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Synthesis, Characterization and Modification of Novel Food Packaging Material from Dimethyl acrylamide/Gelatin and Purple Cabbage Extract

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Abstract: *A novel and functional Multi-Responsive Hydrogel (MRH) was synthesized from N, N dimethyl acrylamide (DMAAm), gelatin, citric acid (CA) and purple cabbage extract (PCE) to be utilized as smart food packaging material. The MRH, which was p(Gelatin-co-DMAAm)/CA-PCE, was synthesized through redox polymerization technique as film form in petri dishes. Mechanical and water resistances of the MRH was improved by addition of citric acid and N, N, methylenebisacrylamide (MBA) as cross linker. PCE was added to the reaction mixture to obtain antimicrobial, antioxidant and anthocyanin properties. Dynamic and Mechanical Analyzer (DMA), Thermo Gravimetric Analyzer (TGA), Fourier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM) were used for the characterizations. FT-IR revealed the existence of bonding between the functional group of PCE and Gelatin, carbonyl groups of DMAAm and carboxylic acid groups of CA. TGA result represented that MRH was stable up to 527°C. SEM results were proved that PCE improved the thermal stability, flexibility and durability as well as pH sensibility of the MRH. Antimicrobial activity of MRH was observed when it tested against Escherichia coli (ATCC 8739), Bacillus subtilis (ATCC 6633) and Staphylococcus aureus (ATCC 6538). Additionally, total antioxidant and anthocyanin activities of MRH were studied at different pH values for monitoring the color change. Furthermore, MRH was applied to the real samples such as whole pasteurized milk and chicken. It exhibited good color indication and antimicrobial activity on pasteurized whole milk and chicken. It was concluded that MRH was a significant candidate to be used in food packaging.*

Keywords: *Functional hydrogel, multi response polymers, p(gelatin-co-DMAAm)/CA-PCE, smart food packaging, antimicrobial effect, antioxidant activity, anthocyanin activity.*

1. INTRODUCTION

Marked-bench or instant monitoring of the food quality is one of the most important issues for costumers, producers and public health care institutions as well [1-2]. Traditional food packaging methods are still commonly utilized for protection, security, convenience, containment, informative transmission, agglomeration and tamper indication in food industry. The package itself is tasked with protection of the food from the deteriorative effects of external environmental circumstances like light, heat, pressure, moisture, microorganisms, vibration, gaseous emissions and so on. The food packaging materials are generally manufactured from glass, metals, plastics [3], papers, paperboards [4], polymers and fabric. Many natural materials such as gelatin, starch and cellulose are also used in food packaging for their biocompatibility, low toxicity and decomposability [5]. For the improving of the packaging quality additional natural compounds such as citric acid [6], pigments from purple cabbage are added to the main materials to the packaging material to gain mechanical strength, antioxidant properties, antimicrobial and anthocyanin properties [7,8]. Furthermore, addition of pigments enables the color change, which is related to the pH shifts, during the food deteriorations [9,10].

Anthocyanins are water-soluble, naturally-colored substances that provide a variety of colors like pink, red, violet, blue and purple to many fruits, vegetables and flowers. They act as natural pH indicator as well [11]. Anthocyanin with red color is called flavylium that occurs at pH 1[12], while colorless carbinol form predominantly occurs around pH 4.5 and blue-green quinoidal anhydrous form can be observed between pH 7 and 8 [13].

Recently, being as a polymeric material, hydrogels are becoming one of the most utilized materials for the food packaging purposes due to their biocompatible, flexible, easy modifiable properties as well as high water absorption capacity and low cost [14]. Furthermore, modifiable hydrogels/multi responsive polymers rapidly and reversibly respond to various physical and chemical conditions and stimuli, such as water, pH, heat, UV light, daylight, electrostatic field, magnetic field and changes in physicochemical and microbiological properties. In recent decades, multi response polymers are being used as catalysts, adsorbents, modification agents of electrodes, wound dressings [15], drug-delivery systems, enriched mediums for microorganisms [16], cell culture substrates and regenerative medicines [17].

The objective of this study is to synthesize, characterize and apply a Multi Responsive Hydrogel (MRH) with addition of natural compounds, which are gelatin, citric acid and purple cabbage extract, being a novel instant food quality monitoring device while used as a food packaging material. MRH, which is p(Gelatin-co-DMAAm)/CA-PCE, was synthesized via redox polymerization as film from in petri dishes from N, N dimethyl acrylamide (DMAAm), gelatin, citric acid (CA) and purple cabbage extract (PCE). PCE was added as the pigment source in which the pigments' colors were changed by medium pH, which is a result of food decomposition. Morphological, surface, thermal and structural characterizations of the MRH were studied by SEM, TGA and FT-IR. The swelling behavior and pH sensitivity of the synthesized MRH was studied. Furthermore, the antimicrobial, antioxidant and color-specific activities of MRH were investigated. Escherichia coli, Bacillus subtilis, Staphylococcus aureus microorganisms were used to investigate the antimicrobial activities of MRH. It was concluded that MRH was exhibited good color indication and antimicrobial activity on pasteurized whole milk and chicken.

2. MATERIAL AND METHOD

2.1. Materials

N, N dimethyl acrylamide (DMAAm), Gelatin (99%), N, N, methylenebisacrylamide (MBA) (99%), ethanol, sodium hydroxide (NaOH) and HCl (36.5-38% v/v) were purchased from Sigma; ammonium per sulfate (APS) (98%) and N, N, N, N-tetramethylenediamine (TEMED) were purchased from Merck. All reagents were of analytical grade of highest purity available and they were used without further purification. Purple cabbage, pasteurized whole milk and chicken were obtained from local suppliers. Distilled water (DI, 18.2 MΩ cm; Millipore Direct-Q3UV) was also used throughout this study.
For antibacterial activity assays, three bacterial strains obtained from the Biology Department at Van Yüzüncü Yıl University and were used. Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 6538) were utilized as gram-positive bacteria, while Escherichia coli (ATCC 8739) was used as gram-negative bacteria.

