Iranian Food Science and Technology Research Journal
Homepage: https://ifstrj.um.ac.ir

Research Article
Vol. 22, No. 1, March- April, 2026, p. 1-16

Development and Characterization of Carboxymethylated Agar-based Film for Food Packaging Applications

F. Falahatgar[1], A. Babakhani[1], E. Zakipour Rahimabadi[1*], H. Rostamzad[1]

1- Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran
(*- Corresponding Author Email: e.zakipoor@guilan.ac.ir)

Received: 12.10.2025
Revised: 31.12.2025
Accepted: 31.01.2026
Available Online: 02.05.2026

How to cite this article:
Falahatgar, F., Babakhani, A., Zakipour Rahimabadi, E., & Rostamzad, H. (2026). Development and characterization of carboxymethylated agar-based film for food packaging applications. *Iranian Food Science and Technology Research Journal*, *22*(1), 1-16. https://doi.org/10.22067/ifstrj.2026.95783.1488

Abstract

The aim of this study was to develop and characterize carboxymethylated agar (CMA) based films crosslinked with citric acid, polyethylene glycol (PEG), and β-cyclodextrin for sustainable food packaging applications. The inherent brittleness, high hydrophilicity, and limited thermal stability of native agar films, which restrict their practical use in food packaging systems were considered as the aim of performing this study. Five distinct film formulations were prepared using different combinations of crosslinking agents, and their physicochemical, morphological, and thermal properties were systematically evaluated. The degree of substitution (DS) of carboxymethyl agar was determined to be 0.69, indicating successful chemical modification. Water contact angle analysis revealed that the CMA-P-β-A film exhibited the highest hydrophobicity, with a contact angle in the range of 80–89°, compared to approximately 67° for the control CMA film, demonstrating enhanced moisture resistance. Differential scanning calorimetry showed a significant increase in glass transition temperature (Tg), reaching 82.08 °C for the CMA-P-β-A formulation, compared to 52.54 °C for CMA, confirming the formation of a highly crosslinked polymer network. FTIR and XRD analyses confirmed successful carboxymethylation and crosslinking, accompanied by reduced crystallinity and improved structural flexibility. SEM micrographs revealed smooth and homogeneous surfaces with interconnected polymer networks. Overall, the integrative carboxymethylation crosslinking strategy effectively enhanced the hydrophobicity, thermal stability, and structural integrity of agar-based films, highlighting the CMA-P-β-A formulation as the most promising candidate for environmentally friendly food packaging applications. These findings provide valuable insights into the design of advanced biopolymer-based packaging materials.

Keywords: Algae, Carboxymethylated agar, Crosslinking, Packaging, Thermal properties

https://doi.org/10.22067/ifstrj.2026.95783.1488

Introduction

Agar is one of the earliest known natural polysaccharides extracted from red marine algae of the order Gracilariales. It is primarily composed of agarose and agaropectin, with agarose being the dominant fraction responsible for its gelling behavior. Agarose is a linear, electrically neutral polysaccharide consisting of repeating agarobiose units formed by alternating 3-O-linked β-D-galactopyranose and 4-O-linked 3,6-anhydro-α-L-galactopyranose residues (Villanueva, Sousa, Gonçalves, Nisson, & Hilliou, 2010). Owing to its biocompatibility, renewability, and film-forming ability, agar has been extensively used in food systems, pharmaceuticals, microbiological media, and biomedical applications (Surendran & Sherje, 2023). However, despite these advantages, native agar films suffer from several intrinsic limitations, including high brittleness, poor mechanical strength, excessive hydrophilicity, and limited thermal stability, which significantly restrict their application in practical food packaging systems (Zhang *et al.*, 2020). To overcome these drawbacks, chemical modifications of agar, such as carboxymethylation, have been explored. Carboxymethylation introduces carboxymethyl functional groups into the polymer backbone, improving the solubility, transparency, flexibility, and stability of polysaccharides, making them more suitable for film-forming applications. Additionally, carboxymethyl cellulose (CMC) is another derivative widely used in the food packaging industry. CMC improves water solubility and film-forming ability, providing excellent moisture resistance and mechanical properties, which are crucial for packaging materials. This study investigates the effects of carboxymethylation of agar and the incorporation of crosslinking agents, including citric acid, polyethylene glycol (PEG), and β-cyclodextrin, on the physicochemical, morphological, and thermal properties of polymer films for sustainable food packaging. The novelty of this work lies in the synergistic derivatization crosslinking strategy, which aims to mitigate the inherent limitations of agar and identify an optimized formulation suitable for environmentally friendly food packaging applications (Chakka & Zhou., 2020; Qi *et al*., 2022).

Materials and Methods

After carboxymethylation of agar, agar-based films were prepared using different crosslinking agents, and their physicochemical, structural, and thermal properties were evaluated to assess their potential application as food packaging materials, following approaches commonly reported for modified polysaccharide-based films (Roy, Kim, & Rhim, 2021; Abdollahi, Damirchi, Shafafi, Rezaei, & Ariaii, 2019).

Agar Extraction

Algae seedlings were collected from their natural habitat in Suru (Bandar Abbas, Iran) and transported to the laboratory for further processing. Agar was extracted from *Gracilaria* algae using an alkaline extraction method. Initially, the collected algae samples were thoroughly washed with distilled water to remove surface impurities, sand particles, and salts, and subsequently dried under ambient laboratory conditions. The dried algae were subjected to alkaline pretreatment using sodium hydroxide solution to facilitate the conversion of sulfate groups to 3,6-anhydrogalactose units and to improve agar gel strength and extraction yield. To remove residual alkali, the algae samples were then repeatedly washed with distilled water until neutral pH was achieved. Subsequently, the pretreated algae were soaked in distilled water and heated to initiate agar extraction. The resulting hot extract was filtered to remove insoluble residues and allowed to cool and form a gel at room temperature. The formed gels were dehydrated, washed with distilled water, and mechanically compressed to remove excess moisture. Finally, the extracted agar was dried in a hot air oven at 60 °C, ground into powder form, and stored in

airtight containers for subsequent analyses (Abraham *et al.*, 2018). The agar yield was calculated using the following equation:

Yield (%) = (Weight of extracted dry agar)/ (Weight of dried algae) × 100

Carboxymethylation of Agar

The preparation of carboxymethylated agar was performed following the method described by Qi *et al.* (2022), with minor modifications. Briefly, 2 g of agar powder extracted from *Gracilaria* algae was dispersed in 100 mL of 80% (v/v) isopropanol and stirred at room temperature (25 °C) for 30 min to ensure uniform swelling. Subsequently, 25 mL of sodium hydroxide solution (5 mol L^{-1}) was added gradually, and the mixture was continuously stirred for 1 h to achieve complete alkalization (Chakka & Zhou, 2020).

