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Smart Dimethylacrylamide/Gelatin–Rosa Hydrogels for Application in Food Packaging Materials

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Abstract: A multifunctional bio-based hydrogel, p(Gelatin-co- N,N-dimethylacrylamide) Citric acid-Rosa Extract (p(Gelatin-co-DMAAm)/CA-RE), was developed as a smart food packaging material. Synthesized via redox polymerization of N,N-dimethylacrylamide, gelatin, citric acid, and Rosa extract (RE), the hydrogel formed robust films with enhanced mechanical strength and water resistance through crosslinking with citric acid and MBA. Entrapped RE imparted antioxidant, antimicrobial, and pH-responsive colorimetric properties, enabling real-time monitoring of food freshness. Characterization by Thermo-gravimetric, Fourier transform infrared spectroscopy, Dynamic mechanical analysis and Scanning Electron Microscopy (DMA, TGA, FT-IR, and SEM) confirmed network formation, thermal stability up to 527°C, and molecular interactions between polymer components and RE. The hydrogel exhibited effective antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, while rapid and visible color changes under varying pH highlighted its sensing capability. Application to pasteurized milk and cheese demonstrated simultaneous microbial inhibition and spoilage detection. The combination of mechanical robustness, bioactivity, and intelligent colorimetric response positions p(Gelatin-co-DMAAm)/CA-RE as a sustainable, multifunctional smart packaging platform with potential extensions to biomedical and other functional material applications.

Keywords: Functional hydrogel, p(Gelatin-co-DMAAm)/CA-RE, Smart food packaging, antimicrobial effect, antioxidant activity, anthocyanin activity.

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1. INTRODUCTION

Traditional packaging systems are primarily designed to perform fundamental functions such as product protection, containment, transportation, and communication between manufacturer and consumer. These conventional materials protect food products from environmental factors such as light, temperature, pressure, humidity, microorganisms, and gaseous emissions, thereby maintaining their quality and safety during storage and distribution. According to the International

Packaging Institute, packaging materials—including films, bags, sachets, boxes, and trays—serve as essential components for preserving product integrity and extending shelf life. However, conventional (passive) packaging mainly relies on the intrinsic barrier properties of the materials used, offering limited functionality beyond basic protection (De Jong et al., 2005; Marsh & Bugusu, 2007; Yam et al., 2005).

In recent years, the growing consumer demand for safer, fresher, and more sustainable foods has led to the development of active and intelligent packaging systems. Active packaging modifies the internal environment of the packaged food to prolong freshness or prevent spoilage, while intelligent packaging involves responsive systems capable of monitoring the condition of the product or its surroundings. These smart systems detect changes in pH, temperature, or gas composition and can communicate this information through visible indicators, thereby improving food safety and traceability throughout the supply chain (Biji et al., 2015; De Jong et al., 2005; Dobrucka & Cierpiszewski, 2014; Kerry et al., 2006; Mahalik, 2009; Marsh & Bugusu, 2007; Yam et al., 2005).

Natural polymer-based hydrogels, particularly those synthesized from materials such as gelatin, starch, and cellulose, have been widely used in intelligent food packaging due to their biocompatibility, biodegradability, and low toxicity. These hydrogels possess dynamic responsiveness to various stimuli—such as water, pH, heat, light, or magnetic fields—enabling them to function as sensors, adsorbents, and carriers in diverse scientific and industrial applications. Moreover, they have been successfully employed in catalysis, adsorption processes (N. Sahiner & Alpaslan, 2014), wound dressings (M. Sahiner et al., 2014), drug delivery systems, microbiological media (Alpaslan et al., 2016), biosensors, and tissue engineering (Shoji et al., 1999), highlighting their versatility and safety for food contact materials (Bot et al., 1996; Kathuria et al., 2009)

To enhance the functional and mechanical performance of such hydrogels, natural additives and organic acids are frequently incorporated into polymer matrices. Citric acid acts as a natural cross-linker and plasticizer, improving the mechanical strength, flexibility, and stability of the composite material (Demitri et al., 2008; Ghanbarzadeh et al., 2011; Rocha-García et al., 2017). Similarly, plant-derived pigments, particularly those obtained from Rosa species, provide antioxidant, antimicrobial, and pH-sensitive colorimetric properties, enabling real-time monitoring of food freshness (Heredia et al., 1998; N. Kumar et al., 2008; J. H. Lee et al., 2011; Shen et al., 2007; Shoji et al., 1999) (16-20). Members of the Rosaceae family are widely utilized in food additives and nutraceuticals due to their anthocyanin-rich composition (Fossen et al., 1998; Giusti & Wrolstad, 2001; Hurtado et al., 2009), which undergoes distinct color transitions in response to environmental pH variations associated with food spoilage (Bondre et al., 2012; Gordillo et al., 2012; N. Kumar et al., 2008; J. H. Lee et al., 2011; Shen et al., 2007; Shoji et al., 1999).

Considering these aspects, the present study focuses on the design, synthesis, and characterization of a novel smart hydrogel film, p(Gelatin-co-DMAAm)/CA-RE, based on gelatin, N,N-dimethylacrylamide (DMAAm), citric acid (CA), and Rosa extract (RE). The hydrogel was synthesized via redox polymerization and evaluated for its morphological, structural, thermal, swelling, pH-responsive, antimicrobial, antioxidant, and colorimetric properties. *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* were selected as model microorganisms to assess antimicrobial performance. Furthermore, the colorimetric response of p(Gelatin-co-DMAAm)/CA-RE was examined in pasteurized milk and cheese samples to evaluate its practical potential as an innovative, eco-friendly, and real-time quality monitoring material for intelligent food packaging applications.

2. MATERIAL AND METHOD

2.1. Material

Natural polymer-based hydrogels, particularly those synthesized. Fresh *Rosa* petals Analytical-grade reagents of the highest available purity were used in this study. N,N-dimethylacrylamide (DMAAm, 99%), gelatin (type A, 99%), N,N′-methylenebisacrylamide (MBA, 99%), ethanol, sodium hydroxide (NaOH), and hydrochloric acid (HCl, 36.5–38% v/v) were supplied by Sigma-Aldrich (USA). Ammonium persulfate (APS, 98%) and N,N,N′,N′-tetramethylethylenediamine (TEMED) were obtained from Merck (Germany). All chemicals were utilized as received, without further purification. Rosa extract **(**RE) was prepared from freshly collected Rosa species obtained from local suppliers in Van, Türkiye. Pasteurized whole milk and cheese samples were also sourced from local markets for application studies. Deionized water (DI, 18.2 MΩ·cm) produced by a Millipore Direct-Q3UV system was used in all experiments.

2.2. Solvent extraction from Rosa

Fresh *Rosa* petals (200 g) were finely ground and extracted using deionized water (DI) under continuous stirring at room temperature to obtain the aqueous pigment solution. The resulting mixture was filtered to remove insoluble residues, and the filtrate was subsequently concentrated using a rotary evaporator until the volume was reduced to approximately 30% (v/v) of the initial

extract. The concentrated Rosa extract (RE) was then stored at 4 °C in amber glass containers to prevent photodegradation and oxidation until further use in antioxidant, antimicrobial, and anthocyanin analysis (Abou-Arab et al., 2011).

