure 6 SEM micrographs of the crosslinked 15P-15A and 15P-30A films at 3.5KX magnification

To evaluate the biosafety of the films, particular attention was given to the 15P-30A formulation, which contained the highest concentration of citric acid. Cell viability assays were performed using African green monkey fibroblast cells (Vero) and immortalized human keratinocytes (HaCaT). Both cell lines exhibited high viability, 95% for Vero and 90% for HaCaT, comparable to the controls (Figure 7(a)). Post-culture analysis of cell morphology and proliferation, presented in Figure 7(b), revealed no observable adverse effects in the presence of 15P-30A. The high viability and preserved cellular morphology highlighted the excellent biocompatibility of the biopolymers, citric acid and glycerol used in the film formulation.



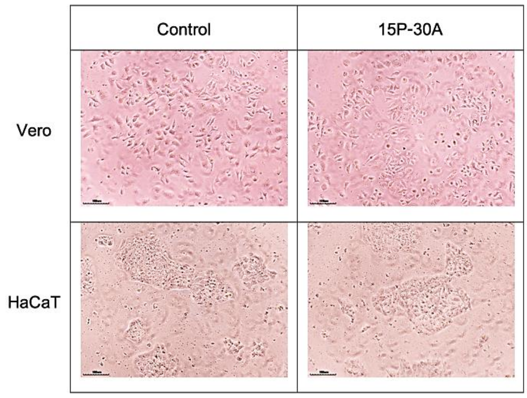


Figure 7 Cell viability of African green monkey fibroblast (Vero) and human keratinocyte immortalized cells (HaCaT) in the presence of 15P-30A film: (a) Percentage of cell viability, (b) Cell Morphology after culture with the film

Discussion

Sodium carboxymethyl cellulose is a renewable, naturally derived polymer with several advantageous characteristics, including biocompatibility and excellent film-forming ability. However, its practical applications are often hindered by its intrinsic water solubility and brittleness. To address these limitations, citric acid was employed as a non-toxic, multifunctional crosslinking agent to improve the properties of CMC, with particular emphasis on enhancing water resistance. The crosslinking mechanism involves esterification reactions between the carboxylic acid groups of citric acid and the hydroxyl groups on the CMC polymer backbone (Figure 8). This reaction promotes the formation of a three-dimensional polymeric network, significantly enhancing film stability and water resistance.

To further improve the flexibility of the films, glycerol was incorporated as a plasticizer to increase chain mobility and reduce brittleness. However, despite the addition of glycerol, the rigid crosslinked structure resulted in films with limited flexibility. Consequently, an additional modification was implemented by blending CMC with pullulan, a natural polysaccharide known for its excellent film-forming and plasticizing properties. Among the formulations tested, the 15P-15A and 30P-15A samples, containing 15% citric acid and 15% or 30% pullulan, respectively, exhibited optimal properties for packaging applications, including improved water resistance, mechanical strength, visual appearance, and flexibility. Cytotoxicity assays were conducted to evaluate the biocompatibility of the films, confirming their biosafety for potential biomedical and food-contact applications. Overall, the results suggest that CMC/pullulan films, crosslinked with citric acid and fabricated using a green, sustainable approach, represent a promising alternative to conventional materials in biodegradable packaging and biomedical applications.

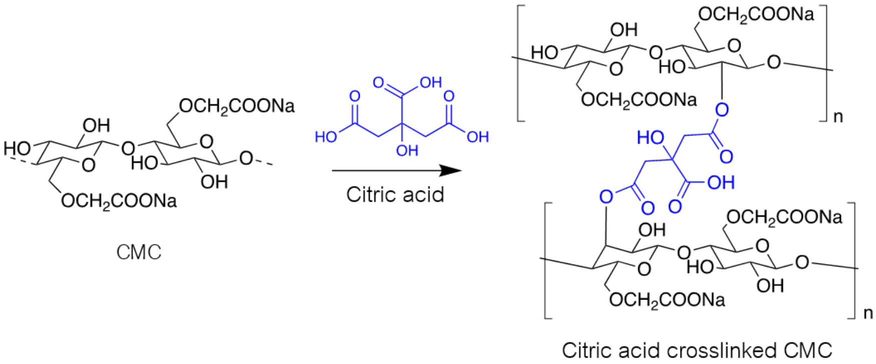


Figure 8 Crosslinking of CMC with citric acid

Conclusions

This study demonstrated the effectiveness of citric acid as a crosslinking agent for enhancing the functional properties of carboxymethyl cellulose-based films by improving water resistance and mechanical strength through ester bond formation. An optimal citric acid concentration of 15% was identified, as higher levels led to reduced mechanical performance due to plasticization. Incorporating pullulan into the CMC matrix further improved the flexibility and tensile behavior of the films. Crosslinked CMC/pullulan blends exhibited superior tensile strength and elongation at break compared to unmodified CMC films, effectively overcoming their inherent brittleness. FTIR and SEM analyses confirmed strong intermolecular interactions and morphological homogeneity, underscoring the compatibility and synergistic integration of the blend components. The optimized 15P-30A film exhibited excellent cytocompatibility with both Vero and HaCaT cells, supporting its potential for biomedical applications. Overall, citric acid-crosslinked CMC/pullulan films represent a promising, biocompatible alternative for use in functional biodegradable materials.

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