After alkalization, 7.5 g of monochloroacetic acid was gradually added, and the reaction mixture was maintained under continuous stirring for 5 h at 55 °C to promote the carboxymethylation reaction. Upon completion, the reaction was terminated by adding 85% methanol, followed by neutralization with 1 mol L^{-1} glacial acetic acid. The resulting product was filtered, washed thoroughly with 60% ethanol to remove residual salts and by-products, dried at 60 °C for 24 h, crushed, and sieved through an 80-mesh sieve to obtain carboxymethyl agar (Im, Lee, Rajabi Abhari, Youn, & Lee, 2018).

Preparation of Films

Agar-based films were prepared by dissolving 2 g of carboxymethyl agar powder in 50 mL of distilled water at 80 °C under continuous stirring until complete dissolution was achieved, following previously reported film-forming procedures for polysaccharide-based materials (Tammina & Rhim, 2023; Abdollahi *et al.*, 2019). Five formulations were prepared as follows: CMA, CMA-P, CMA-P-A, CMA-β-A, and CMA-P-β-A. After the addition of the respective crosslinking agents, the film-forming solutions were homogenized using a digital Ultra-Turrax homogenizer at 65 °C to ensure uniform dispersion and effective interaction among the components. The resulting homogeneous hydrogels were poured onto Petri dishes and dried in a hot air oven at 50 °C for 24 h, as commonly reported for crosslinked biopolymer films (Dharmalingam & Anandalakshmi, 2019).

Determination of Degree of Substitution (DS)

The degree of substitution (DS) of carboxymethyl agar was determined using a neutralization titration method. Carboxymethyl agar (0.1 g) was dissolved in 25 mL of distilled water, followed by the addition of 25 mL of standard NaOH solution (0.1 mol L^{-1}). The mixture was stirred for 10–15 min and subsequently titrated with standard HCl solution (0.1 mol L^{-1}) using phenolphthalein as an indicator until the endpoint was reached. The titration was performed in triplicate, and the average volume of HCl consumed was used for calculations.

The DS was calculated using Equations 1 and 2 (Chakka & Zhou, 2020).

$$DS = \frac{0.306X}{1-0.058X} \quad (1)$$

$$X = \frac{V_0 M_0 - V_1 M_1}{W} \quad (2)$$

Where, 0.306 is the molar mass of the disaccharide unit (g.mol^{-1}), 0.058 is the molar mass of the introduced carboxymethyl group (g.mol^{-1}), V_0 is the volume of NaOH added (mL), M_0 is the concentration of NaOH added (mol.L^{-1}), V_1 is the consumed volume of HCl (mL), M_1 is the concentration of HCl (mol.L^{-1}), and W is the sample weight (g).

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of unmodified agar and carboxymethylated agar were obtained using an FTIR spectrometer (EDX-GP, Shimadzu, Japan) equipped with KBr pellet accessories, following previously reported procedures for polysaccharide-based films (Siripatrawan & Harte, 2010; Lakshmi, Trivedi, & Reddy, 2017). Spectra were obtained in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} to

identify functional groups and confirm chemical modification.

Contact Angle Measurement

The wetting test was carried out using a contact angle measuring (CAM) device. The images were captured using a color industrial camera (Model PG-X, Switzerland). Ultra-high purity water was used as the probe liquid to measure the contact angle at room temperature. Prior to measurement, all film samples (3 cm × 7 cm) were fixed on a movable sample plate and leveled horizontally. A small droplet of pure water was gently deposited onto the surface of each film. For each film, surface hydrophobicity was determined from the initial contact angle values, calculated as the average of contact angles measured on both sides of the droplet almost immediately after deposition, to minimize spreading effects (Rbihi, Aboulouard, Laallam, & Jouaiti, 2020).

X-ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) patterns of the samples were analyzed to determine crystallinity and investigate the internal atomic arrangement using an X-ray diffractometer (Philips, Netherlands) equipped with Ni-filtered Cu–Kα radiation, following the method described by Liu *et al*. (2020). The diffractometer was operated at 40 kV and 30 mA, and diffraction data were collected over a 2θ range of 5° to 60° at a scanning speed of 1° min^{-1}.

Scanning Electron Microscopy (SEM)

A scanning electron microscope (Evo25, Zeiss, Germany) was used to observe the surface and cross-sectional microstructures of the films. The microscope was operated at an accelerating voltage of 12 kV. Prior to analysis, film samples were coated with a thin layer of gold to enhance electrical conductivity. SEM images were recorded at magnifications of 300× and 500× to evaluate film homogeneity and microstructural features (Michler & Lebek, 2016).

Differential Scanning Calorimetry (DSC)

The glass transition temperature (Tg) of the films was determined using differential scanning calorimetry (Diamond DSC, PerkinElmer, USA). Film samples weighing approximately 10–15 mg were prepared using an analytical balance (± 0.002 mg) and sealed in aluminum hermetic pans, with an empty pan used as the reference. The samples were heated from 30 to 300 °C at a heating rate of 10 °C min^{-1} under an argon atmosphere. The Tg was identified as the midpoint of the baseline shift associated with changes in heat capacity during the glass transition (Arancibia, Alemán, López-Caballero, Gómez-Guillén, & Montero, 2015).

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) with SPSS software (version 23, IBM Corporation, Armonk, NY, USA). Differences among treatments were determined using Duncan's multiple range test at a significance level of 5% ($p < 0.05$). All results were expressed as mean ± standard deviation (SD).

Results and Discussion

Degree of Substitution (DS)

The degree of substitution (DS) is a critical parameter that directly influences the physicochemical properties of carboxymethylated polysaccharides, including solubility, viscosity, thermal behavior, and film-forming ability. In the present study, the DS of carboxymethyl agar was determined to be 0.69, confirming the successful introduction of carboxymethyl groups into the agar backbone. This DS value falls within the optimal range reported for commercially functional carboxymethylated polysaccharides and is suitable for film-based applications (Rahman *et al*., 2021; Zhao *et al*., 2025). The introduction of carboxymethyl groups enhances water solubility by increasing electrostatic repulsion between polymer chains and reducing intermolecular hydrogen bonding. Previous studies have shown that carboxymethyl agar exhibits improved solubility at room temperature when the DS exceeds