2.3. Synthesis and preparations of the hydrogels

The p(Gelatin-co-DMAA m)/CA-RE hydrogels were synthesized via a redox polymerization technique. Briefly, 2 mL (0.019M) of N,N-dimethylacrylamide (DMAAm) was blended with 2 g of gelatin in 5 mL of deionized (DI) water under continuous stirring until a homogeneous solution was obtained. Subsequently, 5 mL of Rosa extract (RE) was incorporated into the reaction mixture to enable the immobilization of natural pigments within the polymeric network. To initiate crosslinking, N,N′-methylenebisacrylamide (MBA) was added as a crosslinker at 0.25 mol% relative to the total monomer content. The mixture was then vortexed thoroughly to ensure uniform dispersion. N,N,N′,N′-tetramethylethylene diamine (TEMED, 60 µL) was introduced as an accelerator, followed by the addition of the ammonium persulfate (APS) initiator solution (1 mol% with respect to total monomer concentration) prepared in 100 µL DI water. The resulting solution was vigorously stirred and subsequently poured into plastic Petri dishes (10 mm diameter). Polymerization and crosslinking reactions proceeded under ambient conditions until solid hydrogel films were formed. The resulting films were immersed in DI water, which was refreshed every 8 h for 24 h to remove unreacted monomers and residual impurities. Finally, the purified hydrogels were oven-dried at 40 °C until a constant weight was achieved and then stored at 4 °C for subsequent characterization and application studies. A schematic representation of the hydrogel preparation process is shown in Figure 1.



Figure 1: Hydrogels preparation.

2.4. Swelling behavior of hydrogels

The swelling properties of p(Gelatin-co-DMAAm)/CA-RE hydrogels were determined by a gravimetric method. Pre-weighed dried hydrogel samples were immersed in deionized water at room temperature, and the mass of the swollen samples was recorded at predetermined time intervals. Before each weighing, the surface water on the hydrogels was gently removed using filter paper to ensure accurate measurement. After weighing, the samples were returned to the same swelling medium to continue hydration. The equilibrium swelling ratio (S_max%) was determined after 24 h, corresponding to the point at which no further weight increase was observed. Each measurement was performed in triplicate, and the mean values were reported to ensure reproducibility. The percent swelling degree (S%) as the function time was calculated as

$$S\% = \frac{M_t - M_0}{M_0} \times 100 \qquad \text{(Eq. 1)}$$

Where, M_o and M_t are the initial mass and the mass of hydrogels at time t, respectively (Alpaslan et al., 2016).

2.5. Thermo-gravimetric, FT-IR, and Scanning Electron Microscopy analysis of hydrogels

The thermal stability of the hydrogels and p(Gelatin-co-DMAAm)/CA-RE composites was analyzed using a thermogravimetric analyzer (TG/DSC, Setaram Labsys Evo 1600, France). Approximately 4–6 mg of each sample was placed in ceramic crucibles and heated from 50 to 1000 °C under an argon atmosphere (flow rate: 100 mL min^{-1}) at a constant heating rate of 10 °C min^{-1}. FT-IR spectra of DMAAm and Gelatin-based hydrogels were recorded using a Fourier Transform

Infrared Spectrometer (Thermo Nicolet iS10, USA) equipped with an ATR accessory, in the wavenumber range of 4000–650 cm^{-1} with a resolution of 4 cm^{-1}. The surface morphology of the hydrogels was examined using a field emission scanning electron microscope (FESEM-EDX, Zeiss Ultra, Germany) to observe pore structures and morphological differences among the composites.

2.6. The LC-MS/MS (Q Exactive) analysis

The chemical composition of the RE extract was analyzed using a hybrid quadrupole–orbitrap LC–MS/MS system (Thermo Q Exactive, USA). Separation was achieved on a Hypersil Gold capillary column (50 × 2.1 mm) using helium as the carrier gas at a flow rate of 0.3 mL min^{-1}. The individual components were identified based on analytical standards, including antimicrobial, antioxidant, and anthocyanin compounds.

2.7. Mechanical Strength Test

Dynamic mechanical analysis (DMA) was conducted using a Mettler Toledo Tritone Technology (UK) instrument to evaluate the viscoelastic properties of the hydrogels and p(Gelatin-co-DMAAm)/CA-RE. Samples (1 mm thickness, 4 mm diameter) were analyzed at a frequency of 1 Hz and a heating rate of 5 °C min^{-1} over the temperature range of 30–80 °C. The storage modulus (E′) and loss modulus (E″) were recorded as functions of temperature.

2.8. Antioxidant properties of hydrogels

Thee antioxidant activities of the hydrogels were determined using the Folin-Ciocalteu (Singleton, 1999), ABTS (Childs & Bardsley, 1975)(29) methods following the protocols described in the literature.

2.9. Antimicrobial properties of hydrogels

The antimicrobial activities of the hydrogels were evaluated using the agar dilution and agar dilution and spreading plate method, as described in the literature (Alpaslan et al., 2016). Three bacterial strains were used: *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538) as Gram-positive, and *Escherichia coli* (ATCC 8739) as a Gram-negative representative. Bacterial cultures were maintained on nutrient agar slants at 4 °C and sub-cultured before each assay to ensure viability.

2.10. Anthocyanin properties of hydrogels

Anthocyanin contents of p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA, and p(Gelatin-co-DMAAm)/CA-RE hydrogels were determined according to previously reported method (Brouillard & Delaporte, 1977).

2.11. Colorimetric properties of hydrogels

The pH-dependent color changes of p(Gelatin-co-DMAAm)/CA-RE and control hydrogels were studied according to the method described in the literature (Wrolstad et al., 2005). The color variations were monitored using a UV–Vis spectrophotometer (Analytik Jena Specord S600, Germany) within the 380–770 nm range (Δλ = 2 nm), with distilled water as reference and p(Gelatin-co-DMAAm)/CA as blank. Color parameters were calculated in the CIELab system (L*, a*, b*, C*, h*) using Croma Lab software based on the CIE 1976 standards and the D65 illuminant.:

$$\mathrm{In}\,H_{k-I}^{ki} = \mathrm{In}^{-} + H^{+} \quad \text{(Eq. 2)}$$

$$C^{*} = \left(a^{2} + b^{2}\right)^{\frac{1}{2}} \quad \text{(Eq. 3)}$$

$$h = \arctan\left(\frac{b^{*}}{a^{*}}\right) \quad \text{(Eq. 4)}$$

Color differences (ΔE) between samples at various pH values (1–12) were calculated using the Euclidean distance formula:;

$$\Delta E_{ab} = \left(\left(\Delta L^{*}\right)^{2} + \left(\Delta a^{*}\right)^{2} + \left(\Delta b^{*}\right)^{2}\right)^{\frac{1}{2}} \quad \text{(Eq. 5)}$$