2.2. Solvent Extraction from Purple Cabbage (Brassica oleracea var. capitata f. Rubra)

For each type of hydrogel, 200 g purple cabbage was grounded and extracted with DI water and then concentrated by rotary evaporator (30% vv-1 of PCE). The PCE was stored at 4°C for further analysis like antioxidant, antimicrobial activity and anthocyanin tests [10].

2.3 Synthesis and Preparations of the Hydrogels and MRHs

Redox polymerization technique was utilized for preparation of p(Gelatin-co-DMAAm)/CA-PCE based hydrogels. In order to achieve this synthesis, 2 mL of DMAAm was mixed with 2 g of Gelatin in 5 mL DI water and 5 mL of PCE was added to the reaction mixture. Afterwards, MBA (0.25 mol% with respect to total monomer amount) was mixed through vortex followed by addition of 60 µL TEMED to PCE hydrogel mixture and finally the initiator solution APS (1 mol% with respect to total monomer amount) in 100 µL DI water was added. Then the solution was placed in plastic petri with a 10 mm diameter and was allowed to polymerize and to complete crosslinking at ambient conditions for 24 h. These preparation steps are schematically given in Fig. 1. The synthesized hydrogels were kept in DI water and renewed every 8 h for 24 h to eliminate unreacted monomers. Finally, hydrogels in film form were dried in oven at 40ºC till their weights were stabilized and stored 4ºC for characterization and utilization.

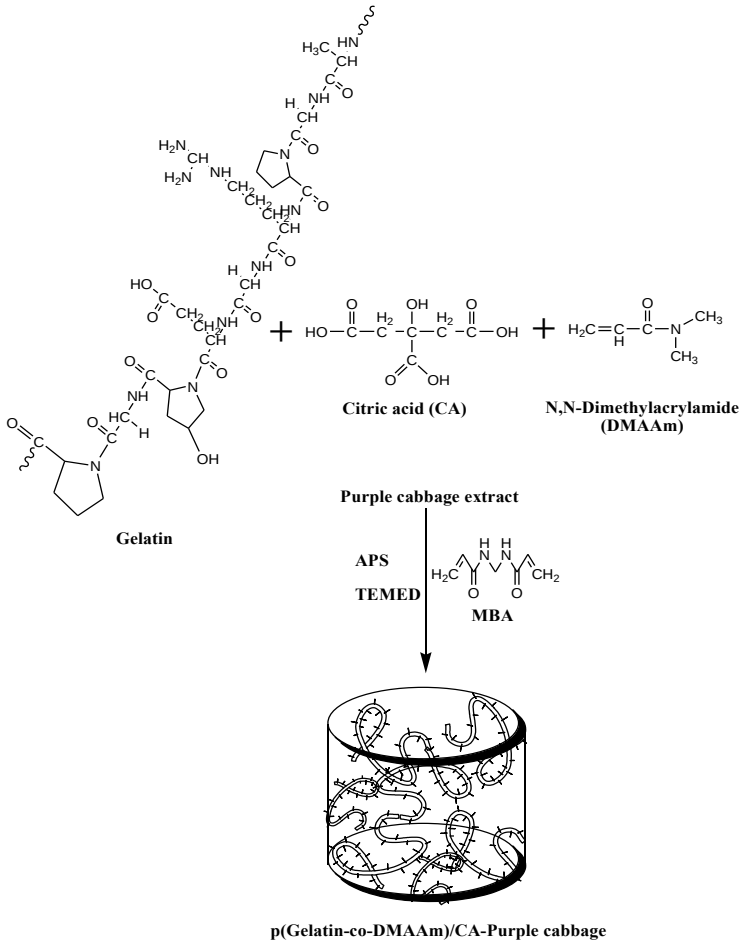

Figure 1. *The hydrogels preparation.*

2.4. Swelling Behavior of the Hydrogels

Swelling properties of hydrogels were carried out in three runs at room temperature by placing certain amounts of dried hydrogels and MRH in DI water. The increase in mass was periodically measured by weighting of the hydrogels and MRH after blot drying with filter paper to remove the superficial water and returned into the same swelling media. The hydrogels and MRH were kept in swelling medium for 24 h to determine the maximum swelling values, Smax%.

The percent swelling degree (S%) as the function time was calculated as:

$$S\% = \frac{M_t - M_0}{M_0} \times 100 \quad (1)$$

Where, Mo and Mt are the initial mass and the mass of hydrogels at time t, respectively [16].

2.5. TGA, FT-IR and SEM Analysis of Hydrogels

Thermal behaviors of the hydrogels and MRH were investigated by a Gravimetric Analyzer (TG/DSC, Setaram Labsys Evo 1600 model, France). Approximately 4-6 mg of samples were

placed in ceramic crucibles and analyzed during heating up to 50-1000°C under Argon atmosphere with 100 mL min-1 flow rate at 10°C min-1 heating rate.

The FT-IR analysis of DMAAm and Gelatin based the hydrogels were investigated using Fourier Transform Infrared Spectroscopy (FT-IR, Thermo Nicolet iS10 FT-IR Spectrometer, USA) using ATR apparatus with 4 cm-1 resolution between 4000 and 650 cm-1.

The surface morphologies of the synthesized materials, which were p(Gelatin-co-DMAAm)/CA and MRH, were monitored by Scanning Electron Microscopy (SEM) (Zeiss Ultra Field emission (FESEM-EDX), Germany).

2.6. Liquid Chromatograph Mass Spectrometer (LC-MS/MS, Q Exactive) Analysis

Liquid Chromatography Mass Spectrometer (Hybrid Quadrupole-Orbitrap Mass Spectrometer, Thermo, USA) was used to determine the PCE composition. The extract samples were renovated by capillary column Hypersil Gold (50 x 2.1 mm) while Helium was used as a carrier gas, at a flow rate of 0.3 mL min-1. Determinations of individual components were identified by analytical standards (antimicrobial, antioxidant and anthocyanin).