approximately 0.55 (Cao, Liu, Luan, & Zhang, 2014). The DS value obtained in this study is therefore expected to facilitate homogeneous film formation and improved compatibility with crosslinking agents. Comparable findings have been reported for carboxymethyl cellulose systems, where DS values in the range of 0.5–0.8 have been associated with favorable mechanical strength and reduced crystallinity (Casaburi, Rojo, Cerrutti, Vázquez, & Foresti, 2018). The DS value achieved in this work indicates a balanced modification level, sufficient to improve functional performance without compromising structural integrity.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was employed to support the successful chemical modification of agar and the formation of intermolecular interactions within the film matrix. Fig. 1 presents the FTIR spectra of unmodified agar and carboxymethyl agar (CMA). The spectrum of native agar exhibited a broad absorption band in the range of 3300–3700 cm^{-1}, corresponding to O–H stretching vibrations of hydroxyl groups, which is characteristic of polysaccharide structures (Mostafa, Ali, El-Wassefy, Saad, & Hussein, 2024). Following carboxymethylation, a noticeable decrease in the intensity of the hydroxyl stretching band was observed, indicating partial substitution of hydroxyl groups by carboxymethyl functionalities. Additionally, new absorption bands appeared at approximately 1439 cm^{-1} and 1372 cm^{-1}, which are attributed to the asymmetric and symmetric stretching vibrations of carboxylate ($–COO^-$) groups and methylene ($–CH_2–$) scissoring vibrations, respectively (Wang *et al.*, 2012). The presence of absorption bands in the region of 1500–1700 cm^{-1} further confirms the successful introduction of carboxymethyl groups into the agar chains. These spectral changes are consistent with previous reports on carboxymethylated polysaccharides and verify the effectiveness of the chemical modification process (Rahman *et al.*, 2021; Bandyopadhyay, Sáha, Sanétrník, Saha, & Sáha, 2021).

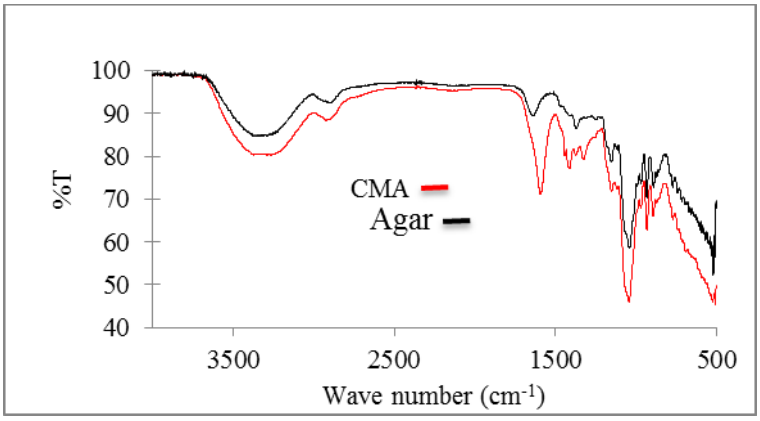

Fig. 1. The FTIR spectra of raw agar and CMA

FTIR Analysis of CMA-based Composite Films

FTIR spectroscopy was employed to investigate the intermolecular interactions and crosslinking behavior within CMA-based composite films containing polyethylene glycol (PEG), citric acid (CA), and β-cyclodextrin (β-CD). Fig. 2 presents the FTIR spectra of CMA film and its modified counterparts. The FTIR spectrum of pristine CMA film exhibited a broad absorption band in the range of 3200–3500 cm^{-1}, which is attributed to the stretching vibrations of hydroxyl (–OH) groups associated with the polysaccharide backbone and absorbed moisture. Upon incorporation of PEG (CMA+P), a noticeable reduction in the intensity of this band was observed, indicating enhanced hydrogen bonding interactions between CMA and PEG chains. Similar behavior has been reported for PEG-plasticized polysaccharide systems and is commonly

associated with improved chain mobility and intermolecular compatibility (Zhang *et al.*, 2020). The addition of citric acid resulted in more pronounced spectral changes. In the CMA+P+A and CMA+B+A films, the appearance and intensification of absorption bands in the region of 1700–1730 cm^{-1} were observed, corresponding to the C=O stretching vibrations of ester groups. This band is a key indicator of esterification reactions between the carboxyl groups of citric acid and the hydroxyl groups of CMA, PEG, and β-CD, confirming the formation of chemical crosslinks within the polymer network. Similar ester-related bands have been widely reported in citric-acid-crosslinked polysaccharide films, including carboxymethyl cellulose and starch-based systems (Smith, 2022; Rahman *et al.*, 2021). Additionally, the bands located at approximately 1400–1450 cm^{-1} and 1320–1380 cm^{-1}, attributed to asymmetric and symmetric stretching vibrations of carboxylate ($–COO^-$) groups and CH2 bending vibrations, exhibited increased intensity and slight shifts in the modified films. These changes further support the involvement of carboxyl groups in crosslinking reactions and enhanced intermolecular interactions, consistent with previous reports on carboxymethylated polysaccharide matrices (Qi *et al.*, 2022; Zhang *et al.*, 2019). In the fingerprint region (1000–1150 cm^{-1}), all composite films displayed intensified and broadened absorption bands corresponding to C–O and C–O–C stretching vibrations. The most significant changes were observed for the CMA+P-B+A film, indicating the formation of a more complex and interconnected network structure. The contribution of β-cyclodextrin to this region has been attributed to its cyclic oligosaccharide structure, which promotes additional hydrogen bonding and network stabilization, as reported in β-CD-based composite films (Velázquez-Contreras *et al.*, 2021). Overall, the progressive spectral evolution from CMA to CMA+P-B+A demonstrates a stepwise formation of hydrogen bonding interactions followed by ester crosslinking, resulting in a chemically integrated and stabilized film matrix. The observed FTIR features are in good agreement with previously reported citric-acid-crosslinked polysaccharide and cyclodextrin-containing film systems, confirming the successful design and optimization of the composite films.

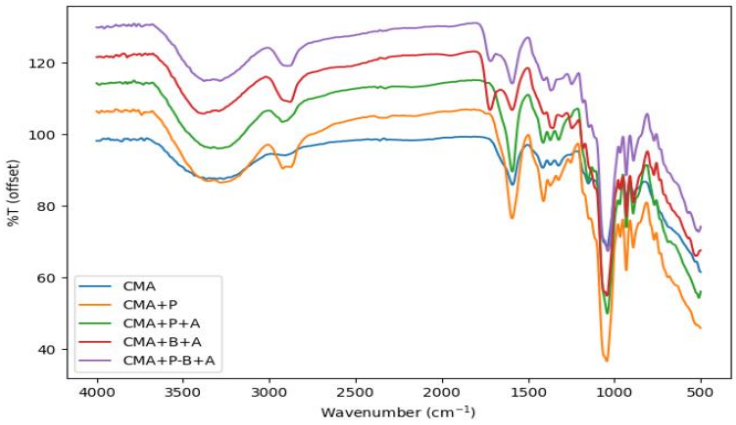

Fig. 2. The FTIR spectra of CMA-based composite films

Water Contact Angle (CA)

The surface wettability of the films was evaluated by water contact angle measurements, which provide insight into surface hydrophilicity and moisture resistance as two key parameters for food packaging materials. As shown in Fig. 3, the control CMA film exhibited a contact angle of approximately

67°, indicating a moderately hydrophilic surface. Among all formulations, only the CMA-P-β-A film exhibited a markedly higher contact angle in the range of 80–89°, demonstrating significantly enhanced surface hydrophobicity. This improvement can be attributed to the synergistic effect of polyethylene glycol, β-cyclodextrin, and citric acid, which promoted effective crosslinking and reduced the availability of hydrophilic functional groups on the film surface. Similar increases in contact angle have been reported for crosslinked polysaccharide films, where ester bond formation and network densification limit water–polymer interactions (Zhu *et al.*, 2022; Balçık Tamer, 2023). The enhanced hydrophobicity observed for the CMA-P-β-A formulation suggests improved resistance to moisture penetration, which is desirable for potentially extending the shelf life of packaged foods.