2.12. Applications to Real Samples

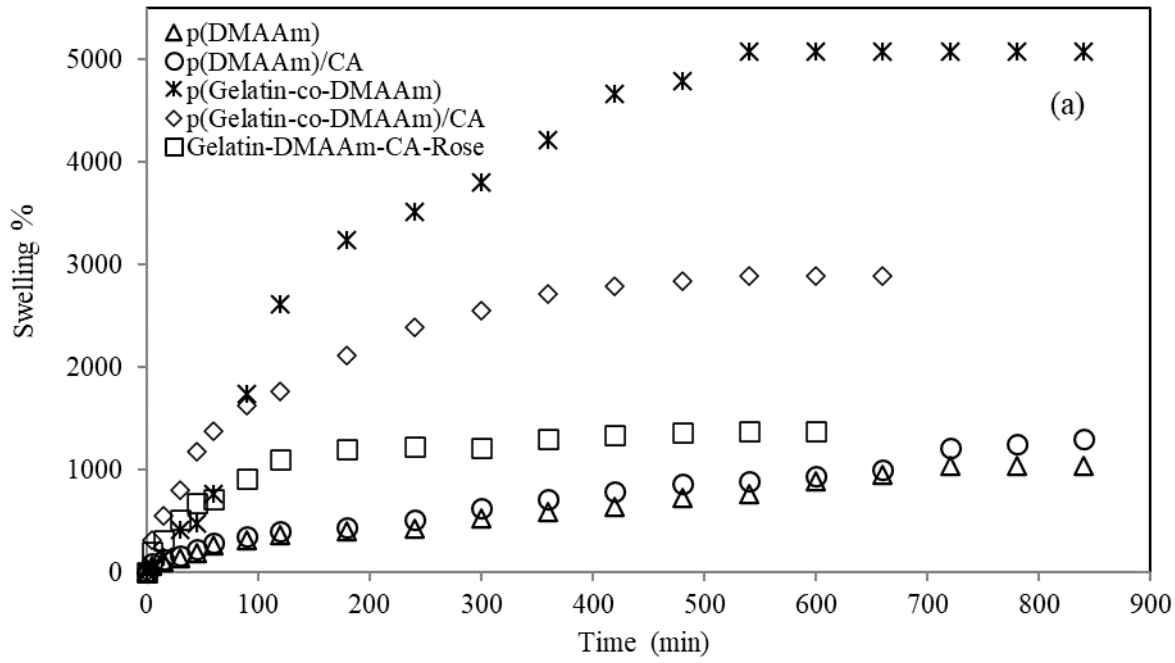
The practical applicability of the hydrogels was tested using whole milk and cheese as real samples. Both samples were intentionally inoculated with *Staphylococcus aureus, Escherichia coli,* and *Bacillus cereus*. Circular hydrogel discs (10 mm diameter) of p(Gelatin-co-

DMAAm)/CA-RE were placed onto 50 mL of milk and 100 g of cheese samples. Treated samples were incubated at 4, 10, 20, 30, and 40 °C for 48 h (Memmert UN 110, Germany). After incubation, the CIELab color parameters (L*, a*, b*, C*, h*) were measured, and untreated samples served as controls.

3. RESULTS AND DISCUSSION

3.1. Characterization of the synthesized hydrogels

The moisture content of a given food product is one of the key parameters determining its shelf life and structural stability. It is well established that polymers synthesized solely from gelatin and DMAAm exhibit limited water resistance and mechanical strength, resulting in easy dissolution in aqueous environments. To overcome these drawbacks, p(Gelatin-co-DMAAm)/CA-RE hydrogels were synthesized by incorporating the biocompatible crosslinker MBA and citric acid (CA) into the polymeric matrix during synthesis (Bylka et al., 2004; K. Y. Lee et al., 2000; Reddy & Yang, 2010). This modification significantly improved both the mechanical integrity and the water resistance of the hydrogels. The swelling degree of p(Gelatin-co-DMAAm)/CA-RE hydrogels reached approximately 941%, while their volume and weight increased by 16% and 6%, respectively, upon water absorption. Notably, the water resistance of p(Gelatin-co-DMAAm)/CA-RE was found to be higher than that of p(DMAAm) and p(DMAAm)/CA hydrogels, both in deionized water and across a wide pH range (Alpaslan et al., 2016; Biji et al., 2015; Dobrucka & Cierpiszewski, 2014, 2014; Kathuria et al., 2009; Kerry et al., 2006; Mahalik, 2009; Marsh & Bugusu, 2007; M. Sahiner et al., 2014; N. Sahiner & Alpaslan, 2014; Yam et al., 2005). Furthermore, the films prepared from p(Gelatin-co-DMAAm)/CA-RE remained stable and non-dissolved in aqueous media for over five months, demonstrating their superior long-term structural durability in aqueous media more than five months



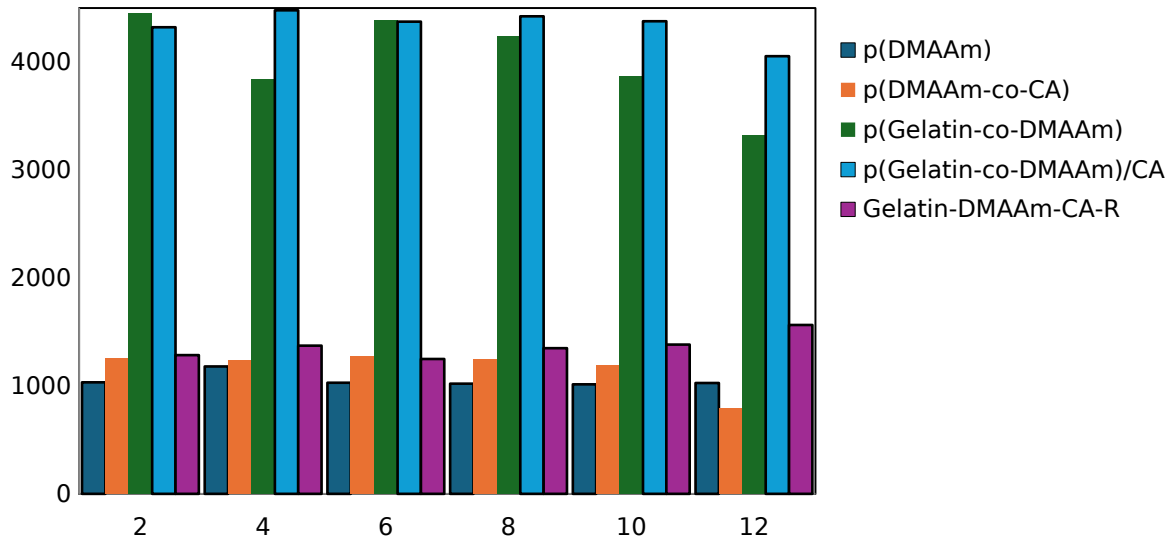


Figure 2: Percent swelling degree in hydrogels with time in **(a)** DI water, (S% vs time) p(DMAAm), p(DMAAm)/CA, p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA and p(Gelatin-co-DMAAm)/CA-RE.

The FT-IR spectra of DMAAm, citric acid, gelatin, p(DMAAm), p(Gelatin-co-DMAAm)/CA, and p(Gelatin-co-DMAAm)/CA-RE are presented in Figure 3. The characteristic absorption bands of p(DMAAm) and p(Gelatin-co-DMAAm)/CA were previously reported by Alpaslan et al., confirming the successful copolymerization of gelatin and DMAAm. Upon incorporation of Rosa extract (RE) into the p(Gelatin-co-DMAAm)/CA matrix, distinct spectral changes were observed, indicating successful physical and chemical interactions between the polymeric network and the extract. Specifically, the broad absorption band attributed to –OH and –NH stretching vibrations (3000–3500 cm^{-1}) became less intense, while the peak around 2912 cm^{-1}, corresponding to C–H stretching, increased in intensity. Furthermore, the appearance of new peaks within the 1600–2500 cm^{-1} region and the disappearance of several peaks between 1000–1500 cm^{-1} suggest structural rearrangements within the hydrogel network. These spectral modifications collectively confirm the formation of new hydrogen-bonding interactions among p(DMAAm), p(Gelatin-co-DMAAm)/CA, and p(Gelatin-co-DMAAm)/CA-RE, demonstrating the successful incorporation of RE into the polymer matrix and potential enhancement of intermolecular interactions.

