Enhanced gelatin based films integrated with xanthan and cellulose derivatives as potential packaging materials Salah A.A. Mohamed^a, Eman A. Ali^b, Abdelmageed M. Othman^c, Alaa E. Elsayed^b

^aPackaging Materials Department, National Research Centre, Chemical Industries Research Institute, ^bPolymer and Pigments Department, National Research Centre, Chemical Industries Research Institute, ^cMicrobial Chemistry Department, Biotechnology Research Institute, National Research Centre, Dokki, Giza, Egypt

Correspondence to Eman A. Ali, PhD, Polymer and Pigments Department, National Research Centre, Chemical Industries Research Institute, 33 El Bohouth Street, Dokki Giza, P. O. 12622, Egypt. Tel: 202 333 224 27; Fax: 202 333 703 31; E-mail: eman.abobakr@outlook.com

Received: 18 June 2023 Revised: 15 July 2023 Accepted: 24 July 2023 Published: 22 November 2023

Egyptian Pharmaceutical Journal 2023, 22:640–649

Background

Films based on gelatin can be useful for pharmaceutical and food packaging, but they have some drawbacks.

Objective

In this study, we aimed to evaluate three series of edible composite films based on gelatin for pharmaceutical and food packaging.

Materials and methods

Three series of composite films based on gelatin were prepared by blending with three different polysaccharides, xanthan, hydroxyethyl methyl cellulose (HEMC), and hydroxypropyl methylcellulose (HPMC). Film composites were prepared by solution casting with glycerol (30% weight). Burst strength, mechanical, contact angel, water vapour permeability rate (WVPR), and air permeability test were tested for the three series of composite films. The antibacterial activity of the produced sheets against Gram-positive bacteria *B. mycoides*, a nonfilamentous fungus called *C. albicans*, and Gram-negative bacteria *E. coli* was examined.

Results

This work reports the successful preparation of stand-alone natural antimicrobial edible composite films with excellent mechanical properties. The addition of HPMC and HEMC had enhanced the thermal stability of gelatin-xanthan composite films. Mechanical properties; tensile strength and elongation percent were investigated. The results showed that the addition of 0.1% of HEMP and HPMC to gelatin-xanthan composite enhanced the elongation% to equal 59.33% and 25.33%, respectively, while the tensile values were 5.570 and 6.617 mPa, respectively. Xanthan addition had improved the antibacterial activity of gelatin films. The results showed that the different composite series have varying relative effects on microbial development effectiveness.

Conclusion

According to the results, these composite films can be considered as promising natural active edible packaging materials.

Keywords:

blend, gelatin, hydroxyethyl methylcellulose, hydroxypropyl methylcellulose, edible packaging, xanthan

Egypt Pharmaceut J 22:640–649 © 2023 Egyptian Pharmaceutical Journal 1687-4315

Introduction

Natural edible films have been emerged as an effective way to extend shelf life and protect food from harmful environmental effects. Additionally, natural edible films can be used to deliver bioactive compounds such as antioxidants, antimicrobials, flavors, and probiotics [1,2]. As a result of global environmental awareness, research into friendly packaging has gained traction [3]. Therefore, the development of new biodegradable polymeric materials has gained great interest [4]. Among all, natural polymers have received more attention. Availability and biodegradability are two characteristics that make natural polymers advantageous over synthetic polymers.

The good film-forming ability of gelatin makes it an ideal packaging material for food protection or shelf-

life extension. Moreover, gelatin acts as protecting food films against light and oxygen exposure [5]. It has been reported that the addition of gelatin with carboxymethylcellulose (CMC) shows a good improvement in mechanical and physical properties as well as in air permeability [6]. Gelatin, the natural hydrocolloid polymer, is produced by hydrolysis of collagen from bones, skin or connective tissue [7]. It is a combination of peptides and proteins. It is extensively utilized in biodegradable films because of good emulsifying and gas barrier properties [7]. Gelatin-based films are commonly developed by

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

solution casting [8]. However, due to gelatin high water solubility, the use of neat gelatin is limited. It is possible to enhance gelatin based films by blending one or more polymers [6,8–10]. Studies demonstrate that adding carboxymethyl cellulose or xanthan gum to gelatin improves its properties as a film material [11]. Another example, gelatin and sodium alginate were used as bio-based packaging composite and the extract of green tea as active agent [12]. Another research reported the mixing of gelatin with chitosan that shows a decrease of solubility and an increase of surface hydrophobicity [7].