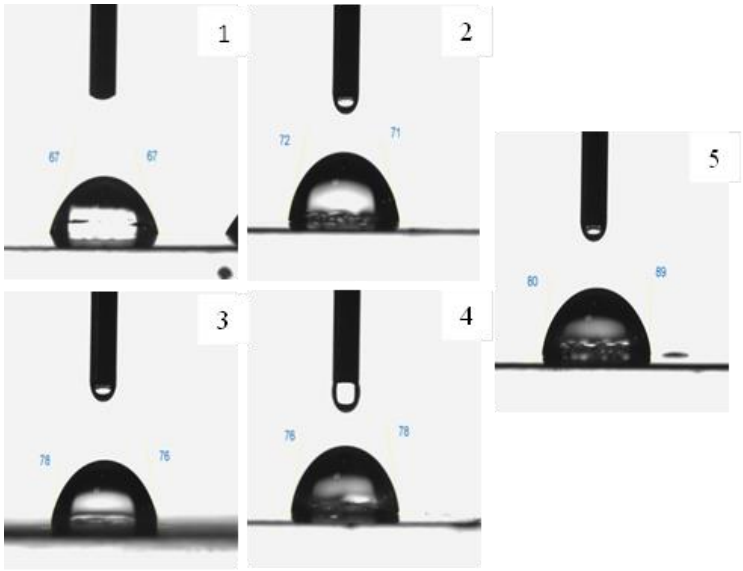

Fig. 3. Water contact angle: 1- Control (CMA), 2- CMA-P, 3- CMA-P-A, 4- CMA-β-A, and 5- CMA-P-β-A

X-ray Diffraction (XRD) Analysis

X-ray diffraction (XRD) analysis was performed to investigate the crystalline structure and structural ordering of carboxymethyl agar (CMA) films and their crosslinked formulations. Fig. 4 shows the XRD patterns of CMA, CMA-P, CMA-P-A, CMA-β-A, and CMA-P-β-A films. The CMA film exhibited a characteristic diffraction peak at approximately $2\theta = 20°$, along with a weaker peak near $2\theta = 12°$, which are typical of semi-crystalline agar-based materials (Casaburi *et al.*, 2018). Upon incorporation of polyethylene glycol, citric acid, and β-cyclodextrin, a noticeable reduction in peak intensity and peak broadening were observed, indicating a decrease in crystallinity and an increase in the amorphous phase of the polymer matrix. This behavior can be attributed to the substitution of hydroxyl groups by carboxymethyl groups and the formation of crosslinked networks that interfere with regular polymer chain packing (Zhu *et al.*, 2022). To ensure reliable interpretation of the diffraction patterns, background noise was minimized during data processing, and the overall trend of peak broadening rather than complete peak disappearance was considered. Similar reductions in crystallinity have been reported for crosslinked polysaccharide-based films, where esterification and hydrogen bonding interactions restrict molecular rearrangement and long-range order (Gieroba, Kalisz, Krysa, Khalavka, & Przekora, 2023; Wang, Xu, Qi, & Chen, 2024).

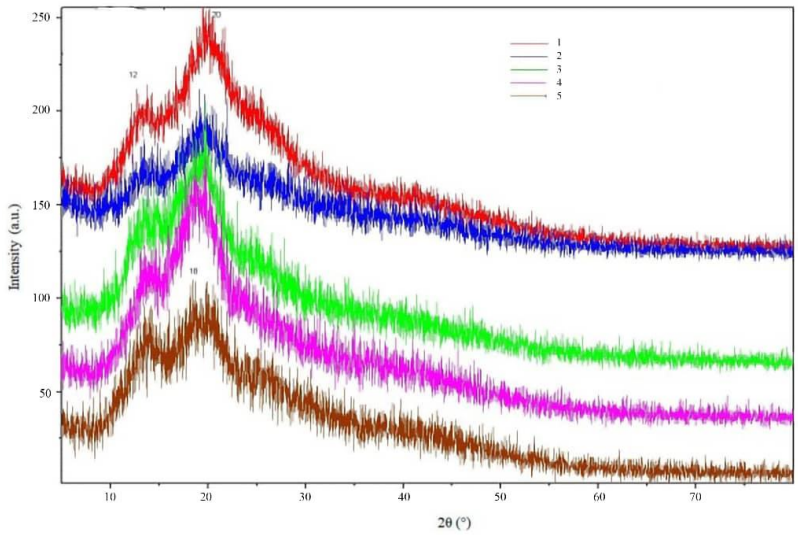

Fig. 4. XRD patterns of 1- Control (CMA), 2- CMA-P, 3- CMA-P-A, 4- CMA-β-A, and 5- CMA-P-β-A

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was used to examine the surface and cross-sectional morphologies of carboxymethyl agar (CMA) films with different formulations (Fig. 5). SEM analysis provides valuable information about film homogeneity, internal structure, and the effectiveness of crosslinking, which directly affect the mechanical, barrier, and thermal properties of biopolymer films (Roy *et al.*, 2021; Mirpoor *et al.*, 2025). The control CMA film exhibited a relatively rough and heterogeneous surface with visible micro-scale irregularities, which are commonly observed in unmodified or weakly modified polysaccharide-based films (Cazón, Velazquez, Ramírez, & Vázquez, 2017). These features can be attributed to limited intermolecular interactions and loosely packed polymer chains. In contrast, films containing polyethylene glycol, citric acid, and β-cyclodextrin (CMA-P, CMA-P-A, CMA-β-A, and CMA-P-β-A) showed smoother and more uniform surfaces, indicating improved compatibility among the film components and stronger intermolecular interactions. The incorporation of citric acid likely promoted ester bond formation between polymer chains, while β-cyclodextrin contributed to additional hydrogen bonding, resulting in a more cohesive polymer network (Zou *et al.*, 2021; Balçık Tamer, 2023). Cross-sectional SEM images further revealed clear structural differences among the formulations. The CMA film exhibited a porous and less compact internal structure, indicating a loosely packed polymer network. By comparison, the CMA-P-β-A film exhibited a dense, continuous, and homogeneous cross-section with significantly reduced porosity, confirming the formation of an interconnected and crosslinked polymer network. This compact structure restricts polymer chain mobility and enhances the overall structural integrity of the film (Rajawasam *et al.*, 2024; Keya, Li, Xu, & Xia, 2025). Similar morphological trends have been reported for citric acid-crosslinked and cyclodextrin-modified biopolymer films, where increased crosslink density leads to improved film uniformity and reduced void formation (Uyanga, Okpozo, Onyekwere, & Daoud, 2020; Escobar *et al.*, 2023). The observed SEM characteristics are consistent with the enhanced hydrophobicity and thermal stability of the CMA-P-β-A formulation, highlighting the strong relationship between microstructural organization and functional performance.