The thermal stability of the synthesized hydrogels was evaluated using a TGA/DSC analyzer, and the results are presented in Figure 4. The thermal degradation behavior of p(DMAAm) and p(Gelatin-co-DMAAm)/CA has been previously discussed by Alpaslan et al. In the present study, the incorporation of Rosa extract (RE) into the p(Gelatin-co-DMAAm)/CA structure significantly influenced the thermal properties of the resulting hydrogel. The TGA curve of p(Gelatin-co-DMAAm)/CA-RE exhibited two distinct weight-loss stages. The first stage, with an approximate mass loss of 4.6%, occurred between 100°C and 217°C, which was primarily attributed to the evaporation of surface and bound water molecules. The second major degradation stage took place between 217°C and 527°C, with an additional mass loss of about 64.5%, corresponding to the decomposition of the polymer backbone and organic constituents of the Rosa extract. The total mass loss reached approximately 69.1% at the end of the thermal process. These results clearly indicate that the incorporation of RE enhanced the thermal resistance of p(Gelatin-co-DMAAm)/CA-RE hydrogels compared to the control samples. The observed improvement in thermal stability can be attributed to the hydrogen bonding and intermolecular interactions between the poly(Gelatin-co-DMAAm) network and the bioactive compounds present in the extract, which strengthened the structural integrity of the hydrogel matrix.

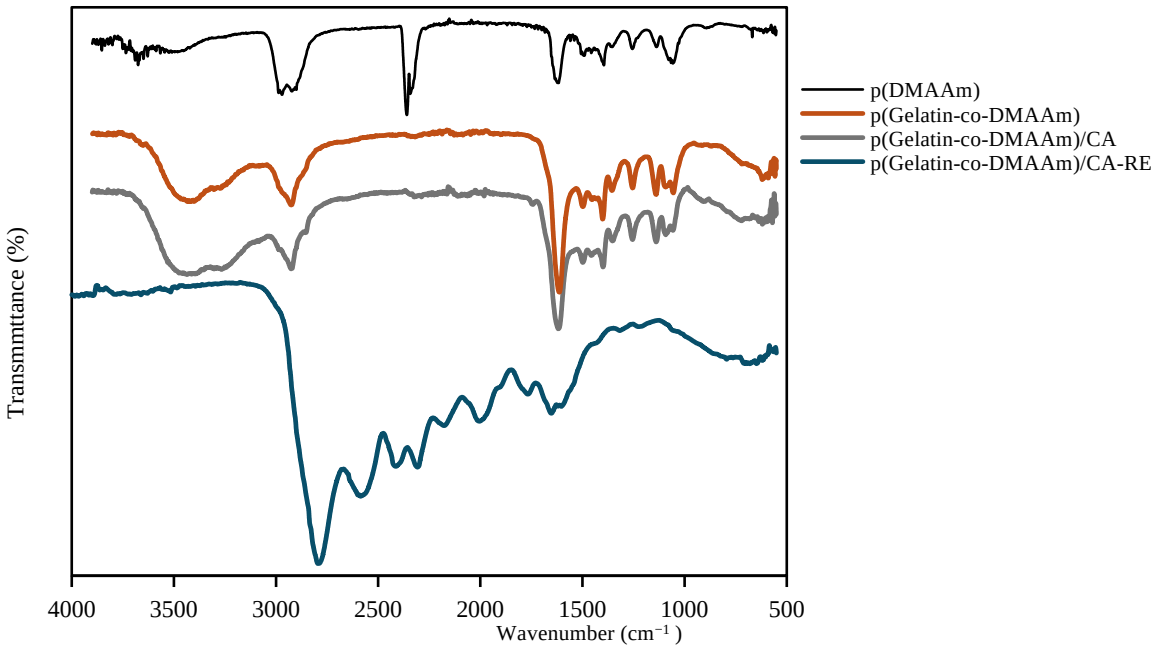


Figure 3. FT-IR analysis of p(DMAAm), p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA and p(Gelatin-co-DMAAm)/CA-RE. The X axis is temperature (°C) and the Y axis is thermal gravimetric analysis.

The surface morphologies of the synthesized hydrogels were examined using Scanning Electron Microscopy (SEM), as shown in Figure 5. The p(Gelatin-co-DMAAm)/CA hydrogel exhibited a rough and wrinkled surface, indicating a heterogeneous polymeric structure. In contrast, the p(Gelatin-co-DMAAm)/CA-RE hydrogel displayed a relatively smoother and more compact morphology, although some undissolved aggregates were still observed within the matrix. The incorporation of Rosa extract (RE) appeared to promote partial aggregation and structural reorganization within the hydrogel network. This modification likely enhanced polymer chain interactions and contributed to the formation of a denser, more uniform surface compared to the unmodified p(Gelatin-co-DMAAm)/CA film. The observed morphological differences suggest that RE acted as a stabilizing and homogenizing agent, facilitating improved compatibility and uniform dispersion within the polymer matrix.

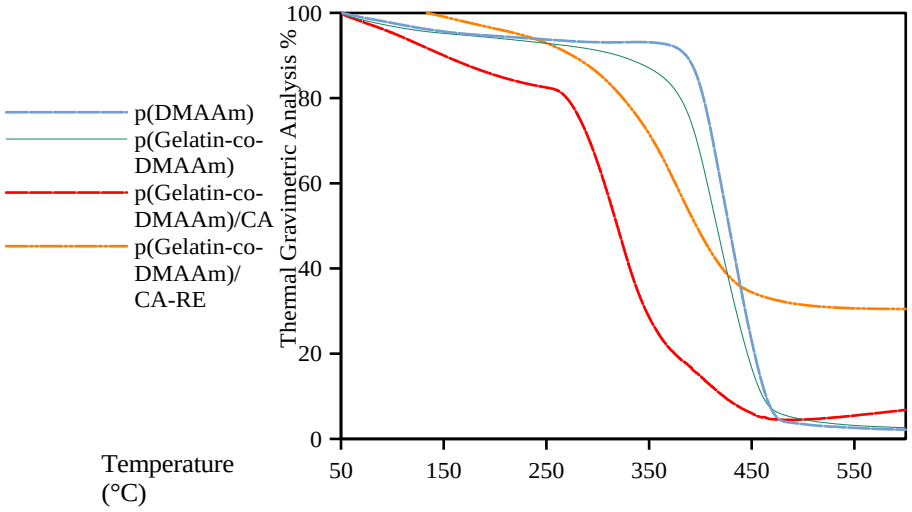


Figure 4. Thermogravimetric analysis of p(DMAAm), p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA and p(Gelatin-co-DMAAm)/CA-RE.

