

# Development of Biodegradable CMC–Collagen Film Enriched with Anthocyanin, Bromelain, and Zinc Oxide Nanoparticle for Active Fruit and Vegetable Packaging

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**Abstract** - This study aimed to develop biodegradable CMC–collagen composite films incorporated with anthocyanin from banana bracts (*Musa spp*) and bromelain from pineapple peels (*Ananas comosus*) for advanced fresh fruits and vegetables packing applications. Anthocyanins and bromelain were extracted from fresh banana bracts and pineapple peels respectively and used for film preparation. The composite films were prepared using 3% carboxymethyl cellulose and 2% collagen with 1% glycerol as plasticizer under heating with constant stirring to form a viscous solution. Anthocyanin and bromelain were added to the polymer matrix, cast in a mold, and oven-dried at 40°C. The dried film was thereafter peeled off the mold and stored for analysis. The resulting films provide antioxidant, pH-responsive, and proteolytic properties, aiding infection monitoring through colour change, and offering a sustainable, Bioactive compounds derived from GF agro-waste resources for active food packaging applications.

**Keywords:** Biodegradable, CMC, Collagen, Composite Films, Anthocyanin, Bromelain, Zinc Oxide Nps, Antioxidant System.

## I. INTRODUCTION

Food loss arising from postharvest deterioration of fruits and vegetables is a major threat to food security. Therefore, this study aimed to develop biodegradable CMC–collagen composite films incorporated with anthocyanin from banana bracts (*Musa spp*) and bromelain from pineapple peels (*Ananas comosus*) for advanced food packaging applications. Increasing postharvest losses of fruits and vegetables, coupled with growing environmental concerns associated with non-biodegradable synthetic plastics, have intensified the demand for sustainable and functional packaging solutions. Conventional packaging materials primarily serve as passive barriers, offering limited protection against microbial contamination, oxidation, and physiological deterioration, thereby contributing to reduced shelf life and significant economic losses. In recent years, the development of biodegradable active packaging systems

incorporating natural bioactive compounds has emerged as a promising strategy to overcome these limitations and enhance food quality and safety. In this biopolymer-based films derived from renewable resources have gained considerable attention due to their biodegradability, biocompatibility, and environmental sustainability. Carboxymethyl cellulose (CMC) is widely recognized for its excellent film-forming ability, mechanical strength, and barrier properties, while collagen contributes to improved flexibility, moisture retention, and structural integrity (Silva *et al.*, 2017). The combination of these polymers provides a suitable matrix for the incorporation of functional bioactive agents.

Anthocyanins, naturally occurring flavonoid pigments extracted from banana bracts (*Musa spp.*), exhibit strong antioxidant activity and unique pH-responsive colorimetric properties, making them ideal candidates for intelligent packaging systems capable of real-time freshness monitoring. Simultaneously, bromelain, a proteolytic enzyme derived from pineapple peels (*Ananas comosus*), offers antimicrobial and anti-biofilm properties, contributing to enhanced microbial safety. Furthermore, zinc oxide (ZnO) nanoparticles have been extensively reported for their broad-spectrum antibacterial activity, primarily through the generation of reactive oxygen species and disruption of microbial cell membranes. The integration of these components into a single composite film results in a multifunctional system combining antioxidant, antimicrobial, and pH-indicating properties. Such synergistic interactions not only improve the preservation efficiency of perishable foods but also align with the principles of circular economy through the valorization of agro-waste materials. Therefore, the present study focuses on the development and evaluation of a biodegradable CMC–collagen composite film enriched with anthocyanin and bromelain for active food packaging applications.

This eco-friendly and cost-effective approach demonstrates significant potential for enhancing shelf life, reducing food waste, and providing intelligent packaging solutions for fresh produce (Sari *et al.*, 2022; Pavan *et al.*, 2012).

## II. MATERIALS AND METHODS

### 2.1. Collection and preparation of Sample

Fresh banana bract (200 g), Citric acid (16 g), Filter paper, Pineapple peel (fresh), Distilled water (aquadest), Ethanol (analytical grade), Ammonium sulfate, Phosphate buffer (0.01 M, pH 7.0), Filter cloth or gauze, Centrifuge, Collagengel (Gelatin), Carboxymethylcellulose (CMC), Plasticizer (glycerine), Zinc acetate dihydrate, NaOH.

### 2.2. Preparation of Anthocyanin

Blend 100 g fresh banana bracts and mix with 200 mL distilled water acidified with 8 g citric acid. Macerate 36 h at room temperature, filter, and centrifuge at 4,000–6,000 rpm for 10–20 min at 4 °C. Collect the clear supernatant as crude anthocyanin extract and further purify using a rotary evaporator (Rosalina *et al.*, 2022 and Sari *et al.*, 2022).