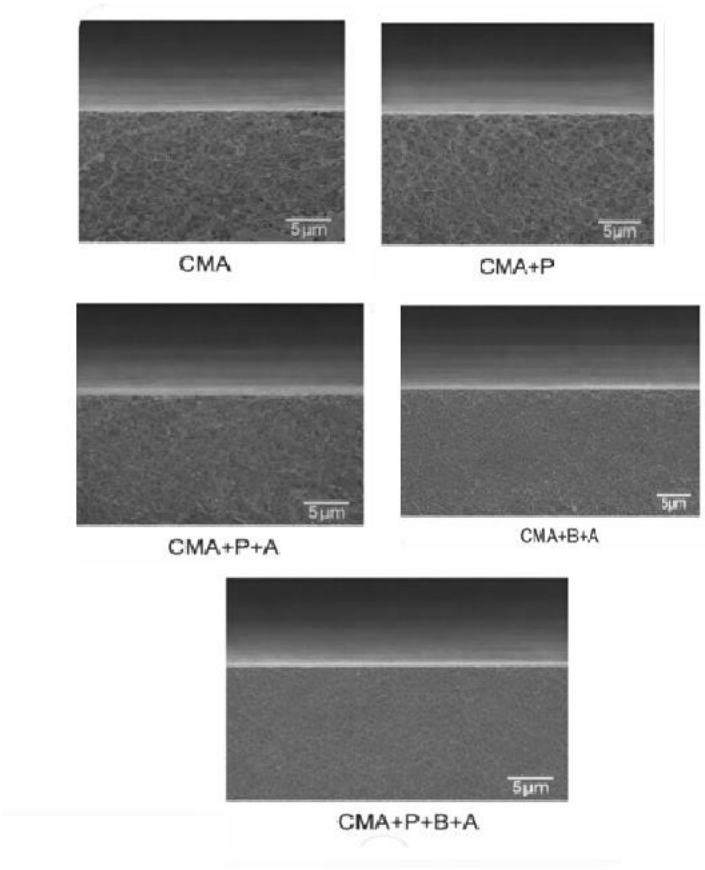

Fig. 5. SEM images of the films

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) was used to evaluate the thermal behavior of CMA and crosslinked films, with particular emphasis on the glass transition temperature (Tg), which is a key parameter reflecting polymer chain mobility and thermal stability (Arancibia *et al.*, 2015; Jin & Torkelson, 2016). The DSC thermograms of the films are presented in Fig. 6. The control CMA film exhibited a Tg of 52.54 °C, which is characteristic of hydrophilic polysaccharide-based films containing a high density of hydroxyl groups (Rahman *et al.*, 2021). The relatively low Tg indicates greater molecular mobility and weaker intermolecular interactions within the polymer matrix, which may contribute to higher water sensitivity and lower thermal resistance. Upon incorporation of polyethylene glycol, citric acid, and β-cyclodextrin, a progressive increase in Tg was observed. The Tg values increased to 58.19 °C for CMA-P, 65.00 °C for CMA-P-A, 68.00 °C for CMA-β-A, and reached a maximum of 82.08 °C for the CMA-P-β-A formulation. This gradual increase in Tg reflects the increasing degree of crosslinking and intermolecular interactions within the polymer network (Uyanga *et al.*, 2020; Startsev, Vapirov, Lebedev, & Kychkin, 2020). The significant increase in Tg for crosslinked films can be attributed to restricted polymer chain mobility resulting from ester bond formation induced by citric acid and enhanced hydrogen bonding interactions associated with β-cyclodextrin. These interactions reduce the free volume within the polymer matrix and limit segmental motion, thereby improving thermal stability (Chen *et al.*, 2025). The absence of other thermal transitions, is related to no distinct melting or degradation peaks within the applied temperature range. This behavior is likely due

to the predominantly amorphous nature of carboxymethylated and crosslinked agar films, where Tg is the dominant thermal transition (Zhu *et al.*, 2022).

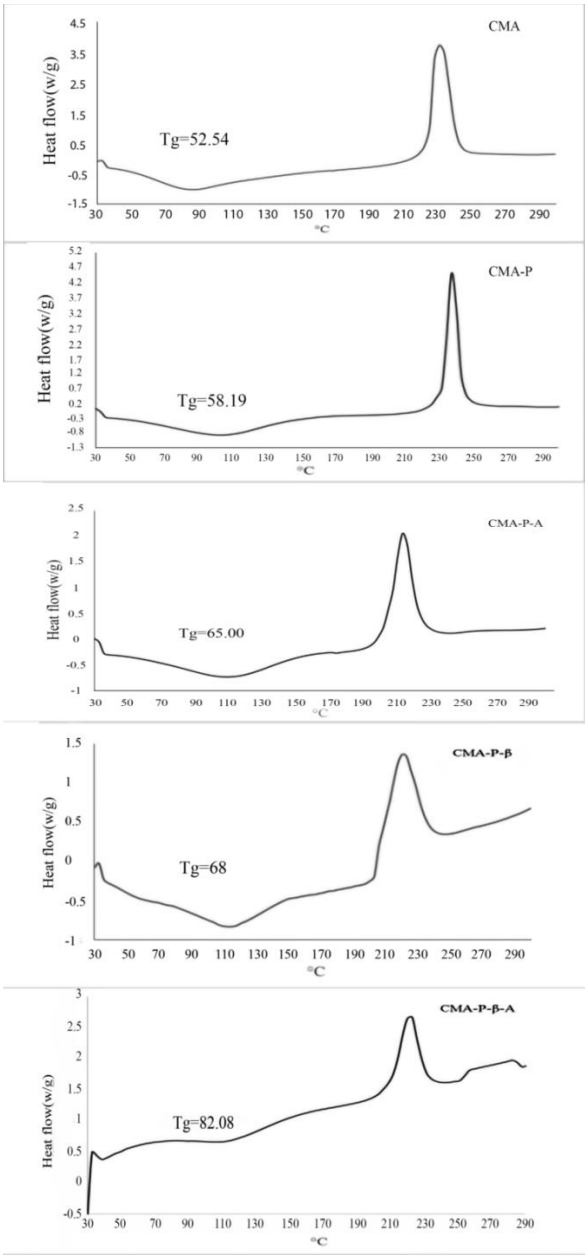

Fig. 6. DSC thermograms of the CMA and treated films

The highest Tg observed for the CMA-P-β-A formulation confirms the synergistic effect of polyethylene glycol, β-cyclodextrin, and citric acid on enhancing thermal stability, which is consistent with previous studies on citric acid-crosslinked and cyclodextrin modified biopolymer films (Han, Sathiyaseelan, Lu, Kim, & Wang, 2024; Torche *et al.*, 2025). Although the primary focus was on the glass transition temperature (Tg), other thermal transitions, such as melting and degradation points, were also considered. However, as mentioned earlier no distinct peaks for melting or degradation were clearly observed within the applied temperature range, likely due to the predominantly amorphous nature of the

carboxymethylated and crosslinked agar films. This behavior highlights the predominant role of Tg as the key thermal transition in the modified films.