3.2. The LC-MS/MS (Q Exactive) analysis of the RE

The Rosa extract was analyzed using Liquid Chromatography–Mass Spectrometry (LC–MS/MS, Q Exactive) to identify its bioactive constituents responsible for the functional properties of the synthesized hydrogels. The analysis revealed that Rosa contains a variety of phenolic acids, flavonoids, terpenes, and anthocyanin compounds that exhibit potent antioxidant, antimicrobial, and colorimetric activities. Specifically, the identified compounds included Rutin, Quercetin, Myricetin, Quercitrin, Kaempferol, Gallic Acid, Citronellol, Geraniol, Linalool, Nerol, Nonadecane, Coumaric Acid Hexoside Isomer-1, (−)-Epicatechin, (+)-Catechin, Peonidin-3-glucoside, Eugenol, Methyl Eugenol, Phenylethyl Alcohol, α-Pinene, and α-Terpineol. Among these, gallic acid, quercetin, coumaric acid, catechins, and myricetin are well known for their antioxidant and antimicrobial potential, while cyanidin, delphinidin, and pelargonidin derivatives contribute significantly to anthocyanin-based colorimetric activity. The composition of rosa extract as stated in the literature; Phenolic acids (Gallic acid, Coumaric acid derivatives) 20–30% of total phenolics, Flavonoids (Quercetin, Rutin, Quercitrin, Myricetin, Kaempferol, (+)-Catechin, (−)-Epicatechin) 40–55% of total phenolics, Anthocyanins (Cyanidin-, Delphinidin-, Pelargonidin-3-glucoside) derivatives 5–15% of total phenolics, Monoterpene alcohols, (Citronellol, Geraniol, Nerol) 50–70% of volatile oil fraction), volatile compounds (Linalool, Phenylethyl alcohol, α-Terpineol, Eugenol, Methyl eugenol) 10–20% of volatile oil fraction. (Bylka et al., 2004; Khezerlou et al., 2025; Koponen et al., 2007; Shameh et al., 2018; Xiao et al., 2006).

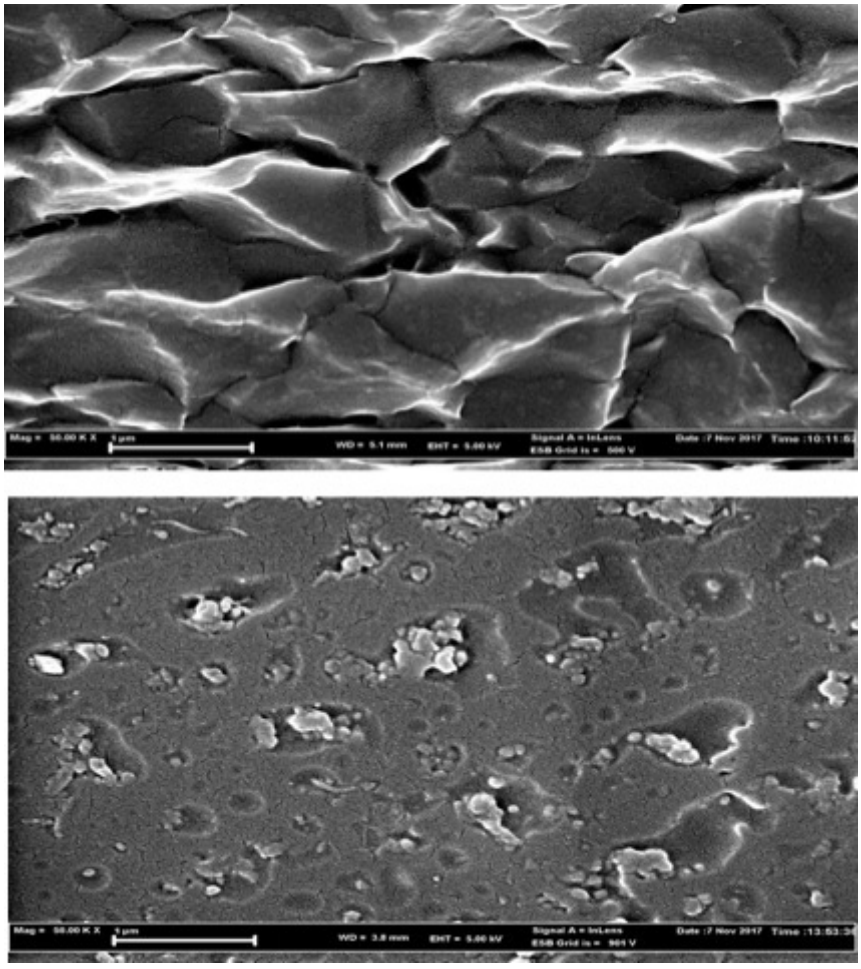


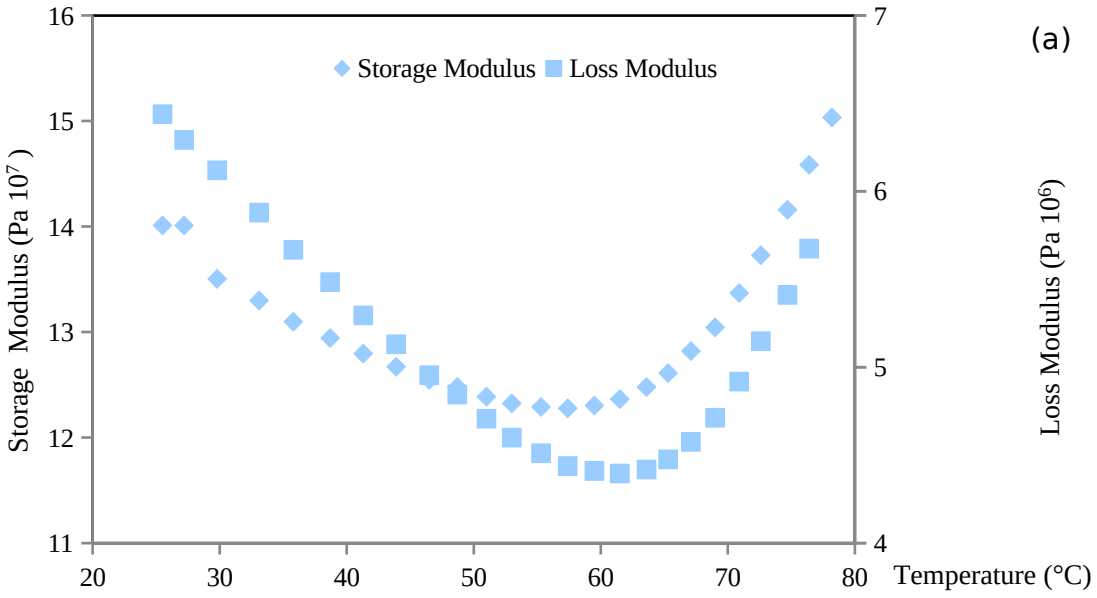
Figure 5. SEM images of p(Gelatin-co-DMAAm)/CA and p(Gelatin-co-DMAAm)/CA-RE.

Furthermore, the presence of copigmentation cofactors—such as phenolic acids, flavonoids, and particularly flavonol and flavone derivatives—enhances the stability and color intensity of the anthocyanins through copigmentation mechanisms (Baranac et al., 1996; Boulton, 2001). These compounds collectively endow Rosa extract with multifunctional bioactivity, making it an excellent

natural additive for pH-responsive smart hydrogel-based food packaging materials.