Xanthan is biopolymer obtained from the bacteria Xanthomonas campestris [13]. This biopolymer has a unique structure consists of (1/4)- β -D-glucose units as the main chain with a trisaccharide side chains at every second unit (Scheme 2). This side chains containing D-glucurunic acid units surrounded by two Dmannoses [14,15]. Xanthan outstanding properties, counting solubility in water, affordability, and biocompatibility, have made it the highest anionic natural heteropolysaccharide applied in the pharmaceutical and food industry. For instance, xanthane was used for the preparation of cryogels that possess antioxidant and antimicrobial activity [16]. Another research prepare a edible blend films of xanthan and curdlan [14] to enhance the poor mechanical characteristics of xanthan [17,18]. Also, edible films were prepared from a blend of xanthan, curdlan and gelatin polymers [19].

Hydroxyethyl methylcellulose (HEMC) is a viscous soluble fibre that is frequently used in the food,

cosmetics, and pharmaceutical sectors as a thickening and gelling agent [20]. According to Su *et al.* study, HEMC has a feature of preventing and treatment of high fat diet-induced hyperlipidemia [21].

Because of hydroxypropyl methyl cellulose (HPMC) environmental attractiveness, low cost, elasticity, and transparency, it has been considered as a promising polymer in the food industry [22]. It is a derivative of cellulose where some of the hydroxyl groups were substituted by hydroxypropyl groups. Because of its biocompatibility, and nontoxicity, HPMC has been approved as Generally Recognized as Safe (GRAS) for food by Food and Drug Administration (FDA) [23]. Moreover, HPMC's forming gas barrier and tasteless have made it an excellent natural polymer in many applications. For example, HPMC were incorporated with xanthan gum to enhance shelf life of banana [24].

Herein, the study describes the successful preparation of new three groups of edible composite films based on gelatin that was blended with xanthan, hydroxyl methyl cellulose and hydroxyl propyl cellulose. To the best of our knowledge, preparation of these green composite films has not been reported. The aim of this study is to improve gelatin film's biological properties along with exhibiting good mechanical properties. The possibility of their potential application as edible packaging materials was carried out by studying of physicochemical properties including air permeability, burst strength, the thermal stability, surface morphology, and mechanical properties.



Scheme 2



Schematic representation of xanthane structure.

Materials and methods Materials

Gelatin was purchased from a local company (El-Nasr Pharma Chem. Co.). Xanthan (XA), hydroxyethyl methylcellulose (HEMC), hydroxypropyl methyl cellulose (HPMC) and glycerol were purchased from Merck.

Preparation of blend solution for the composite films

Gelatin (G) and Xanthan (XA) were dissolved separately in distilled water by 2% and 5%, respectively, and then, they were heated at 60°C. Films with different ratios of gelatin to XA were prepared; 1 : 0.08, 1 : 0.16, 1 : 0.25 w/w, respectively. In another experiment, HEMC and HPMC were dissolved in distilled water and added in ratios as represented in Table 2 with respect to XA (Scheme 1). All the prepared solution contained 30% glycerol as plasticizer. Then, the composite films were dried at 40°C in an oven for 12 h with circulating air.

Physicochemical properties of the prepared films Thickness

Thickness of the prepared sheets $(3 \times 3 \text{ cm})$ was measured using a digital micrometer (Mitutoyo, model C112EXB, USA, accuracy 0.001 mm). For each sample, 10 separate sites were measured. The findings were presented as the sample's average value and standard deviation [25].