Conclusion

This study demonstrated that chemical modification of agar through carboxymethylation, combined with a multi-agent crosslinking strategy, is an effective approach to overcome the inherent limitations of native agar films for food packaging applications. Carboxymethyl agar with a degree of substitution (DS) of 0.69 was successfully synthesized, providing improved solubility and compatibility with crosslinking agents. The incorporation of polyethylene glycol, citric acid, and β-cyclodextrin significantly influenced the structural, surface, and thermal properties of the films. Among all formulations, the CMA-P-β-A film exhibited the most favorable performance. This formulation showed a markedly higher water contact angle in the range of 80–89°, compared to approximately 67° for the control CMA film, indicating enhanced surface hydrophobicity and improved resistance to moisture penetration. SEM analysis confirmed the formation of a dense, homogeneous, and interconnected polymer network for this formulation, while XRD results revealed a pronounced reduction in crystallinity, suggesting increased amorphous character and improved flexibility. Thermal analysis further supported these findings, as the glass transition temperature (Tg) increased from 52.54 °C for CMA to 82.08 °C for the CMA-P-β-A film, reflecting restricted polymer chain mobility due to effective crosslinking. These results are consistent with previous reports on citric acid-crosslinked and cyclodextrin-modified biopolymer films, which have shown similar improvements in thermal stability and structural integrity (Balçık Tamer, 2023; Venkatesan, Vetcher, Al-Asbahi, & Kim, 2024). Overall, the synergistic effect of carboxymethylation and multi-component crosslinking resulted in films with enhanced hydrophobicity, reduced crystallinity, and superior thermal stability. Based on the combined physicochemical, morphological, and thermal evaluations, the CMA-P-β-A formulation was identified as the most promising candidate for environmentally friendly food packaging applications.

Scope of the Study and Limitations

The mechanical properties of the prepared films, including tensile strength and elongation at break, as well as barrier properties such as water vapor permeability and oxygen permeability, have been reported and discussed in our previously published studies. Therefore, to avoid redundancy and overlap with earlier publications, these properties were not included in the present manuscript. Accordingly, the scope of this study was intentionally limited to the structural, surface, and thermal characterization of carboxymethyl agar-based films. In addition, the thermal analysis primarily focused on the glass transition temperature (Tg), as other thermal transitions were not clearly distinguishable due to the predominantly amorphous nature of the modified films. Further investigations on film food interactions and biodegradation behavior under different environmental conditions remain important for comprehensive evaluation of practical packaging applications.

Author Contributions

F. Falahatgara: Conceptualization, Methodology, Data Curation, Writing–Original Draft. **A. Babakhania:** Methodology, Supervision, Data Curation, Visualization. **E. Zakipour Rahimabadi:** Conceptualization, Supervision, Project administration, Writing–review & editing. **H. Rostamzada:** Methodology, Data Curation.

Founding Source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Abdollahi, M., Damirchi, S., Shafafi, M., Rezaei, M., & Ariaii, P. (2019). Carboxymethyl cellulose-agar biocomposite film activated with summer savory essential oil as an antimicrobial agent. *International Journal of Biological Macromolecules*, *126*, 561-568. https://doi.org/10.1016/j.ijbiomac.2018.12.115
2. Abraham, A., Afeweki, B., Tsegay, B., Ghebremedhin, H., Teklehaimanot, B., & Reddy, K.S. (2018). Extraction of agar and alginate from marine seaweeds in Red Sea region. *International Journal of Marine Biology and Research*, *3*, 1–8. https://doi.org/10.15226/24754706/3/2/00126
3. Arancibia, M.Y., Alemán, A., López-Caballero, M.E., Gómez-Guillén, M.C., & Montero, P. (2015). Development of active films of chitosan isolated by mild extraction with added protein concentrate from shrimp waste. *Food Hydrocolloids*, *43*, 91–99. https://doi.org/10.1016/j.foodhyd.2014.05.006
4. Balçık Tamer, Y. (2023). Development of citric acid crosslinked biodegradable chitosan/hydroxyethyl cellulose/organo-modified nanoclay composite films as sustainable food packaging materials. *Polymer-Plastics Technology and Materials*, *62*(9), 1138–1156. https://doi.org/10.1080/25740881.2023.2195908
5. Bandyopadhyay, S., Sáha, T., Sanétrník, D., Saha, N., & Sáha, P. (2021). Thermo compression of thermoplastic Agar-Xanthan gum-carboxymethyl cellulose blend. *Polymers (Basel)*, *13*(20), 3472. https://doi.org/10.3390/polym13203472
6. Cao, M., Liu, X., Luan, J., & Zhang, X. (2014). Characterization of physicochemical properties of carboxymethyl agar. *Carbohydrate Polymers*, *111*, 449–455. https://doi.org/10.1016/j.carbpol.2014.04.036
7. Casaburi, A., Rojo, Ú.M., Cerrutti, P., Vázquez, A., & Foresti, M.L. (2018). Carboxymethyl cellulose with tailored degree of substitution obtained from bacterial cellulose. *Food Hydrocolloids*, *75*, 147–156. https://doi.org/10.1016/j.foodhyd.2017.09.002
8. Cazón, P., Velazquez, G., Ramírez, J.A., & Vázquez, M. (2017). Polysaccharide-based films and coatings for food packaging: A review. *Food Hydrocolloids*, *68*(5), 1-10. https://doi.org/10.1016/j.foodhyd.2016.09.009
9. Chakka, V.P., & Zhou, T. (2020). Carboxymethylation of polysaccharides: Synthesis and bioactivities. *International Journal of Biological Macromolecules*, *165*, 2425–2431. https://doi.org/10.1016/j.ijbiomac.2020.10.178
10. Chen, J., Wu, J., Veldhuis, T., Picchioni, F., Raffa, P., & Koning, C.E. (2025). Mechanistic study on citric acid–based esterification: A versatile reaction for preparation of hydrophilic polymers. *ACS Sustainable Chemistry & Engineering*, *13*(1), 559-570. https://doi.org/10.1021/acssuschaemeng.4c07569
11. Dharmalingam, K., & Anandalakshmi, R. (2019). Fabrication, characterization and drug loading efficiency of citric acid crosslinked NaCMC-HPMC hydrogel films for wound healing drug delivery applications. *International Journal of Biological Macromolecules*, *134*, 815–829. https://doi.org/10.1016/j.ijbiomac.2019.05.027
12. Escobar, K., Garrido-Miranda, K.A., Pulido, R., Naveas, N., Manso-Silván, M., & Hernandez-Montelongo, J. (2023). Coatings of cyclodextrin/citric-acid biopolymer as drug delivery systems: A review. *Pharmaceutics*, *15*(1), 296, https://doi.org/10.3390/pharmaceutics15010296
13. Gieroba, B., Kalisz, G., Krysa, M., Khalavka, M., & Przekora, A. (2023). Application of vibrational spectroscopic techniques in the study of the natural polysaccharides and their cross-linking process. *International Journal of Molecular Sciences*, *24*(3), 2630. https://doi.org/10.3390/ijms24032630