3.3. Application of mechanical strength test

The mechanical properties of the synthesized hydrogels were evaluated using Dynamic Mechanical Analysis (DMA) to assess their viscoelastic behavior, as shown in Figure 6. The storage modulus (E′), representing the elastic energy stored during deformation, was found to decrease at lower temperatures, indicating that molecular chains possess greater mobility and free volume under such conditions (Menard & Menard, 2020). The p(Gelatin-co-DMAAm)/CA hydrogel exhibited lower storage modulus values compared to p(Gelatin-co-DMAAm)/CA-RE, suggesting that it is more flexible and deformable. As shown in Figure 6a, the storage modulus of p(Gelatin-co-DMAAm)/CA increased gradually between 40°C and 60°C, implying reduced chain mobility within this temperature range. Conversely, for p(Gelatin-co-DMAAm)/CA-RE (Figure 6b), the storage modulus significantly increased between 50 °C and 70 °C, indicating an enhancement in mechanical strength and rigidity upon incorporation of Rosa extract (RE).Hydrogels exhibiting broad loss modulus (E″) peaks typically possess a non-homogeneous crosslinking structure, whereas a sharper and more vertical curve**.** denotes a uniform crosslinking density. The loss modulus analysis demonstrated that p(Gelatin-co-DMAAm)/CA-RE exhibited a lower tendency for energy dissipation, confirming its improved internal structural integrity. The intrinsic mechanical weakness of gelatin was effectively compensated by the incorporation of DMAAm, citric acid (CA), and MBA as comonomer, crosslinker, and reinforcement agents, respectively. Furthermore, the inclusion of Rosa extract acted as a molecular bridge, forming hydrophilic interactions with DMAAm and gelatin chains, thereby improving both mechanical strength and flexibility of the hydrogel (Koponen et al., 2007; Loetscher et al., 2013; Menard & Menard, 2020).



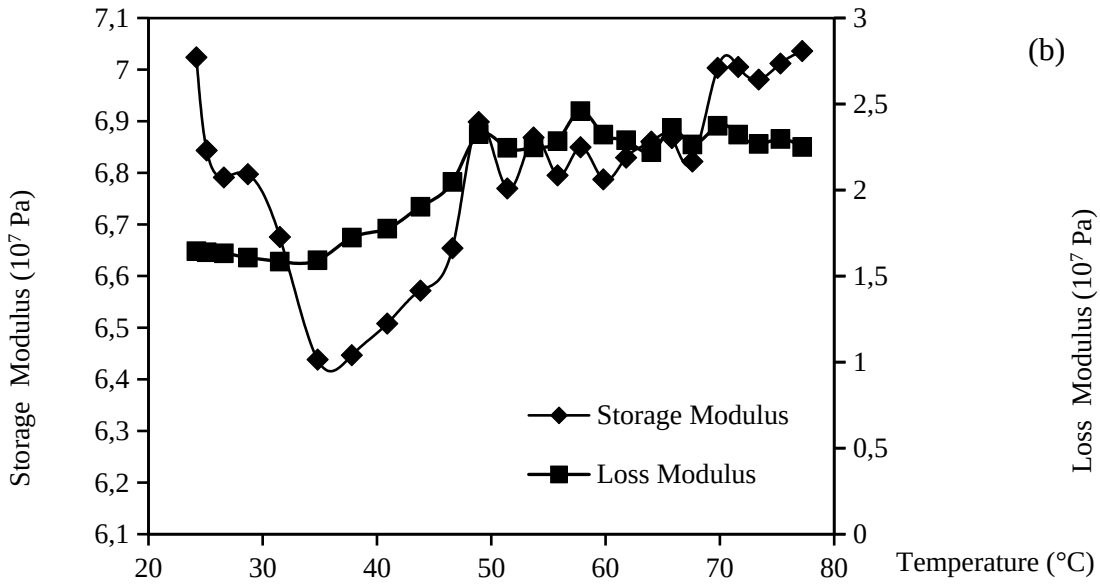


Figure 6. DMA analysis of (a) p(Gelatin-co-DMAAm)/CA, (b) p(Gelatin-co-DMAAm)/CA-RE.

3.4. Antioxidant properties of the hydrogels

The antioxidant activity of RE, p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA, and p(Gelatin-co-DMAAm)/CA-RE was evaluated using the TEAC (Trolox Equivalent Antioxidant Capacity) and ABTS methods. The gallic acid equivalent values of the hydrogels are presented in Table 1. The results indicate that both RE and the hydrogels exhibit substantial antioxidant activity, comparable to previously reported values in the literature (Angelov et al., 2014; De Jong et al., 2005; Guerrero C et al., 2011; Ignat et al., 2011; Ilyasoğlu, 2014; Loetscher et al., 2013; Özkan et al., 2004; Öztürk Yılmaz & others, 2011; Roman et al., 2013; Schmitzer et al., 2010; Shen et al., 2007; Su et al., 2007; Vinokur et al., 2006; Yi et al., 2007). A significant portion of this activity can be attributed to the phenolic compounds present in the extract, as confirmed by Q Exactive analysis of the hydrogel structures. Consequently, the release of antioxidant substances from p(Gelatin-co-DMAAm)/CA-RE can effectively mitigate the harmful effects of free radicals.

Table 1. Comparison of total phenol content (gallic acid equivalent phenol content) and TEAC (Trolox equivalent antioxidant capacity) values of RE, p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA, p(Gelatin-co-DMAAm)/CA-RE and similar RE appeared in the literature.

	TEAC Value (mM Trolox equivalent g^{-1})
RE	2605
p(Gelatin-co-DMAAm)	93
p(Gelatin-co-DMAAm)/CA	310
p(Gelatin-co-DMAAm)/CA-RE	8143
	Total phenol (mg g^{-1})
RE	4186
p(Gelatin-co-DMAAm)	93
p(Gelatin-co-DMAAm)/CA	310
p(Gelatin-co-DMAAm)/CA-RE	881

3.5. Antimicrobial properties of the hydrogels

The antimicrobial activity of hydrogels is a critical feature, encompassing both the production of bioactive compounds and the suppression of pathogenic microorganisms. The antimicrobial effects of RE and p(Gelatin-co-DMAAm)/CA-RE were evaluated against three common bacterial strains, with the results summarized in Table 2. p(Gelatin-co-DMAAm)/CA-RE was exposed to the bacteria for 72 h, and the Minimal Inhibitory Concentration (MIC) was determined as 100 mg mL^{-1} for

Staphylococcus aureus and >100 mg mL^{-1} for *Escherichia coli* and *Bacillus cereus*. The Minimal Bactericidal Concentration (MBC) values for RE, p(Gelatin-co-DMAAm)/CA, and p(Gelatin-co-DMAAm)/CA-RE were all above 200 mg mL^{-1} for the tested strains. These results indicate that the hydrogel exhibits significant antimicrobial activity, consistent with previous reports (Kumarasamy et al., 2002; Özkan et al., 2004; Öztürk Yılmaz & others, 2011; Yi et al., 2007). These results indicate that the hydrogel exhibits significant antimicrobial activity, consistent with previous reports. The high acidity and phenolic content of Rosa extracts likely contribute to the observed inhibitory effects, suggesting potential applications in food preservation, agriculture, and traditional medicine (Loetscher et al., 2013). When applied appropriately, p(Gelatin-co-DMAAm)/CA-RE can reduce or inhibit microbial growth in environmental and food systems. Microbial and chemical deterioration in foods often results from complex interactions involving temperature, water content, pH, and other environmental factors, all of which can be catalyzed by microbial proliferation (Huis In'T Veld, 1996; Koponen et al., 2007). Moreover, the hydrogel's colorimetric response provides a visual indication of environmental pH changes, allowing real-time monitoring of potential microbial activity and ensuring enhanced food safety.