2.7. Mechanical Strength Test

Dynamic and mechanical behaviors of the synthesized and modified materials were carried out by DMA (Mettler Tolledo Tritone Technology, UK) device. The samples were prepared in approximately 1 mm height and 4 mm diameter. DMA measurements were recorded at 1 Hz frequency and at a heating rate of 5ºC min-1while the temperature ranged from 30 to 80ºC. The modulus properties such as storage modulus and loss modulus were recorded as a function of temperature.

2.8. Antimicrobial Properties of the Hydrogels

The agar diffusion method was used to evaluate the antimicrobial activity as explained in the literature [18]. Escherichia coli (ATCC 8739), Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 6538) bacteria were used for the test. The test was run for three different media, which are PCE, p(Gelatin-co-DMAAm)/CA and MRH monitored for 24 h. The numbers of bacteria were counted in CFU.

2.9. Antioxidant Properties of the Hydrogels

Total phenolic substances were determined according to ABTS method [19]. The absorbances of samples in phosphate buffered saline (PBS) at 734 nm were recorded against the blank. The observed absorbance values indicated of the reduction in the ABTS radicals in the media. The impact of PCE against the ABTS radicals in the media was calculated using the following equation:

$$ABTS\ Activity\% = \frac{A_0 - A_1}{A_0} \times 100 \quad (2)$$

Where, A0 is absorbance of control sample and A1 is absorbance of the sample.
In Folin-Ciocalteu method, phenolic compounds oxidize Folin-Ciocalteu reagent in an alkaline media while the color of the media changes. Gallic acid equivalent of the phenolic compounds

according to this concentration were then calculated from linear regression equations derived from standard curves that were prepared with gallic acid. Total amounts of phenolic compounds in PCE were reported as mg gallic acid per litter. In this analysis, saturated sodium carbonate solution was prepared by dissolving 35 g Na2CO3.10H2O in 100 mL of DI water and left to stand overnight. Crystallization was initiated by addition of Na2CO3 solution and saturated carbonate solution was prepared by filtering through glass wool after crystallization ended. One mL of Folin-Ciocalteu agent was then added to 0.1 mL of each sample. The mixture was then allowed to rest for 4 min followed by addition of 1.25 mL of saturated sodium carbonate solution. Samples were then allowed to stand for 120 min and the absorbance was recorded at 720 nm wavelength using a Thermo UV-Vis spectrophotometer (Genesis 10S UV-Vis, USA) [20].

2.10. Anthocyanin Properties of the Hydrogels

Anthocyanin properties of hydrogels which are p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA and MRH, were measured as indicated in the literature [21]. The absorbance of each dilution was then determined at 516 nm and total absorbance of turbidity at 700 nm. Anthocyanin activity was obtained from maximum absorbance value by subtracting from the total value. This procedure was repeated for each MRH

Anthocyanins activity of extracts and hydrogels were calculated with the following equation;

$$Activity\% = \frac{\left|\left[(A_{516} - A_{700})pH_1 - (A_{516} - A_{700})pH_{4,5}\right] \times 1000 \times MW\right|}{[(l) \times \delta]} \quad (3)$$

where, cyaniding-3-glucoside (cyn-3-glue) MW is 449.2 g, ε (molar absorptivity coefficient) is 13000 and, l is 1 cm; pelergonidine-3-glucoside MW is 433.2 g, ε (molar absorptivity coefficient) is 22440 and, l is 1 cm, malvidine-3-glucoside MW is 493.2 g, ε (molar absorptivity coefficient) is 22440 and, l is 1 cm [22].

2.11. Colorimetric Properties of the Hydrogels

The effect of the medium pH on MRH and the hydrogels were studied according to literature [23]. Generally, the indicator is a weak acid (In H), which is in equilibrium with the surrounding pH according to below equation:

$$\text{In } H^{ki}_{k-I} = In^- + H^+ \quad (4)$$

Where Hkik-I is acidic color intensity and In- is alkali color intensity. The variation of the medium colors with variation of medium pH was measured within the visible region while p(Gelatin-co-DMAAm)/CA hydrogel was used as the blank sample.

The absorption spectra (380−770 nm) of all solutions were recorded at constant intervals (Δλ = 2 nm) with a (Analytikjena, Spercord S 600, Germany spectrophotometer), using 5 mm path length quartz cells and distilled water as a reference. The CIELab parameters were calculated from the absorption spectra by using the original software Croma Lab, following the recommendations of the Commission International de L'Eclariage: CIE 1976 and the Standard Illuminant D65, corresponding to natural daylight. The color identifications were made by CIE 1976 (L*a*b*) (CIELab) uniform color diagram. a* and b* are the axis of the 2D coordinate system, in which a* takes positive values for reddish colors and negative values for greenish ones while b* takes

positive values for yellowish colors and negative for the bluish ones and L* defines lightness, ranging from black to white according to the CIELab uniform diagram a psychometric index. Other color parameters were obtained by equations 5 and 6; hue angle (h) and (C), which stand for color and color intensity respectively [24,25].

The L* defines lightness, C* specifies chroma and h* denotes hue angle, an angular measurement.

$$C^* = (a^2 + b^2)^{1/2} \quad (5)$$

$$h = \arctan\left(\frac{b^*}{a^*}\right) \quad (6)$$

The CIELab parameters (L*, a*, b*, C*, h*) were determined for 5 mL solutions of each hydrogel at different pH values, ranging from 1 to 12. ΔE (Euclidean). The values were calculated from the initial pH value (pH 1) and after each increase of pH, considering the Euclidean distance between the two color points. Euclidean distance of hydrogels was calculated with the following equation;

$$\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2} \quad (7)$$

2.12. Real Samples Applications

Whole milk and chicken breast were used as the real samples. Firstly, those real samples were infected by Staphylococcus aureus, Escherichia coli and Bacillus cereus than treated with MRH, which were cut into 10 mm in diameter, were placed on 50 mL of whole milk and to 100 g chicken. MRH exposed samples were kept in a temperature controlled incubator (Memmert UN 110, Germany) at different temperature, which were 4ºC, 10ºC, 20ºC, 30ºC and 40ºC, for 48 h. The color (CIELab) parameters (L*, a*, b*, C*, h*) were calculated for each sample while untreated whole milk and chicken were used as the blanks.