14. Han, K., Sathiyaseelan, A., Lu, Y., Kim, K.M., & Wang, M.H. (2024). Agar/carboxymethyl cellulose composite film loaded with hydroxyapatite nanoparticles for bone regeneration. *Cellulose*, *31*(15), 9319-9334. https://doi.org/10.1007/s10570-024-06148-5
15. Im, W., Lee, S., Rajabi Abhari, A., Youn, H.J., & Lee, H.L. (2018). Optimization of carboxymethylation reaction as a pretreatment for production of cellulose nanofibrils. *Cellulose*, *25*, 3873-3883. https://doi.org/10.1007/s10570-018-1853-9
16. Jin, K., & Torkelson, J.M. (2016). Enhanced Tg-confinement effect in cross-linked polystyrene compared to its linear precursor: Roles of fragility and chain architecture. *Macromolecules*, *49*(14), 5092–5103. https://doi.org/10.1021/acs.macromol.6b01042
17. Keya, K.N., Li, Z., Xu, L., & Xia, W. (2025). Exploring cross-link density and additive effects on mechanical and morphological behaviors of cross-linked polymers. *Macromolecular Materials and Engineering*, *310*(5), 1-12. https://doi.org/10.1002/mame.202400383
18. Lakshmi, D.S., Trivedi, N., & Reddy, C.R.K. (2017). Synthesis and characterization of seaweed cellulose derived carboxymethyl cellulose. *Carbohydrate Polymers*, *157*, 1604-1610. https://doi.org/10.1016/j.carbpol.2016.11.042
19. Liu, S., Xiao, Y., Shen, M., Zhang, X., Wang, W., & Xie, J. (2020). Effect of sodium carbonate on the gelation, rheology, texture and structural properties of maize starch-mesona chinensis polysaccharide gel. *Food Hydrocolloids*, *87*, 943–951. https://doi.org/10.1016/j.foodhyd.2018.09.025
20. Michler, G.H., & Lebek, W. (2016). Electron microscopy of polymers. In: *Polymer Morphology: Principles, Characterization, and Processing*. Pp. 37-53. Guo, Q. Eds., John Wiley & Sons, inc. https://doi.org/10.1002/9781118892756.ch3
21. Mirpoor, S.F., Massironi, A., Winning, D., Lignou, S., Ghawi, S.K., Trotta, F., & Charalampopoulos, D. (2025). Agar/Carboxymethyl cellulose blended films with green-synthesised silver nanoparticles as a sustainable alternative for food packaging applications. *Polymers*, *17*(23), 3126. https://doi.org/10.3390/polym17233126
22. Mostafa, S.I., Ali, M.A., El-Wassefy, N.A., Saad, E.M., & Hussein, M.H. (2024). Adsorption and interaction studies of methylene blue dye onto agar-carboxymethylcellulose-silver nanocomposite in aqueous media. *Biomass Conversion and Biorefinery*, *14*(7), 1-10. https://doi.org/10.1007/s13399-022-02505-1
23. Qi, B., Yang, S., Zhao, Y., Wang, Y., Yang, X., Chen, S., Wu, Y., Pan, C., Hu, X., Li, C., & Wang, L. (2022). Comparison of the physicochemical properties of carboxymethyl agar synthesized by microwave-assisted and conventional methods. *Gels*, *8*(3), 162. https://doi.org/10.3390/gels8030162
24. Rahman, M.S., Hasan, M.S., Nitai, A.S., Nam, S., Karmakar, A.K., Ahsan, M.S., Shiddiky, M.J., & Ahmed, M.B. (2021). Recent developments of carboxymethyl cellulose. *Polymers*, *13*(8), 1345. https://doi.org/10.3390/polym13081345
25. Rajawasam, C.W., Dodo, O.J., Weerasinghe, M.S.N., Raji, I.O., Wanasinghe, S.V., Konkolewicz, D., & Watuthanthrige, N.D.A. (2024). Educational series: Characterizing crosslinked polymer networks. *Polymer Chemistry*, *15*(3), 1-47. https://doi.org/10.1039/d3py00914a
26. Rbihi, S., Aboulouard, A., Laallam, L., & Jouaiti, A. (2020). Contact angle measurements of cellulose based thin film composites: Wettability, surface free energy and surface hardness. *Surfaces and Interfaces*, *21*, 100708. https://doi/org/10.1016/j.surfin.2020.100708
27. Roy, S., Kim, H.J., & Rhim, J.W. (2021). Effect of blended colorants of anthocyanin and shikonin on carboxymethyl cellulose/agar-based smart packaging film. *International Journal of Biological Macromolecules*, *183*, 305-315. https://doi.org/10.1016/j.ijbiomac.2021.04.162