Thickness = $\frac{(\text{Sum of measured values})}{10}$

Attenuated total reflectance-Fourier transform infrared spectroscopy

(Bruker VERTEX 80, Germany) combined platinum diamond ATR was used for recording ATR-FTIR data. The frequency range was 400-4000 cm⁻¹ with a resolution of 4 cm⁻¹.

Thermal analysis

Thermal gravimetric analysis (TGA)

The composite film samples were examined using thermal gravimetric (TGA) apparatus (Shimadzu TGA-50). Under inert nitrogen atmosphere, the temperature was raised from 0 to 1000° C at a heating rate of 10° C/min.

Differential scanning calorimetry (DSC)

To determine the glass transition temperature (Tg), differential scanning calorimetry (DSC) was used. The analysis was performed in a temperature range 0 to 170° C at a rate of 10° C/min.

Morphology analysis prepared

Scanning electron microscopy (SEM) (JEOL Model JSM-T20) was used to study the morphology of the prepared films.

Mechanical properties

Mechanical properties of the films were studied by measuring the tensile using the Zwick/Roell machine (Germany). Dumbbell shape strips of 1.5 cm width and 8 cm length were used for the tensile measurements at a crosshead speed of 2 mm/min at 25° C. The results were averaged of three replicates measurement of each sample.

Burst strength

Burst strength was investigated using TAPPI Standard test method 403 om-97 using Mullen (Perkins, Chicopee, MA, USA).

The contact angles

The contact angles were estimated by analyzing the images of water drop of $10\,\mu$ l by Image J1.52 h, USA program.

Air permeability test

BENDTSEN, Smoothness and Porosity Tester (Model 5, No. 11772, Andersson and Sorensen, Copenhagen), was used for studying air permeability.

Water vapor permeability rate (WVPR)

The WVPR (g/m²h) of films was detected using the ASTM standard E96- 95 (ASTM Standards, 1995b) with a modification [26,27]. Film specimens were mounted on glass vials containing a predetermined weight of distilled water (RH=100%). These vials were located in a chamber at 25°C and RH=50%. Every day, the vials weights were recorded for a total of 8 days.

Microbiological analyses

Microorganisms and media

In order to assess the antibacterial efficacy of the produced sheets, Gram positive and negative bacterial strains (Bacillus mycoides and Escherichia coli), and a nonfilamentous fungus strain (Candida albicans) were utilized to exhibit various microbial populations. The three microorganisms were grown on slants of modified nutritional agar medium (g/L), containing the following ingredients: Peptone, 3, Yeast Extract, 1.5, Meat Extract, 1.5, Glucose, 0.5, NaCl, 0.25, and Agar, 20.0 at pH 7.0. After having fully grown, the inoculated microbial culture was preserved at 4°C in agar slants. The used microbial strains were seeded and cultivated at 37°C on nutritional agar medium (70148 Nutrient Agar, Fluka, Spain) with the following components (g/L): Yeast extract, 2.0; peptone, 5.0; sodium chloride, 5.0; and agar, 15.0. Meat extract, 1.0. In order to make a nutrient agar medium, 28 g of the ready medium was suspended in 1.0 L of distilled water, and the pH level was then adjusted to 7.0. Then, it was heated until it was entirely dissolved. Afterward, it was autoclaved for 20 min at 121°C with 1.5 atmospheres to sanitize them [28,29]. The used compounds were of high purity and analytical grade.

Agar diffusion technique for measuring antibacterial activity

To test the produced sheets' efficacy as antimicrobial agents, microbial cultures were seeded in nutritional agar medium (70148 Nutrient Agar, Fluka) using 100 μ L (1×10⁷ CFU) of overnight re-suspended culture as follows: After incorporating the designated microbial inoculums into the melted medium at around 45°C, the nutrient agar medium was then poured into a series of Petri dishes. Plates were allowed to harden at room temperature before discs (15 mm) from ready-made films were adhered to the infected agar plates' surface. These plates were cultured overnight at 37°C, and the generated inhibitory zones were evaluated [30–32].