3. RESULTS AND DISCUSSION

3.1. Characterization of the Synthesized Hydrogels

The moisture content of a given food is one of the most important parameters for its shelf life and structural degradation. It is known that polymers prepared form gelatin and DMAAm have not water resistance, mechanical strength and dissolved easily in an aqueous media. Therefore, water resistance and mechanical strength of MRH were adjusted by addition of biocompatible MBA as cross linker and CA to the polymer mixture during the synthesis [26]. Water resistance of MRH, which was p(Gelatin-co-DMAAm)/CA-PCE, was observed higher than those of p(DMAAm), p(DMAAm)/CA, p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA in DI as shown in Fig. 2 and at different pH values ranging from 2-12. The films prepared form MRH were non-dissolved and stable in aqueous media more than five months. Relatively high water absorption of MRH to the conventional food packaging materials enables it to fast response to the pH change in the media.

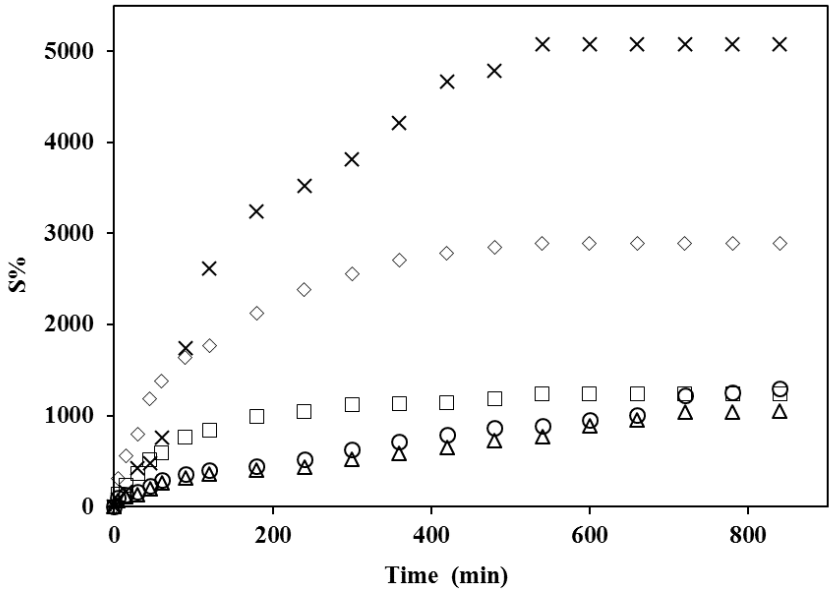

Figure 2. *Swelling behavior of prepared hydrogels; △ p(DMAAm), O p(DMAAm)/CA, X p(Gelatin-co-DMAAm), ◇ p(Gelatin-co-DMAAm)/CA and □ MRH.*

As given in Fig. 3, the FTIR spectra for p(DMAAm) showed the characteristic peak belonging to amide at 1616 cm-1 and the peaks between 1398 cm-1 and 1250 cm-1 were related to C–N stretching. Other peaks corresponding to p(Gelatin-co-DMAAm)/CA hydrogel were missing, which can be justified by the new bonds intervened during crosslinking process. When PCE was added, the broad peak between 3649 cm-1 and 3080 cm-1 was reduced and stress of the peaks at 2359 cm-1 and 2342 cm-1 wave numbers were decreased. In other words, those appeared new bonds and structural variances demonstrated the existence of a hydrogen-bonding interaction between p(DMAAm), p(Gelatin-co-DMAAm)/CA and MRH.

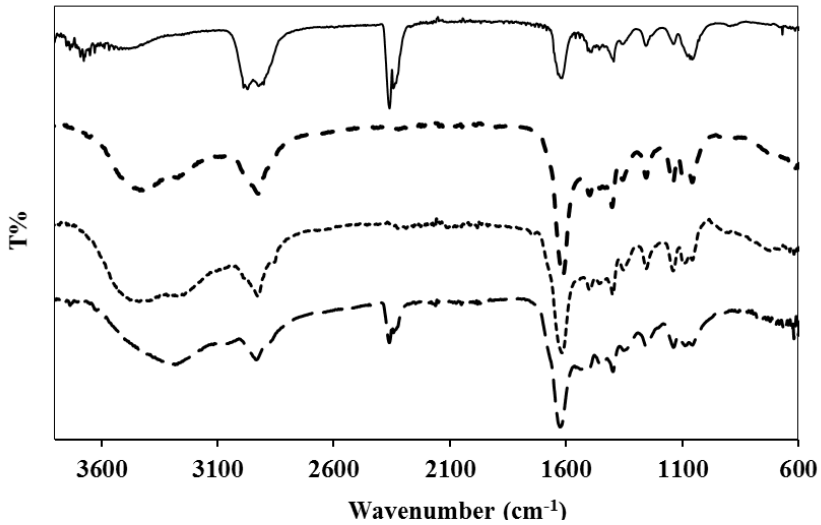

Figure 3. *FT-IR analysis of — p(DMAAm), - -p(Gelatin-co-DMAAm), -----p(Gelatin-co-DMAAm)/CA and — — MRH.*

Thermal stabilities of synthesized hydrogels were investigated using a TGA/DSC analyzer. In Fig. 4, the thermal degradation of p(DMAAm) hydrogel has one step and during this stage, the total mass loss was about 97% while temperature reached 500°C. This can be explained with accelerated mass loss of hydrogel due to the main chains and cross-linked networks in p(DMAAm) getting destroyed [27]. p(Gelatin-co-DMAAm)/CA hydrogel degradation has three steps. At the initial stage, the first mass loss around 15% (205°C) was due to evaporation of surface water and of the remaining small molecules. The second step began when the temperature was 250°C and at 359°C temperature, mass loss became approximately 57.8%. Third step began 359°C and the mass loss, which was about 19.8%, tended to be constant till the temperature of 465°C. Thermal degradation of MRH was observe in two steps as shown in Fig. 4. The mass loss of MRH at the first stage was approximately 12.8% because of the evaporation of the surface water while the temperature increased to 196°C. The second step began when the temperature was 196°C and average mass loss was approximately 75.3% from 196°C to 527°C. The total mass loss was reached to 88.1% during decomposition of MRH.