### 2.3. Preparation of Bromelain

Fresh pineapple peels (*Ananas comosus*) were homogenized in 0.01 M phosphate buffer (pH 7.0) at 1:2 (w/v). The extract was filtered through gauze and

Whatman No. 1 paper. Ammonium sulfate (50% saturation) was added under cold stirring, centrifuged at 8,000 rpm for 15 min at 4 °C, and the pellet redissolved to obtain partially purified bromelain (Pavan *et al.*, 2012).

### 2.4. Preparation of zinc nanoparticle

Zinc oxide nanoparticles were synthesized by direct precipitation for wound dressing applications. Zinc acetate dihydrate (6.7 g/150 mL) reacted with sodium hydroxide (2.5 g/50 mL) at pH 10–12 under stirring. The mixture was heated at 60–80 °C for 2–3 h, centrifuged, washed, and dried at 100–120 °C, yielding 2–2.5 g (~20–50 nm) ZnO nanoparticles (Kumar *et al.*, 2017).

### 2.5. Antimicrobial Activity (Agar Well Diffusion Method)

Antimicrobial activity against *Staphylococcus aureus* was evaluated using the agar well diffusion method. Inoculated Mueller–Hinton agar plates were loaded with 50 µL of extract and incubated at 37°C for 24 h. Zones of inhibition were measured in mm. Streptomycin was used as the positive control.

### 2.6. Film Preparation

Biodegradable composite films were prepared by the solvent casting method based on the specified formulation ratios mentioned in the given Table 1.

Table 1: Composite of Formulation of film

Component	Amount	Component	Amount
CMC	2 g	Bromelain extract	5ml
Gelatin	1.5 g	Acetic acid	1 mL
ZnO nanoparticles	1 g	Distilled water	Up to 100 mL
Anthocyanin extract	5 mL	Glycerol	1ml

A 2% (w/v) CMC solution and 1.5% (w/v) gelatin solution were prepared separately, mixed after neutralization, and plasticized with 1% (v/v) glycerine. Anthocyanin and bromelain were added

after cooling as shown in Fig 1. The degassed mixture was cast and dried at 40°C to form film, The final pH was 7.5, indicating near-neutral conditions suitable for wound applications.

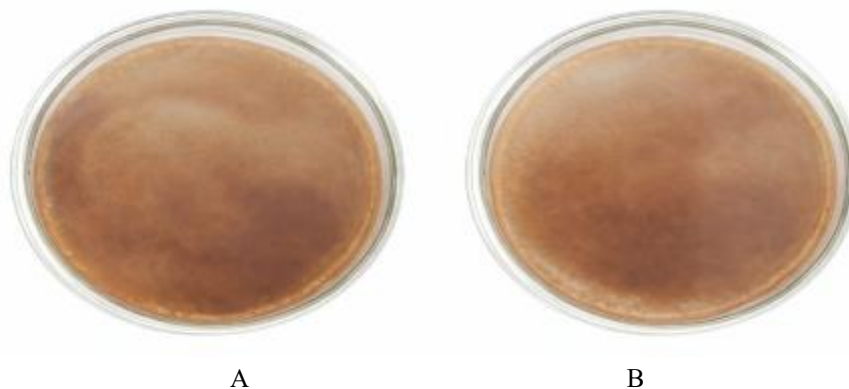


Fig:1 A Control film, B Film incorporated with anthocyanin and bromelain

### 2.7. HPLC analysis of anthocyanin

HPLC was carried out using a water (A) acetonitrile (B) gradient system. The run began at 90:10 (A:B), increased to 30% B at 20 min and 50% B at 30 min, and was returned to 80:20 at 40 min for column re-equilibration (Bunea, *et al* 2011). Flow Rate: 1.0 mL/min, Injection Volume: (Typically 10–20 µL, adjust as needed), Column: C18 reversed-phase column (e.g., 250 × 4.6 mm, 5 µm), Column Temperature: 25–30°C (or as required)

### 2.8. HPLC analysis of Bromelain

Chromatographic separation was carried out in isocratic mode at room temperature using a ODSC18 (250x4.6mm, 5µ) column. The mixture of 0.1% OPA: Acetonitrile 50:50 v/v at a flow rate 1.0 ml/min was used as a mobile phase. The injection volume was 20µl and eluents was monitored at 257nm using PDA

detector for bromelain, the run time was 10min. (Goda *et al* 2019).