Results and discussion

The spectrum of gelatin showed in Figure 1a. Very strong, broad peaks were observed at of 3281.94 cm^{-1} and 3080 cm^{-1} , which are assigned to O-H stretch and N-H vibration. The Amide I peak at 1632.89 is related

Figure 1



FTIR (a) Gelatin, (b) Xanthan, (c) HEMC, (d) HEMP, (e) XAGHEP9, (f) XAGHEP6, and (g) XAGHEP3.

to C=O stretch and N-H bond bending. Amide II band in the region of 1550-1400 cm⁻¹ is assigned to the deformation of N-H bonds [33].

Due to stretching vibrations of the O-H group, the xanthan (Fig. 1b) had a broad peak at 3300.70 cm^{-1} . Another peak at 1719.35 cm⁻¹ is caused by C=O stretching vibrations. Peaks at 1602.14 cm⁻¹ and 1406.80 cm⁻¹ are caused by asymmetrical and symmetrical vibrations of COO⁻ groups, respectively [34].

HPMC is functionally very similar to hydroxyethyl methylcellulose (HEMC). Both HEMC and HPMC are methylcellulose derivatives with ethyl and propyl group replacements, respectively [21]. Fig. 1e-g shows the composite spectra. The broadness of the (OH) band observed in the 3000-3500 cm⁻¹ region designates the presence of free hydroxyl groups and numerous of hydrogen bonded (O-H) stretching vibration. These individual bands observed at 2921 and 2880 cm⁻¹ result from antisymmetric and symmetric stretching bands of the CH2 groups, respectively. In Figure 1, the peak intensities and positions of the bands, as well as the appearance of new overlapping between bands, are the only differences apparent between composite samples and pure gelatin.

Thermal analysis

TGA analysis was performed on samples XAGHEP 6 and XAGHEP 9 to evaluate the effect of adding 0.3% of HEMC and HPMC, respectively, to gelatinxanthan composite film (Table 2). Two major phases were detected in the samples based on these thermograms. The first phase, which occurs between





TGA of the prepared films.

25 and 150°C, may be associated with residual water volatilization. The heating decomposition is responsible for the second phase of the thermal process, which occurs between 250 and 400°C. This includes thermal decomposition of glycerol, the main backbone of xanthan, as well as gelatin protein components [12] (Fig. 2).

Figure 2 shows that the thermal stability of XAGHEP 9 is better than XAGHEP 6. The decomposition started at 317°C for XAGHEP 9, whereas XAGHEP 6 decomposition started at 254.25°C. The residual weight percentages at 500°C were 47.09 and 50.8% for XAGHEP 6 and XAGHEP 9, respectively. Compared with pure gelatin films, XAGHEP 6 and XAGHEP 9 possess a high residual weight percentage at 500°C.

Differential scanning calorimetry (DSC)

In terms of material properties, the glass transition temperature (Tg) is of particular importance in polymer manufacturing processes as well as quality control in food and pharmaceutical industry [35].

Figure 3





Below Tg, the molecular mobility is frozen and the elastomer becomes rigid and glassy. Therefore (Tg) is the temperature at which molecular mobility begins to take place. Figure 3 illustrates the DSC curves of XAGHEP 6, XAGHEP 9 and XAGHEP 10. It reveals that the thermal transitions were varied in the range from room temperature up to 450°C. The maximum peaks, Tg, were obtained at 93.4 and 84.17°C for XAGHEP 6 and XAGHEP 9, respectively, whereas for pure gelatin (XAGHEP 10) was observed at 62.9°C. After Tg peaks, endothermic peaks were observed for XAGHEP 6, XAGHEP 9 and XAGHEP 10 as a result of the ordered triple helical structure of gelatin chains. There is an exothermic peak found in DSC of pure gelatin at 296.2°C which is attributed to intermolecular side chain breaking down

Table 1 Thermal analysis of the prepared films

[36]. Table 1 summarizes glass temperatures and the variations of enthalpy (Δ H), energy released or absorbed during the phase transition, of pure gelatin and the composite samples.