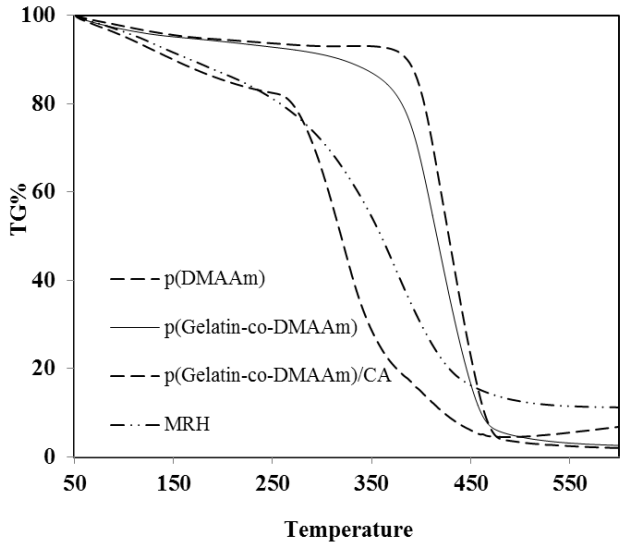

Figure 4. *Thermogravimetric analysis of p(DMAAm), p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA and MRH.*

Surface morphologies of synthesized materials were monitored using SEM and represented in Fig. 5a and b. Fig. 5a shows the surface image of p(Gelatin-co-DMAAm)/CA hydrogel prepared with gelatin, DMAAm and CA. As seen clearly, roughness, cracks and cracks were formed on the surface after the polymerization was completed. This image shows that the structure is not tunable and elastic. However, when PCE added the polymerization reaction to synthesized MRH the cracks and fragilities were lost and the structure became smoother and more elastic as shown in Fig. 5b. Furthermore, after the PCE is added, it was verified by DMA analyzes that MRH was more elastic than p(Gelatin-co-DMAAm)/CA. It was concluded that the homogeneously dispersed PCE in the hydrogel structure acted as a bond in the structure, removing cracks and brittleness, and made the structure more flexible and smooth.

a) b)

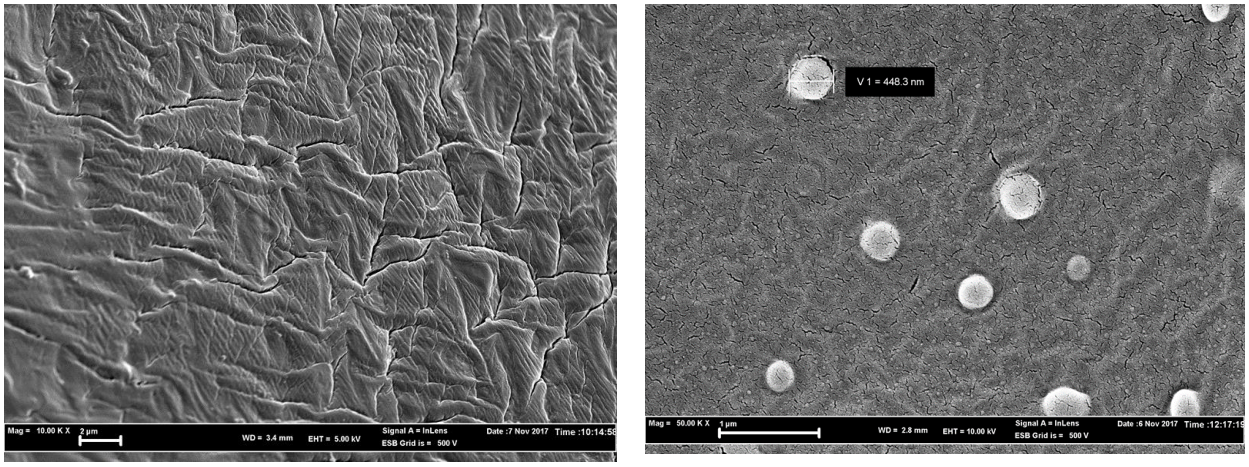

Figure 5. *SEM images of (a) p(Gelatin-co-DMAAm)/CA and (b) MRH.*

3.2. Liquid Chromatography Mass Spectrometer (LC-MS/MS, Q Exactive) analysis

Apparently, purple cabbage includes some compounds, which are act as indicators when medium pH changes. Purple cabbage extracts include quercetin, rutin, coumaric acid, caffeic acid, ferullic acid and naringenin substance, all having antioxidant, antimicrobial activities and copigments and pelargonidin substance with even higher anthocyanin activities [28]. Copigmentation cofactors (copigments) involves the anthocyanin glucosides, phenolic acids, flavonoids and in particular the derivatives of the flavonol and flavone subgroups [29,30]. To determine those substances, purple cabbage was extracted with water and analyzed by a Q Exactive. The detected compounds known for their antioxidant, antimicrobial and anthocyanin activities were as follows; digalloyl-glucose izomer-1, digalloyl-glucose izomer-2, digalloyl-glucose izomer-3, quercetin hexoside-isomer-1, quercetin hexoside-isomer-2,quercetin-3-o-galactoside, quercetin-3-o-glucoside, kaempferol-3-galactoside, kaempferol-3-glucoside, coumaric acid hexoside-isomer-1, coumaric acid hexoside-isomer-2, coumaric acid hexoside-isomer-3, 3-p-coumaroylquinic acid, 4-p-coumaroylquinic acid, caffeic acid, quercetin-3-o-rutinoside, isorhamnetin-3-o-rutinoside, naringenin, rutin, ferullic acid and pelargonidin [28].