28. 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*, 770–775. https://doi.org/10.1016/j.foodhyd.2010.04.003
29. Smith, B.C. (2022). Infrared spectroscopy of polymers, IX: Pendant ester polymers and polycarbonates. *Spectroscopy*, *37*(11), 16-19. https://doi.org/10.56530/spectroscopy.xn9369p8
30. Startsev, O.V., Vapirov, Y.M., Lebedev, M.P., & Kychkin, A.K. (2020). Comparison of glass-transition temperatures for epoxy polymers obtained by methods of thermal analysis. *Mechanics of Composite Materials*, *56*, 227-240. https://doi.org/10.1007/s11029-020-09875-5
31. Surendran, G., & Sherje, A.P. (2023). Biomedical applications of agar and its composites: A mini-review. *Natural Products Journal*, *13*(5), 23-30. https://doi.org/10.2174/2210315512666220615113320
32. Tammina, S.K., & Rhim, J.W. (2023). Carboxymethylcellulose/agar-based functional film incorporated with nitrogen-doped polyethylene glycol-derived carbon dots for active packaging applications. *Chemosphere*, *313*, 137627. https://doi.org/10.1016/j.chemosphere.2022.137627
33. Torche, A., Chouana, T., Akachat, B., Rekbi, F.M.L., Rahmani, Y., El Hadj, M.D.O., Uzun, Ş.A., Parlak, M.E., D'Elia, M., Rastrelli, L., & Sarıcaoğlu, F.T. (2025). Cassava starch films with green-synthesized ZnO nanoparticles from date palm pits for active packaging. *Food Chemistry*, *495*, 146392. https://doi.org/10.1016/j.foodchem.2025.146392
34. Uyanga, K.A., Okpozo, O.P., Onyekwere, O.S., & Daoud, W.A. (2020). Citric acid crosslinked natural bi-polymer-based composite hydrogels: Effect of polymer ratio and beta-cyclodextrin on hydrogel microstructure. *Reactive and Functional olymers*, *154*, 104682. https://doi.org/10.1016/j.reactfunctpolym.2020.104682
35. Velázquez-Contreras, F., Zamora-Ledezma, C., López-González, I., Meseguer-Olmo, L., Núñez-Delicado, E., & Gabaldón, J.A. (2021). Cyclodextrins in polymer-based active food packaging: A fresh look at nontoxic, biodegradable, and sustainable technology trends. *Polymers*, *14*(1), 104. https://doi.org/10.3390/polym14010104
36. Venkatesan, R., Vetcher, A.A., Al-Asbahi, B.A., & Kim, S.C. (2024). Chitosan-based films blended with tannic acid and *Moringa oleifera* for application in food packaging: The preservation of strawberries (*Fragaria ananassa*). *Polymers*, *16*(7), 937. https://doi.org/10.3390/polym16070937
37. Villanueva, R.D., Sousa, A.M.M., Gonçalves, M.P., Nisson, M., & Hilliou, L. (2010). Production and properties of agar from the invasive marine alga, *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta). *Journal of Applied Phycology*, *22*(2), 211–220. https://doi.org/10.1007/s10811-009-9444-7
38. Wang, Y., Mo, Q., Li, Z., Lai, H., Lou, J., Liu, S., & Mao, J. (2012). Effects of degree of carboxymethylation on physicochemical and biological properties of pachyman. *International Journal of Biological Macromolecules*, *51*(5), 1052–1056. https://doi.org/10.1016/j.ijbiomac.2012.08.022
39. Wang, Z., Xu, C., Qi, L., & Chen, C. (2024). Chemical modification of polysaccharides for sustainable bioplastics. *Trends in Chemistry*, *6*(6), 314-331. https://doi.org/10.1016/j.trechm.2024.04.009
40. Zhang, C., An, D., Xiao, Q., Weng, H., Zhang, Y., Yang, Q., & Xiao, A. (2020). Preparation, characterization, and modification mechanism of agar treated with hydrogen peroxide at different temperatures. *Food Hydrocolloids*, *101*, 105527. https://doi.org/10.1016/j.foodhyd.2019.105527
41. Zhang, X., Chen, T., Lim, J., Gu, F., Fang, F., Cheng, L., Campanella, O.H., & Hamaker, B.R. (2019). Acid gelation of soluble laccase-crosslinked corn bran arabinoxylan and possible gel formation mechanism. *Food Hydrocolloids*, *92*, 1–9. https://doi.org/10.1016/j.foodhyd.2019.01.032

42. Zhao, C., Li, L., Xiao, H., Huang, Y., Zhao, Y., Ma, L., & Tian, Y. (2025). Physicochemical properties and structural characterization of crosslinking modifications of carboxymethyl cellulose with varying degrees of substitution. *Food Chemistry*, *496*(1), https://doi.org/10.1016/j.foodchem.2024.146617
43. Zhu, W., Zhang, D., Liu, X., Ma, T., He, J., Dong, Q., Din, Z.U., Zhou, J., Chen, L., Hu, Z., & Cai, J. (2022). Improving the hydrophobicity and mechanical properties of starch nanofibrous films by electrospinning and cross-linking for food packaging applications. *LWT- Food Science and Technology*, *169*, 114005. https://doi.org/10.1016/j.lwt.2022.114005
44. Zou, Y., Yuan, C., Cui, B., Sha, H., Liu, P., Lu, L., & Wu, Z. (2021). High-amylose corn starch/konjac glucomannan composite film: Reinforced by incorporating β-cyclodextrin. *Journal of Agricultural Food Chemistry*, *69*(8), 2493–2500. https://doi.org/10.1021/acs.jafc.0c06648

نشریه پژوهشهای علوم و صنایع غذایی ایران
https://ifstrj.um.ac.ir

مقاله پژوهشی
جلد ۲۲، شماره ۱، فروردین - اردیبهشت ۱۴۰۵، ص. ۱۶-۱

تولید و بررسی خصوصیات فیلم‌های بر پایه آگار کربوکسی متیله‌شده برای استفاده در بسته‌بندی محصولات غذایی

فریده فلاحتگر[۱]- آریا باباخانی[۱]- اسحق زکی‌پور رحیم‌آبادی[۱]*- هانیه رستم‌زاد[۱]

تاریخ دریافت: ۱۴۰۴/۰۷/۲۰
تاریخ پذیرش: ۱۴۰۴/۱۱/۱۱

چکیده

این مطالعه به‌منظور تعیین ویژگی‌های فیلم‌های کربوکسی متیل آگار با استفاده از عوامل اتصال عرضی شامل اسید سیتریک، بتا-سیکلودکسترین و پلی‌اتیلن گلیکول انجام شد. پنج نوع فیلم با عوامل اتصال دهنده عرضی مختلف تهیه گردید. در این تحقیق، ویژگی‌های مورفولوژیکی، زاویه تماس و خواص حرارتی فیلم‌ها برای استفاده در بسته‌بندی مواد غذایی مورد ارزیابی قرار گرفتند. نتایج زاویه تماس نشان داد که فیلم بهینه، متشکل از کربوکسی متیل آگار همراه با پلی‌اتیلن گلیکول، اسید سیتریک و بتا-سیکلودکسترین، زاویه تماس بالاتری نسبت به سایر فیلم‌ها نشان داد. آگارز با موفقیت کربوکسی کتیله شده و میزان کریستاله شدن آن کاهش یافته و این امر سبب افزایش انعطاف‌پذیری و بهبود خواص سدکنندگی فیلم‌ها گردید. دمای گذار شیشه‌ای به ۸۲/۰۲ درجه سانتی‌گراد رسید که نشان‌دهنده افزایش سختی ساختاری و کاهش جذب آب است که با آنالیز DSC تأیید گردید. این تحقیق نشان داد که فیلم بر پایه آگار کربوکسی متیله شده پتانسیل قابل توجه‌ای به‌عنوان جایگزین بسته‌بندی سازگار با محیط‌زیست دارد.

واژه‌های کلیدی: آگار کربوکسی متیله شده، اتصالات عرضی، جلبک، خصوصیات حرارتی

۱- گروه شیلات، دانشکده منابع طبیعی، دانشگاه گیلان، صومعه سرا، ایران
(*- نویسنده مسئول: Email: e.zakipoor@guilan.ac.ir)

https://doi.org/10.22067/ifstrj.2026.95783.1488