Morphological study

Figure 4 shows SEM images of the surface the gelatin composite films. Figure 4a represents the surface morphology of the pure gelatin film as flat and dense surface with some protrusions. As shown in Figure 4b the addition of xanthane improves the surface appearance. It shows a smooth flat surface with no protrusions or pits. Mainly, this behavior is likely attributed to polysaccharides ability to change the protein surface through inhibition of protein network structure [7]. Both XAGHEP 3 and XAGHEP 6

| | Thermogravimetric analysis | | | | | | | Differential scanning calorimetric analysis | |
|-----------|----------------------------|-------------|----------|--------------|-------------|----------|------------------|---|--|
| | First peak | | | Maximum peak | | | Glass Transition | | |
| Sample | Onset (°C) | Offset (°C) | Max (°C) | Onset (°C) | Offset (°C) | Max (°C) | Tg (°C) | ΔH (J/g) | |
| XAGHEP 6 | 36.61 | 150.38 | 96.59 | 194.14 | 356.50 | 254.14 | 93.07 | 151.07 | |
| XAGHEP 9 | 31.50 | 138.60 | 87.66 | 276.21 | 356.47 | 317.06 | 84.17 | 259.47 | |
| XAGHEP 10 | 43.00 | 144.6 | 90.00 | 316.46 | 338.27 | 325.36 | 62.91 | 460.31 | |

Table 2 Composition a mechanical properties of the prepared films

| Sample | GELATIN %w/v | XANTHAN % w/v | HEMC % w/v | HPMC % w/v | Thickness (mm) | Elongation % | Air permeability (ml/sec) |
|-----------|--------------|---------------|------------|------------|----------------|--------------|------------------------------|
| XAGHEP 1 | 1.2 | 0.1 | 0 | 0 | 0.15 | 13.00 | Nil |
| XAGHEP 2 | 1.2 | 0.2 | 0 | 0 | 0.15 | 14.67 | Nil |
| XAGHEP 3 | 1.2 | 0.3 | 0 | 0 | 0.15 | 15.00 | Nil |
| XAGHEP 4 | 1.2 | 0.2 | 0.1 | 0 | 0.15 | 25.33 | Nil |
| XAGHEP 5 | 1.2 | 0.2 | 0.2 | 0 | 0.15 | 50.00 | Nil |
| XAGHEP 6 | 1.2 | 0.2 | 0.3 | 0 | 0.15 | 31.00 | Nil |
| XAGHEP 7 | 1.2 | 0.2 | 0 | 0.1 | 0.15 | 59.33 | Nil |
| XAGHEP 8 | 1.2 | 0.2 | 0 | 0.2 | 0.15 | 14.33 | Nil |
| XAGHEP 9 | 1.2 | 0.2 | 0 | 0.3 | 0.15 | 19.00 | Nil |
| XAGHEP 10 | 1.2 | 0 | 0 | 0 | 0.15 | 293.33 | Nil |

Figure 4



SEM images of composite film (a) XAGHEP 10, (b) XAGHEP 3 and (c) XAGHEP 6 with X300 magnification.

(Fig. 4b and c) composite films shows a smooth, homogenous and flat appearance referring to the good compatibility between polymer blends.

Mechanical properties

Film elongation investigation is aimed to measure the competency of samples to resist changes of shape with crack-free.

By adding different ratios of xanthan, a decrease in elongation percentage and tensile value (Table 2 and Fig. 5) compared with the blank gelatin film was observed. This may attributed to the reduction of the movement of the gelatin chains due to physical crosslinking (electrostatic attraction) [13].

According to Table 2 and Figure 5, an improvement in elongation percentage and tensile value is shown by the addition of HEMC to gelatin-xanthan composite. The same behavior was observed when HPMC incorporated in gelatin-xanthan composite. Nevertheless, the results (Table 2 and Fig. 5) showed a better performance of HEMC compared with HPMC.

Air permeability

Air permeability is an important parameter in packaging. It illustrates the resistance of the film to gas flow. Table 2 showed the effect of film composition on an air permeability property. No air permeability was observed for all gelatin–xanthan samples because of the good interaction between gelatin and xanthan through electrostatic attraction and hydrogen bonding [5]. This performance did not change by incorporation of either HEMC or HPMC.