3.3. Application of Mechanical Strength Test

The mechanical properties of hydrogels were studied by a Dynamic Mechanical Analysis (DMA) for their elasticity and given in Fig. 6. Decreasing storage modulus values at low temperatures, which are interpreted as molecules moving easily because of having more free volume [32]. It was obtained that p(Gelatin-co-DMAAm)/CA was more flexible and shapeable than the MRH according to decreasing storage modulus values. It was observed that the storage energy of p(Gelatin-co-DMAAm)/CA increased between 40°C and 60°C and molecules move with more difficultly between these temperature values (Fig. 6a). In Fig. 6b, the energy storage of MRH decreased between 30°C and 80°C as the cross-linking property of hydrogel was increased. The loss modulus was observed that MRH has reduced likelihood of losing energy. Because of the poor mechanical strength of gelatin, DMAAm, CA, MBA were added to the polymerization mixture to improve their mechanical strength and the flexibility. MRH possessed stronger mechanical properties than p(Gelatin-co-DMAAm)/CA. It was concluded that PCE worked as a bridge molecule, which interacted the DMAAm by hydrophilic interaction and promotes a better mixing with gelatin [32].

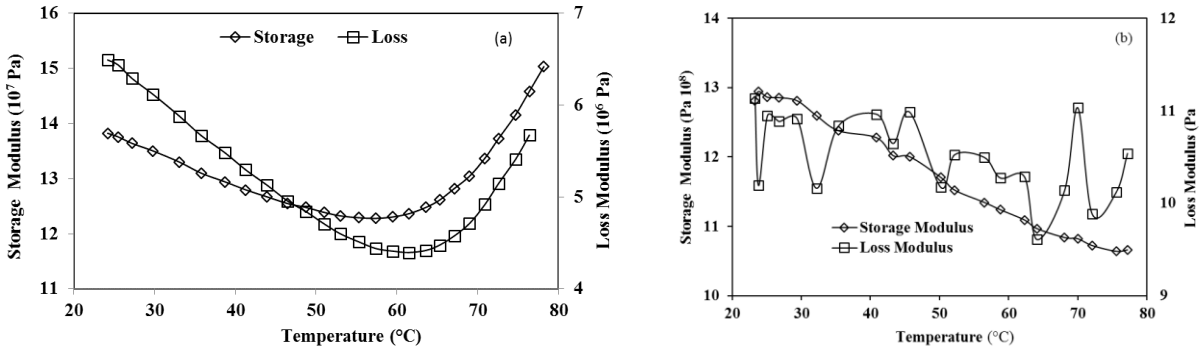

Figure 6. *DMA analysis (a) p(Gelatin-co-DMAAm)/CA and (b) MRH.*

3.4. Determination of Antimicrobial Properties of the Hydrogels

The antimicrobial activity is an important feature that includes the production of the antimicrobial substance and competitive dismissal of pathogens microorganisms. The antimicrobial activity for PCE and MRH were tested against three common bacteria and the obtained data were given in Table 1. MRH was treated with the bacteria for 72 h. and the MIC (Minimal Inhibition Concentration) values were determined as 100 mg mL-1 against Staphylococcus aureus and over 100 mg mL-1 against Escherichia coli and Bacillus cereus. MIB (Minimal Bactericidal Effect) values of PCE, p(Gelatin-co-DMAAm)/CA and MRH were observed over 200 mg mL-1 against Staphylococcus aureus, Escherichia coli and Bacillus cereus. As presented in Table 1, the antimicrobial effects of hydrogel are very well when compared to those of literature [7,33]. Antimicrobial activity of purple cabbage's extract is beneficial for food, agriculture and medicinal applications [33]. The antimicrobial effect of MRH will reduce and/or inhibit the microbial growth in the environment when utilized properly. Many important microbial and chemical deteriorative changes occur with the reactions within the food. In addition to temperature, other environmental factors such as water, color and pH induced deleterious changes in foods that are catalyzed by microbial growth [34,35]. And if microbial growth still occurs in the environment of MRH, the change in pH of the medium will be monitored by color change.

Table 1. *Comparison of the MIC and MIB values of PCE, p(Gelatin-co-DMAAm)/CA and MRH and PCE by diffusion method appeared in the literature (Agar Well Diffusiona (mm), Micro Dilutionb).*

Substance	*Escherichia coli*		*Bacillus cereus*		*Staphylococcus aureus*		
	MIC (mg mL^{-1}- mg g^{-1})	MIB (mg mL^{-1}- mg g^{-1})	MIC (mg mL^{-1}- mg g^{-1})	MIB (mg mL^{-1}- mg g^{-1})	MIC (mg mL^{-1}- mg g^{-1})	MIB (mg mL^{-1}- mg g^{-1})	
PCE	100	>200	100	>200	100	>200	[This study]
p(Gelatin-co-DMAAm)/CA	>100	>200	>100	>200	>100	>200	[This study]
MRH	>100	>200	100	>200	100	>200	[This study]
PC	<0.25^{b}	------	<0.5^{b}	------	<0.25^{b}	------	
	12.5^{a}				14.2^{a}		[7]
	14.4^{a}		------		16.1^{a}		[33]

3.5. Determination of Antioxidant Properties of the Hydrogels

Total antioxidant activities of PCE and MRH were measured by ABTS method and calculated according the following equation;

$$ABTS\ \ Activity\% = \frac{A_0 - A_1}{A_0} \times 100 \quad (8)$$

Troloks equivalent value (TEAC) of PCE, p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA and MRH were calculated by Equation 9 and given in Table 2.

$$TEAC = \frac{A}{3.4396} \times f \quad (9)$$

Where, A is slope of measurement, 3.4396 is the slope of standard and f is dilution factor.