## III. RESULTS AND DISCUSSION

### 3.1. HPLC analysis of anthocyanin

HPLC analysis showed six prominent peaks, confirming the presence of multiple anthocyanin components. The highest peak was observed at RT 30.008 min with 53.41% area, indicating it as the major anthocyanin compound. The second highest peak appeared at 32.100 min (19.67%), followed by 28.012 min (8.74%). Additional peaks were detected at 34.630 min (7.12%), 26.172 min (4.42%), and 1.300 min (1.79%). These six peaks collectively represent the principal anthocyanin constituents in the extract, with the dominant compound eluting at 30.008 min as shown in table 2

Table 2: Major Anthocyanin Peaks Identified by HPLC Analysis

Rank	RT (min)	Area	Area %	Probable Compound Class
1	30.008	54576.25	53.41	Malvidin-3-O-glucoside
2	32.100	20096.90	19.67	Peonidin-3-O-glucoside
3	28.012	8935.25	8.74	Petunidin-3-O-glucoside
4	34.630	7276.98	7.12	Acylated anthocyanin
5	26.172	4516.64	4.42	Cyanidin-3-O-glucoside

The multi-peak anthocyanin profile observed in the present study is consistent with earlier reports, where plant-derived anthocyanin extracts typically exhibit a complex mixture of flavonoid compounds. Studies by (Zhang *et al.*, 2023) and (Roy *et al.*, 2023) identified dominant peaks corresponding to malvidin and cyanidin derivatives in extracts from fruit and floral waste, confirming the heterogeneous nature of anthocyanins.

However, the proportion of the major anthocyanin compound (53.41%) in this study is comparatively higher than previously reported values (30–45%), indicating improved extraction efficiency and pigment concentration. This enhancement may be attributed to the citric acid-assisted extraction method, which effectively stabilizes anthocyanin structures and minimizes degradation. Additionally, the use of banana bracts as a raw material highlights their potential as a rich and underutilized source of anthocyanins, in contrast to commonly studied sources such as berries and grape skins. The detection of acylated anthocyanins further suggests improved

thermal and pH stability, which is advantageous for packaging applications. Similar findings have been reported in previous studies, where acylated derivatives contributed to enhanced colour stability and functional performance of anthocyanin-based films. From a functional perspective, (Li *et al.*, 2025) demonstrated that anthocyanin-incorporated films improve oxidative stability in food systems by scavenging reactive oxygen species. The present findings align with this observation, supporting the role of anthocyanins in extending shelf life and maintaining product quality.

### 3.2. HPLC analysis of Bromelain

High-performance liquid chromatography coupled with diode array detection (HPLC–DAD) at 214, 254, 280, 365, and 425 nm exhibited a single, sharp, and symmetrical peak at a retention time (Rt) of approximately 2–3 min. No additional significant peaks were observed throughout the chromatographic run, indicating the presence of one predominant compound with high purity and

negligible detectable impurities under the applied analytical conditions as shown in table 3

Table 3: HPLC Peak Profile of Bromelain Protease Components

Rank	RT (min)	Area	Height	Height%	Area%	Probable Compound Class
1	1.176	9789.27	930.66	86.20	88.0	Major cysteine protease
2	0.925	1211.26	122.45	11.34	10.89	Minor cysteine protease
3	1.565	43.05	7.17	0.66	0.39	Ananain
4	3.018	34.11	7.15	0.66	0.31	Comosain
5	2.051	25.67	7.74	0.69	0.23	Peptide fragment
6	2.359	10.82	2.99	0.28	0.10	Trace protein

(Singh *et al.*, 2022) reported bromelain purity levels of 70–85% in biopolymer systems, reflecting moderate extraction efficiency. In comparison, the higher purity (~88%) achieved in the present study indicates improved extraction efficiency and reduced enzymatic denaturation, thereby enhancing functional stability and proteolytic activity. Recent studies further support that optimized extraction from pineapple waste can significantly improve bromelain recovery and stability.

Although bromelain has been extensively investigated for biomedical and therapeutic applications, its utilization in food packaging remains relatively limited. Emerging research suggests that the incorporation of natural enzymes into biodegradable films can enhance antimicrobial properties and extend shelf life. In this context, the present study offers a novel approach by integrating bromelain into a CMC–collagen composite film. The higher purity and retained enzymatic activity are expected to enhance antimicrobial efficacy, highlighting its potential for application in active and sustainable food packaging systems.

### 3.3. Anti-Bacterial activity

The CMC–collagen composite film containing anthocyanin, bromelain, and ZnO nanoparticles showed clear antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The effectiveness increased with concentration, with the highest inhibition observed at 0.100 mL (Fig. 2). The standard antibiotic, streptomycin, showed slightly higher inhibition, while the blank film showed no antibacterial effect, confirming that the activity is due to the added bioactive components (Table 4).