Figure 5





Burst strength

This test evaluates the tolerance of packaging film to withstand internal pressurization until it bursts. The results (Fig. 6) show that the composite that contained 0.2% of HEMC is better than the other ratios of HEMC.

Figure 6 shows that the bursting strength increased with increasing HPMC content in gelatin-xanthan composite. The highest bursting strength was achieved for sample XAGHEP 9 where the gelatin-xanthan composite is blended with 0.3% HPMC.

The contact angle

Despite the benefits of the hydrophilic character of gelatin in many applications [9,10,37], in packaging applications, it is essential to suppress the hydrophilicity of the polymeric material to improve food shelf life [13].

Hydrophobic and hydrophilic surfaces can be determined by computing the contact angle of water droplets. Generally, the substrate is termed hydrophobic when the angle between the material surface and the droplet surface extends from 90° [38].

Figure 7 shows the effect of the film compositions on the contact angle. All samples show a hydrophilic character which is attributed to the abundance of hydroxyl and amino groups in composit films. However, XAGHEP 3 and XAGHEP 9 had a much higher contact angle, 86° and 86.25°,





The effect of composite film's composition on burst strength.



respectively, compared with pure gelatin (82.9°). Electrostatic attraction between gelatin and xanthan and hydrogen bonding may have contributed to this improvement [12].

Water vapor permeability rate

The water vapor permeability rate was evaluated by observing the loss of weight against time. The linear regression of the sample curves was used to determine the slope in g/h. The water vapour permeability rate was calculated using the following equation [26,27],

$$WVPR = \frac{slope}{f\,ilm\,area\,(m^2)}$$

Figure 8 shows the effect of the film composition on WVPR of the prepared films. The pure gelatin film had WVPR of 61.04 g/m^2 h. A slight increase of WVPR had been observed by adding xanthan, except for the sample XAGHEP 2 where WVPR equals 58.39 g/m^2 h. The addition of HEMC to gelatin-xanthan composite had decreases WVPR of the samples XAGHEP4 and XAGHEP5, but increasing HEMC content (XAGHEP 6) leads to a higher value of WVPR. Compared with gelatin-xanthan composite, the WVPR values declined along with increasing the HPMC content in the film. This behavior may be a result of good compatibility between HPMC and the two main components of the composite due to the strong hydrogen bonds between HPMC and gelatin-xanthan composite and the good moisture barrier properties of HPMC [39].

Antimicrobial activity

Agar diffusion technique for measuring antibacterial activity The antibacterial activity of the produced sheets against Gram-positive bacteria *B. mycoides*, a nonFigure 8



Water vapor permeability rates of the prepared samples.

filamentous fungus called C. albicans, and Gramnegative bacteria E. coli was examined in the current work. The growth inhibition zone surrounding the discs was measured and converted into millimeters to evaluate the antibacterial activity. The findings were listed in Table 3 which shows how effective various produced sheets are as antibacterial agents. The results show that various forms of treatments and mixing percentages have various relative impacts on the effectiveness towards microbial development, and which was discovered to be dependent on the contents proportions. In this regard, sample 2 demonstrated the highest Gram-negative antibacterial activity against E. coli (36 mm), whereas sample 9 demonstrated the highest antimicrobial activities as a function of inhibition zone diameter against B. mycoides (31 mm) as a Gram-negative bacteria and against C. albicans (35 mm) as a nonfilamentous fungus. The strongest antibacterial activity of the studied samples was demonstrated in this respect against E. coli, in contrast to that observed against B. mycoides and C. albicans. Sample 10 had no antibacterial effects on any of the tested microorganisms (Table 3). Sample 10 (without xanthan) had no antibacterial effects on any of the tested microorganisms (Table 3), which declares the efficiency of xanthan as a bioactive compound. Many factors, including the presence of hydroxyl groups, the amount of unmethylated uronic acid (Scheme 2), the percentage of glucose, viscosity, solubility rate, configuration changes, molecular size, surface area, and others, can affect the antibacterial activity of polysaccharides, which demonstrated effective bactericidal activity against a wide range of gramnegative and gram-positive bacterial infections [40].