Table 2. *Comparison of total phenol content (gallic acid equivalent phenol content) and TEAC (Trolox equivalent antioxidant capacity) values of PCE, p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA, MRH and PCE appeared in the literature.*

Substance	Antioxidant	
	Total phenol (mg g^{-1})	TEAC (µmol g^{-1})
PCE	3020	36 [This work]
p(Gelatin-co-DMAAm)	93	----- [This work]
p(Gelatin-co-DMAAm)/CA	310	1 [This work]
MRH	1297	16 [This work]
Brassica oleraceae L. var. acephala DC	0.0013	----- [36]
Brassica oleraceae	1.91	----- [37]
Brassica oleraceae	1.71	15 [38]

Total phenol amount of PCE and MRH were calculated using Folin-Ciocalteu method at 760 nm wavelength and inhibition % from the following equation;

$$Absorbance_{(760nm)} = 0.009[Gallic\ acid\ ] + 0.1034 \quad (10)$$

Gallic acid equivalent value of PCE, p(Gelatin-co-DMAAm), p(Gelatin-co-DMAAm)/CA and MRH were calculated as displayed in Table 2. It is indicated that total antioxidant activity of PCE and hydrogels are very well when compared to those of literature [36,38]. A significant proportion of the antioxidant activity originates from phenolic substances in extract and hydrogel structures were analyzed from Q Exactive analysis. Therefore, the antioxidant substance releasing of MRH minimize the negative effects of free radicals.

3.6. Determination of Anthocyanin Properties of the Hydrogels

As shown in Table 3, total anthocyanin activities of PCE and MRH were determined for cyaniding-3-glucoside, pelergonidine-3-glucoside and malvidin-3-glucoside. It is indicated that total anthocyanin activities of PCE and hydrogels are very promising when compared to those of literature [12,39,40]. There are numerous physical and chemical factors, which can have a negative impact on the stability of anthocyanin, the most important of which are increases or decreases in the temperature, light, oxygen concentration and pH, along with presence or absence of ascorbic acid and metal ions.

Table 3. *Comparison of anthocyanin values of PCE, MRH and Brassica oleraceae appeared in the literature.*

Substance	Anthocyanin (mg mL^{-1}- mg g^{-1})		
	Malvidin-3-glucoside	Cyanidin-3-glucoside	Pelergonidine-3-glucoside
PCE	1.9	6.67 [This study]	6.7
MRH	1.1	3.6	1.2
Brassica oleraceae	---	0.7 [39]	---
Brassica oleraceae	---	1.14 [40]	---
Brassica oleraceae	---	0.76 [19]	---

3.7. Determination of Colorimetric Properties of the Hydrogels.

The colorimetric interpretation of copigmentation based on the CIELab color diagram has demonstrated to be practical interest because both quantitative and qualitative color changes can be better understood. It has been demonstrated that pH, copigment structure and concentration have significant influences on the copigmentation process, which induced different absolute and relative color changes in anthocyanin solutions [41,42]. Table 4 represents the chromatic characteristics of MRH according to CIELab color diagram [37,38]. Each color has its own distinct appearance, based on three elements: hue (a), chroma (b) and value (lightness) (L) [8,9]. The color change of MRH was measured by immersing that into water for 5 min. in which pH values ranging from 1 to 12. A visible color change was detected instantly after contact of MRH and water. The colorimetric parameters ΔL^* Δa^* Δb^* for MRH were calculated by Equation 6 while pH varying from 1 to 12. ΔE^* was calculated to be 134.81. It is reported that about 3-unit change in ΔE^* can be easily detected by an average human eye [42]. According to the method, a* value of -12.83 indicates greener or less red while b*value of 21.85 indicates yellow or less blue. As clearly indicated in Table 4, when pH varying from 1 to 12, ΔL^*, ΔC^* and ΔH^* values calculated to be -88.88, 101.38 and 1.07 respectively. It means that MRH at pH 1 is lighter than MRH at pH 12. PCE was utilized by Chen and Gu, as absorption-type pH sensors to determine purple cabbage pigmentation in sol-gel film [12]. Walkowiak-Tomczak and Czapski, observed the color change in red cabbage extract by varying pH [25]. It was concluded that MRH easily responded pH changes caused by chemical and/or microbiological activities in the media.

Table 4. *Color Parameters (CIELab) L*, a*, b* measured for MRH in different pH solutions.*

pH intervals	L*	a*	b*	ΔE	ΔC	ΔH
1-2	67.03	-12.83	21.85	71.66	25.33	-1.04
2-3	3.89	13.60	4.69	14.90	14.38	0.33
3-4	18.16	5.43	-6.86	20.16	8.75	-0.90
4-5	-56.59	-3.32	-13.68	58.31	14.08	1.33
5-6	-18.97	0.11	7.55	20.41	7.55	1.56
6-7	-0.76	0.17	0.12	0.79	0.21	0.63
7-8	-2.41	0.61	-8.00	8.37	8.02	-1.49
8-9	15.76	-0.78	5.21	16.61	5.26	-1.42
9-10	-14.22	1.14	-1.16	14.31	1.63	-0.79
10-11	5.69	0.76	-3.94	6.96	4.01	-1.38
11-12	71.29	0.69	13.28	72.52	13.30	1.52
1-12	-88.88	-48.76	-88.86	134.81	101.36	1.07

3.8. Utilization of MRH for Real Samples.

It is difficult to verify pH changes and inhibition effect on microorganisms existing in a food. The MRH was applied to various foods such as chickens and dairy products, which are frequently consumed and known as perishable foods [43,44]. Fig. 7a shows the rate of change in color of MRH exposed to spoiling whole milk at room temperature. Color change of MRH was similar in spoiled milk containing Staphylococcus aureus, Escherichia coli and Bacillus cereus. Prior to the first 8 h, no color change was detected visibly. Thereafter, the color of MRH was steadily changed from red to green (8–24 h). However, after 24 h more intense color change was observed visibly allowing a better visualization of the occurrence of spoilage.