Table 2. Comparison of the MIC and MIB values of RE, p(Gelatin-co-DMAAm)/CA and p(Gelatin-co-DMAAm)/CA-RE and RE by diffusion method appeared in the literature (Agar Well Diffusion[a] (mm), Micro Dilution[b]).

	MIC (mL mL^{-1} -mg mg^{-1})		
Microorganisms	*Rose*	*p(Gelatin-DMAAm)/CA*	*p(Gelatin-DMAAm)/CA-R*
Escherichia coli	100	>100	>100
Bacillus subtilis	100	>100	>100
Staphylococcus aureus	100	>100	>100
	MIB (mL mL^{-1} -mg mg^{-1})		
Escherichia coli	>200	>200	>200
Bacillus subtilis	>200	>200	>200
Staphylococcus aureus	>200	>200	>200

3.6. Anthocyanin properties of hydrogels

The total anthocyanin content of RE and p(Gelatin-co-DMAAm)/CA-RE was quantified for cyanidin, delphinidin, and pelargonidin, as presented in Table 3. Both RE and the hydrogels exhibit robust anthocyanin activity, consistent with values reported in the literature (Denny et al., 2008; Giusti & Wrolstad, 2001; Guerrero C et al., 2011; Jaakola et al., 2002; Koponen et al., 2007; S. Kumar et al., 2013; J. H. Lee et al., 2011; Schmitzer et al., 2010; Shen et al., 2007; Shoji et al., 1999; Zupka et al., 2015). Anthocyanin stability, however, is highly sensitive to environmental factors such as temperature fluctuations, light exposure, oxygen levels, pH, and the presence of stabilizing or destabilizing agents including citric acid, ascorbic acid, and metal ions. Importantly, the p(Gelatin-co-DMAAm)/CA-RE hydrogel leverages the inherent colorimetric properties of anthocyanins, enabling it to function as a visual pH indicator. This feature allows the hydrogel to serve as a smart sensor, providing real-time monitoring of environmental changes that may signal microbial growth or food spoilage, thereby enhancing its potential applications in intelligent food packaging systems.

Table 3. Comparison of anthocyanin values of RE and *Rosa* appeared in the literature.

Anthocyanin (mg/L)	**Rosa extract**	**p(Gelatin-co-DMAAm)/CA-Re**
Pelargonidin	350	391
Peonidin	189	211
Cyanidin	1045	1168

3.7. Determination of colorimetric properties of the smart hydrogels

The colorimetric behavior of anthocyanin copigmentation, interpreted via the CIELab color space, provides valuable insights by enabling both quantitative and qualitative evaluation of color changes. It has been well established that pH, copigment structure, and concentration significantly influence the copigmentation process, resulting in distinct absolute and relative color variations in anthocyanin solutions (Gordillo et al., 2012, 2014; Martínez et al., 2001) (24, 64, 65). Table 4 summarizes the chromatic parameters of p(Gelatin-co-DMAAm)/CA-RE measured according to the CIELab system, where each color is expressed through lightness (L*), red–green axis

(a*), and yellow–blue axis (b*) (Alpaslan, 2019; Bobelyn et al., 2006; Heredia et al., 1998). To evaluate pH responsiveness, the hydrogel was immersed in aqueous solutions ranging from pH 1 to 12 for 5 minutes. A rapid and visually detectable color transition occurred immediately upon contact. According to the calculated colorimetric differences, the hydrogel exhibited the highest total color change between pH 7–8 (ΔE* = 42.19), exceeding the ~3-unit threshold perceivable to the human eye (Gordillo et al., 2012). Notably, the strong shift in chroma at this interval (ΔC* = 351.83) and the marked hue variation (ΔH* = –122.55) indicate substantial molecular reorganization of the p(Gelatin-co-DMAAm)/CA-RE in response to pH. Across the entire pH range, ΔE* values varied from 5.74 (pH 2–3) to 68.70 (pH 12–1), demonstrating that the hydrogel remains highly responsive even under moderate pH fluctuations. The negative a* and b* values observed at several intervals—such as pH 6–7 (a* = –6.34; b* = –38.90)—reflect clear shifts toward green and blue tones, respectively, whereas intervals such as pH 12–1 (L* = 14.34; b* = 67.12) indicate a transition to a significantly more yellow appearance. To assess performance in real food matrices, the hydrogel was also evaluated in whole milk and cheese inoculated with common spoilage and pathogenic bacteria. In whole milk, bacterial activity produced noticeable color changes, with ΔE* values of 41.78 (*S. aureus*), 40.65 (*B. subtilis*), and 36.25 *(E. coli).* The corresponding ΔC* and ΔH* values—particularly the large hue shift in *E. coli*-contaminated milk (ΔH* = 214.86)—confirm that microbial metabolism significantly alters the chemical environment surrounding the hydrogel. Even stronger chromatic responses were observed in cheese samples, where ΔE* reached 55.39 for *B. subtilis* and ΔH* values exceeded 200 for *S. aureus* and *B. subtilis*, indicating pronounced hydrogel sensitivity to biochemical changes in solid-state dairy matrices. Together, these results reveal that p(Gelatin-co-DMAAm)/CA-RE provides rapid, stable, and highly distinguishable color signals in response to both pH variations and microbiological activity (Loetscher et al., 2013).

3.8. Utilization of p(Gelatin-co-DMAAm)/CA-RE for real samples

Monitoring pH changes and microbial activity in food products is often challenging. In this study, p(Gelatin-co-DMAAm)/CA-RE was applied to perishable foods such as dairy and cheese, which are highly consumed and prone to spoilage (O.-H. Lee & Lee, 2010; Yoshida et al., 2014) (68, 69). Figure 7a illustrates the color evolution of p(Gelatin-co-DMAAm)/CA-RE when exposed to spoiling whole milk at room temperature. The hydrogel displayed similar color changes in milk contaminated with *S. aureus, E. coli,* and *B. subtilis*. No visible color change was observed during the first 8 hours; thereafter, the hydrogel gradually shifted from red to purple over 8–24 hours, with a more pronounced change after 24 hours, providing a clear visual indication of spoilage.

Table 4. Color Parameters (CIELab) L*, a*, b* measured for p(Gelatin-co-DMAAm)/CA-RE in different pH solutions.

pH intervals	L*	a*	b*	ΔE	ΔC	ΔH
1-2	2.31	3.10	-16.20	16.66	131.04	16.9
2-3	-0.25	-3.14	-4.80	5.74	50.36	2.5
3-4	-30.90	1.82	16.44	35.05	164.84	26.9
4-5	4.09	0.10	-8.29	9.25	81.66	6.6
5-6	18.88	-0.43	-2.66	19.07	26.87	0.71
6-7	-7.92	-6.34	-38.90	40.20	393.96	153.65
7-8	23.29	5.10	34.81	42.19	351.83	122.55
8-9	9.47	-1.83	19.60	21.85	151.04	22.43
9-10	-10.27	4.72	-28.45	30.61	237.36	55.51
10-11	10.63	-1.25	11.11	15.43	110.63	12.11
11-12	-33.67	1.25	17.28	37.87	173.25	29.72
12-1	14.34	-3.10	67.12	68.70	30.34	3.59

Milk spoilage is typically triggered when its temperature rises above 4–7 °C, leading to microbial proliferation and lactic acid accumulation, which decreases the pH (O.-H. Lee & Lee, 2010; Yoshida et al., 2014). The colorimetric response of p(Gelatin-co-DMAAm) /CA-RE offers a simple and effective method for detecting and monitoring such spoilage. Comparable results were obtained for milk treated with *S. aureus, E. coli, and B. subtilis* (Figure 7a), where color changes began at 10 hours and stabilized after 24 hours. Quantitative colorimetric measurements are presented in Figure 8b (Hurtado et al., 2009).