Similar antimicrobial activity has been reported by (Kumar *et al.*, 2024), where ZnO nanoparticle-based biodegradable films exhibited inhibition zones in the range of 1.2–1.5 mm against *E. coli* and *S. aureus*. The slightly higher inhibition observed in the present study (up to 1.6 mm) suggests enhanced antimicrobial efficacy, likely due to the combined effect of enzymatic (bromelain) and phytochemical (anthocyanin) components. Furthermore, (Koshy *et al.*, 2023) demonstrated that anthocyanin–ZnO composite films possess improved antibacterial properties; however, those systems lacked enzymatic functionality. In contrast, the present study integrates bromelain, providing an additional mechanism of microbial inhibition through protein degradation and biofilm disruption.

Table 4: Anti-Bacterial Analysis with *S. aureus* and *E. coli*

Sample	Test Organism	Conc. (mg/mL)	Zone of Inhibition (mm)
Streptomycin (Positive Control)	<i>Staphylococcus aureus</i>	Standard	1.7 mm
Streptomycin (Positive Control)	<i>Escherichia coli</i>	Standard	1.8 mm
CMC–Collagen + Anthocyanin + Bromelain + ZnO NPs	<i>Staphylococcus aureus</i>	0.50 mL	1.4 mm
CMC–Collagen + Anthocyanin + Bromelain + ZnO NPs	<i>Staphylococcus aureus</i>	0.100 mL	1.6 mm

CMC–Collagen + Anthocyanin + Bromelain + ZnO NPs	<i>Escherichia coli</i>	0.50 mL	1.2 mm
CMC–Collagen + Anthocyanin + Bromelain + ZnO NPs	<i>Escherichia coli</i>	0.100 mL	1.5 mm

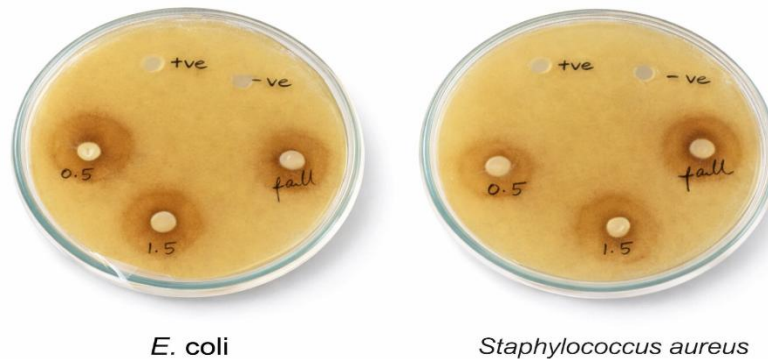


Fig 2: Zone of Inhibition of Composite Film Against *E. coli* and *S. aureus*

Overall, the results indicate that the developed composite film exhibits improved antibacterial performance compared to previously reported systems, highlighting its potential for application in active and multifunctional food packaging.

### 3.4. Freshness Preservation Study

After three days of storage under ambient conditions, the film-wrapped tomato, grapes, and jamun

(*Syzygium cumini*) showed reduced moisture loss, maintained better firmness, and retained their natural color. No visible fungal growth or surface spoilage was observed on the wrapped fruits. These findings indicate that the CMC–collagen bioactive film effectively preserved freshness and delayed spoilage during the storage period.



**Fruits coated with Film**

Tomato Jamun Grape

Fig 3: Fruit and vegetable wrapped in film



**Post packaged Fruits**

Tomato Jamun Grape

Fig 4: Post wrapping after 3 days

Similar findings have been reported by (Li *et al.*, 2023), who demonstrated that anthocyanin-based smart films effectively preserve fruit quality by reducing oxidative degradation and microbial proliferation. Likewise, (Ma *et al.*, 2026) reported that intelligent packaging systems incorporating natural pigments could maintain freshness for up to 2–3 days under ambient conditions. The results of the present study are in close agreement with these findings; however, the enhanced preservation

efficiency observed here can be attributed to the synergistic effects of antioxidant (anthocyanin), enzymatic (bromelain), and nanoparticle-mediated (ZnO) antimicrobial mechanisms.

Overall, the developed composite film exhibits effective freshness preservation and demonstrates strong potential as a multifunctional active packaging material for extending the shelf life of perishable fruits.