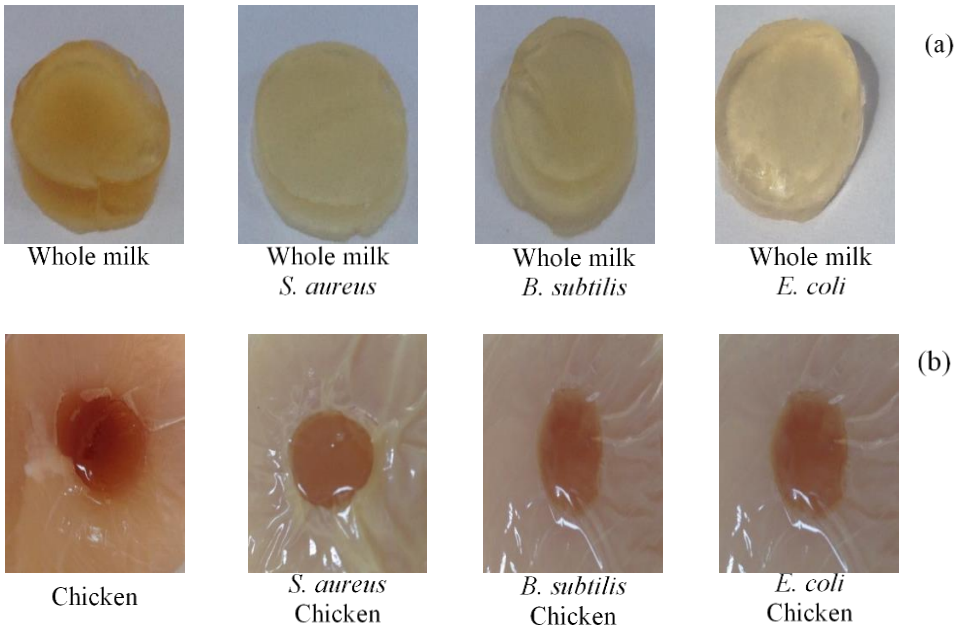

Figure 6. *MRH color response in contact with (a) Whole milk, Whole milk S. aureus, Whole milk B. Subtilis and Whole milk E. coli and (b) Chicken, Chicken S. aureus, Chicken B. Subtilis and Chicken E. coli at 20 ºC.*

When whole milk reaches to temperature above of 4-7ºC, it begins to decompose and the medium pH changes. The pH change in whole milk with microbial growth was monitored at room

temperature and the pH of whole milk changed from 6.81 to 4.8. (48 h). Many kinds of factors such as microbial contamination and subsequent accumulation of lactic acid due to microbial metabolism can cause to a decrease of pH of milk [43,44]. Thus, whole milk spoilage can be detected and monitored through a colorimetric monitoring system as in the MRH developed in the present work. Similar results were obtained in the whole milk treated with Staphylococcus aureus, Escherichia coli and Bacillus cereus (Fig. 7a). Color of MRH gradually changed from 10 h up to the 24 h after which no further color change was observed. The colorimetric measurements are given in Table 5, as indicated in literature [11].

Table 5. *Total color change (ΔE) parameters (CIELab) after from upon contact with whole milk and chicken of MRH.*

MRH	ΔE	ΔC	ΔH
Whole milk	570.8	221.29	1.51
Whole milk- *S. aureus*	143.15	135.95	1.38
Whole milk- *B. subtilis*	430.79	138.42	1.43
Whole milk- *E. coli*	408.63	147.14	1.40
Chicken	454.92	273.91	0.62
Chicken- *S. aureus*	141.27	20.75	-0.46
Chicken- *B. subtilis*	127.18	24.37	-1.11
Chicken- *E. coli*	182.81	131.47	1.07

Raw chicken is highly perishable. Under aerobic packaging conditions the shelf life of the refrigerated product is limited by the growth of microorganisms and changed the medium pH [43,44]. Fig. 7b displays the rate of change in color of MRH exposed to spoiling chicken at different temperatures like 4ºC, 10ºC, 20ºC, 30ºC and 40ºC and to different microorganisms like Staphylococcus aureus, Escherichia coli and Bacillus cereus. Prior to the first 8 h, slight color changes were detected by bare eye. The colorimetric measurement values of these results are tabulated in Table 5, as indicated in literature [11].

The board antimicrobials, antioxidants, anthocyanins and the distinguishable color change properties of PCE at different pH contributed to the MRH for whole milk and chicken. Moreover, the MRH should have wide application in kinds of food as meat, vegetable and fruits products. For example, many of microorganisms during spoilage of meat, vegetable and fruits products can be inhibited and the color changed can be recorded. This study results demonstrated that MRH may function as a quality indicator and preservative for various food products.

4. CONCLUSION

MRH, which was synthesized through redox polymerization technique from DMAAm, gelatin, CA and PCE, was a biocompatible polymer, which indicate non-toxic components and safe indicators for food coverage. MRH has a high potential to be used to increase the lifetime of foods on the markets. In the present study, developed MRH has excellent spectroscopic and physicochemical properties to be utilized as smart food packaging while it rapidly responds pH changes in the environment simultaneously reflect its color in a wide spectrum from green to red. It provides a cheap and simple way for directly detected if a food would face any physical and chemical decomposition and spoiled. MRH was tested on chicken, whole milk against three

microorganisms, which are Staphylococcus aureus, Escherichia coli and Bacillus cereus results were promising. It is claimed that MRH can be very effective in terms of anthocyanin, antimicrobial and antioxidant properties and safe as food packaging materials.

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