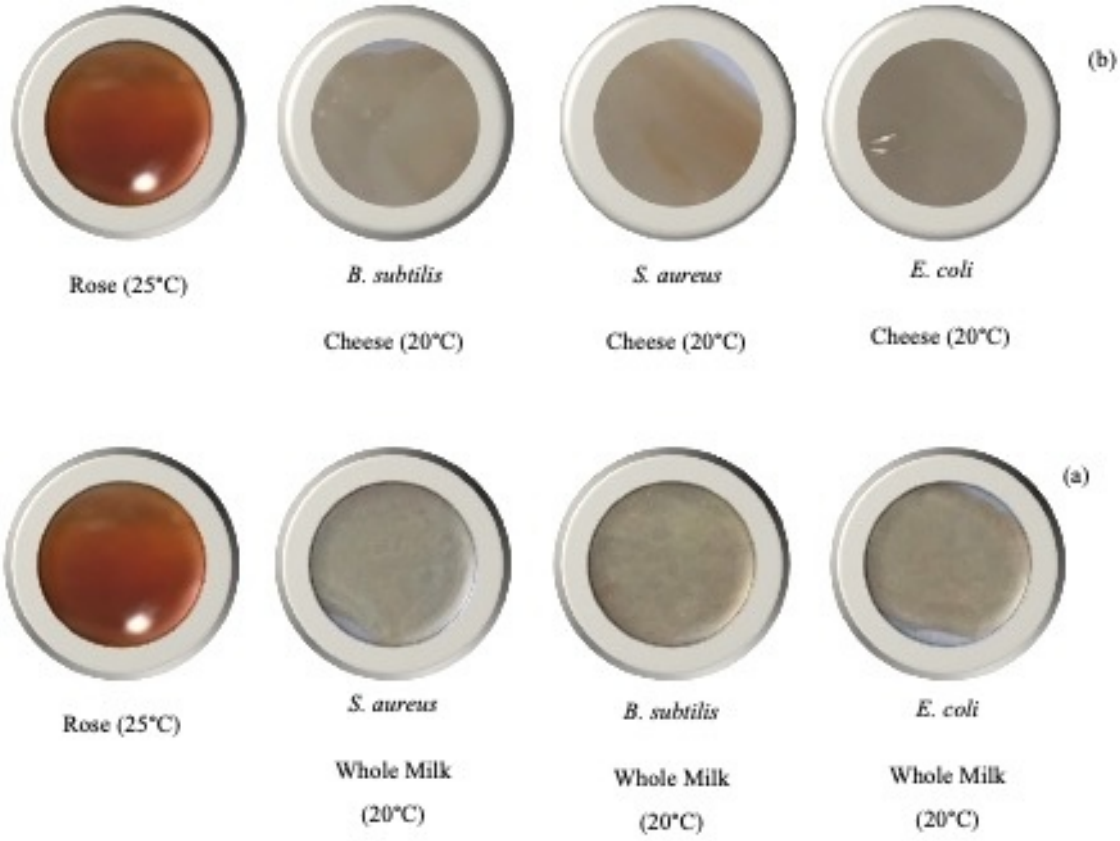


Figure 7. p(Gelatin-co-DMAAm)/CA-RE color response in contact with (a) Whole milk, Whole milk *S. aureus,* Whole milk *B. Subtilis* and Whole milk *E. coli* and (b) Chicken, Chicken *S. aureus*, Chicken *B. Subtilis* and Chicken *E. coli* at 20ºC.

Cheese, being highly perishable, is typically protected through strategies such as pH reduction, preservative addition, controlled microflora, water activity reduction, water removal, oxygen-limited packaging, and freezing. Factors including temperature, light exposure, microbial growth, and pH shifts predominantly influence its shelf life. Figure 6a shows the color change kinetics of p(Gelatin-co-DMAAm)/CA-RE applied to cheese at various temperatures (4ºC, 10ºC, 20ºC, 30ºC, and 40ºC) and in the presence of different microorganisms (*S. aureus, E. coli, and B. cereus)*. Minimal changes were observed in the first 8 hours, and detailed colorimetric values are summarized in Table 5 (Hurtado et al., 2009).

Table 5. Total color change (ΔE) parameters (CIELab) after from upon contact with whole milk and cheese of p(Gelatin-co-DMAAm)/CA-RE.

	L*	a*	b*	ΔE	ΔC	ΔH
Whole milk	25.04	23.88	19.54			
Whole milk-*S. aureus*	27.90	24.33	19.36	41.78	30.37	44.37
Whole milk-*B. subtilis*	31.54	23.89	9.32	40.65	20.64	231.77
Whole milk-*E. coli*	25.69	23.69	9.65	36.25	20.96	214.86
Cheese	25.04	23.88	19.54			
Cheese-*S. aureus*	-45.73	18.04	0.01	49.161	25.02	216.00
Cheese-*B. subtilis*	-49.53	24.65	-2.72	55.39	30.09	482.97
Cheese-*E. coli*	-0.02	20.33	1.15	20.36	27.31	-21.44

The multifunctional properties of RE—including antimicrobial, antioxidant, and anthocyanin-based colorimetric responses—contribute significantly to the performance of p(Gelatin-co-DMAAm)/CA-RE in milk and cheese. Beyond dairy products, this hydrogel shows potential for monitoring spoilage

and microbial activity in a wide range of perishable foods, including meat, vegetables, and fruits. The results highlight that p(Gelatin-co-DMAAm)/CA-RE can serve both as a quality indicator and as a preservative, providing real-time visual feedback and potentially extending the shelf life of diverse food

4. CONCLUSION

The bio-based hydrogel p(Gelatin-co-DMAAm)/ CA-RE exhibits a compelling suite of functional properties that position it as a next-generation smart food-packaging material. The incorporation of Rosa extract significantly amplifies its performance by imparting robust antioxidant capacity, broad-spectrum antimicrobial activity, and highly responsive anthocyanin-based colorimetric sensing. Owing to its high polyphenol content, the hydrogel effectively suppresses oxidative degradation, thereby delaying quality loss in perishable foods. Simultaneously, its strong inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus* in both milk and cheese underscore its potential to enhance microbial safety in real food matrices. T

The anthocyanin components enable an exceptionally sensitive and rapid pH-responsive color transition, with total color differences reaching ΔE* = 68.70 across pH 1–12—far beyond the threshold easily detectable by the human eye. This immediate visual shift allows real-time recognition of biochemical changes associated with spoilage, including acidification, proteolysis, and microbial proliferation. Application tests in milk and cheese unequivocally demonstrated that the hydrogel's colorimetric response directly tracks shifts in pH and microbial activity, providing a reliable and continuous visual indicator of freshness loss during storage.

Taken together, these results firmly establish p(Gelatin-co-DMAAm)/CA-RE as a biodegradable, multifunctional, and highly responsive active packaging platform. Its simultaneous roles—antioxidant protection, antimicrobial defense, and real-time spoilage detection—underscore its strong potential for extending shelf life, improving food safety, and delivering consumer-friendly visible quality monitoring in a wide range of perishable foods..

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