#### IV. CONCLUSION

The developed biodegradable CMC–collagen composite film, enriched with anthocyanin derived from *Musa spp.*, bromelain from *Ananas comosus*, and ZnO nanoparticles, demonstrates considerable potential for application in active food packaging systems. The incorporation of these bioactive components imparts multifunctional properties to the film, including strong antioxidant activity and effective antibacterial action against *Staphylococcus aureus*. These functional attributes indicate the film's ability to reduce surface microbial load, inhibit the growth of spoilage-causing microorganisms, and retard oxidative degradation. Consequently, the composite film contributes to improved preservation of fresh fruits and vegetables by delaying spoilage and maintaining product quality. Overall, the integration of natural extracts and nanoparticles within a biodegradable polymer matrix highlights a sustainable and efficient approach for enhancing food safety and extending shelf life.

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#### REFERENCE

- [1] Bayat, S., Rabbani Zabihi, A., Amel Farzad, S., Movaffagh, J., Hashemi, E., Arabzadeh, S., & Hashemi, M. (2021). Evaluation of debridement effects of bromelain-loaded sodium alginate nanoparticles incorporated into chitosan hydrogel in animal models. *Iranian Journal of Basic*
- [2] Goday, S., Abdulrahman, S. K., & Prameelaranid, A. (2019). Development and validation of stability indicating RP-HPLC method for determination of bromelain, trypsin, rutoside and diclofenac in bulk and pharmaceutical dosage forms. *International Journal of Life Science and Pharma Research*, 9(4), 19–27.
- [3] Koshy, J. T., Mathew, S., & Thomas, S. (2023). Development of anthocyanin–ZnO nanoparticle incorporated biopolymer films for multifunctional applications. *Polymers*, 15(10), 2372.
- [4] Kumar, R., Verma, A., & Singh, S. (2024). ZnO nanoparticle-based biodegradable films for food packaging applications. *Journal of Food Engineering*, 350, 111345.
- [5] Kumar, S., & Sahare, P. (2017). Simple synthesis of ZnO nanoparticles by precipitation method and study of their properties. *Materials Today: Proceedings*, 4(9), 9693-9697.
- [6] Li, X., Chen, Y., & Wang, Z. (2025). Optimization of anthocyanin-based smart films for food packaging applications. *Food Packaging and Shelf Life*, 51, 101585.
- [7] Li, X., Wang, L., & Zhao, Y. (2023). Smart packaging films incorporated with natural pigments for real-time monitoring of food quality. *Trends in Food Science & Technology*, 134, 245–258.
- [8] Ma, P., Liu, Y., & Zhang, Q. (2026). Intelligent anthocyanin-based packaging films for food freshness monitoring. *Food Chemistry X*, 34, 103634.
- [9] Mogoşanu, G. D., Mihai, M., Purcăreanu, I. L., Lazău, R., Părvu, O., Mărginean, C., Musuc, A. M., & Ficai, D. (2021). Collagen-carboxymethylcellulose biocomposite wound-dressings with antimicrobial activity. *Materials*, 14(6), 1384.
- [10] Rosalina, Y., Warsiki, E., Fauzi, A. M., & Sailah, I. (2022). Study of anthocyanin extraction from red banana (*Musa sapientum* L. var *Rubra*) waste and characteristics of light effects. *Science and Technology Indonesia*, 7(4).
- [11] Roy, S., Banerjee, S., & Das, A. (2023). Natural pigments in food packaging: Applications and future perspectives. *Foods*, 12(21), 4011.
- [12] Sari, D. K., & Santoso, B. (2022). Study of anthocyanin extraction from red banana (*Musa sapientum* L. var. *Rubra*) waste and characteristics of light effects. *Jurnal Sains dan Teknologi Indonesia*, 24(2), 45–52.
- [13] Silva, N. H. C. S., Vilela, C., Marrucho, I. M., Freire, C. S. R., Pascoal Neto, C., & Silvestre, A. J. D. (2017). Bacterial nanocellulose loaded with bromelain. *Journal of Nanomedicine & Nanotechnology*, 8(5), 1000469.
- [14] Singh, P., Sharma, K., & Gupta, R. (2022). Bromelain-based biopolymer composites for antimicrobial and biomedical applications. *International Journal of Biological Macromolecules*, 209, 1725–1735.

- [15] Tenorová, K., Masteiková, R., Pavloková, S., Kostelanská, K., Bernatonienė, J., & Vetchý, D. (2022). Formulation and evaluation of novel film wound dressing based on collagen/microfibrillated carboxymethylcellulose blend. *Pharmaceutics*, *14*(4), Article 782.
- [16] Zhang, Y., Chen, H., & Liu, J. (2023). Development of anthocyanin-based intelligent films for food packaging applications. *Food Chemistry*, *405*, 134847.