| Table 3 Assessment of antibacterial activity | y of prepared file | ms using the agar | diffusion method |
|--|--------------------|-------------------|------------------|
|--|--------------------|-------------------|------------------|

| XAGHEP | GELATIN % | XANTHAN % | HEMC % | HPMC % | Inhibition zone *(mm) | | |
|--------|-----------|-----------|--------|--------|-----------------------|------------|------------|
| | | | | | E. coli | B.mycoides | C.albicans |
| 1 | 1.2 | 0.1 | 0 | 0 | 30 | 15 | 15 |
| 2 | 1.2 | 0.2 | 0 | 0 | 36 | 15 | 15 |
| 3 | 1.2 | 0.3 | 0 | 0 | 35 | 30 | 15 |
| 4 | 1.2 | 0.2 | 0.1 | 0 | 35 | 30 | 19 |
| 5 | 1.2 | 0.2 | 0.2 | 0 | 35 | 28 | 19 |
| 6 | 1.2 | 0.2 | 0.3 | 0 | N.D. | N.D. | N.D. |
| 7 | 1.2 | 0.2 | 0 | 0.1 | 35 | 26 | 0 |
| 8 | 1.2 | 0.2 | 0 | 0.2 | 32 | 24 | 20 |
| 9 | 1.2 | 0.2 | 0 | 0.3 | 33 | 31 | 35 |
| 10 | 1.2 | 0 | 0 | 0 | 0 | 0 | 0 |

One hundred micro-liters $(1 \times 10^7 \text{ CFU})$ of overnight re-suspended culture were used to seed microbial cultures in nutritional agar medium. On the surface of the infected agar plates, 15-mm discs from pre-made films were adhered. Culture plates were incubated at 37 °C for an overnight period, and the inhibitory zones produced were assessed.

In this regard, a xanthan-oligosaccharide exhibited antibacterial efficacy against *S. aureus*, according to Wang *et al.* [41], with an inhibitory zone of 33.51 mm. They showed that xanthan's mechanism of action on *S. aureus* indicated that it not only influenced cell membrane permeability but also prevented the growth of biofilms and hindered Ca^2 ⁺-Mg²⁺-ATPase activity on the cytomembrane of the pathogen [41].

Conclusion

Three groups of green biopolymer films were successfully prepared based on gelatin. Xanthane was incorporated in three different ratios with gelatin, then the optimum ratio was further modified by two cellulose derivatives, HEMC and HPMC. The prepared films were characterized by AT-IR, thermal analysis and differential gravimetric scanning calorimetry. The composite films show different behaviour through mechanical properties, burst water vapor permeability rate, strength, air permeability tests. The addition of HEMC and HPMC has improved the mechanical, moisture barrier properties and thermal stability of gelatinxanthan composites. However, the thermal stability XAGHEP 9 (0.3 of HPMC) is better than XAGHEP 6 (0.3 of HMEC). The residual weight percentages at 500°C were 47.09 and 50.8% for XAGHEP 6 and XAGHEP 9, respectively, which were higher than pure gelatin film. Finally, these improvements showed that the addition of xanthan; hydroxyethyl methyl cellulose (HEMC), and hydroxyl propylmethyl cellulose (HPMC) to gelatin film enhanced its properties. The addition of xanthan to gelatin had a great impact to improve gelatin antibacterial activity. The antibacterial study of the composite films showed the potentiality of these natural composites to be used as edible active packaging.

Acknowledgments

The authors gratefully acknowledge the provided facilities by the National Research Centre.

Statements and Declarations

Author Contributions: Material preparation, data collection, and analysis were performed by Salah A. A. Mohamed, Eman A. Ali, and Alaa E. Elsayed. The antimicrobial study was performed by Abdelmageed M. Othman. The first draft of the manuscript was written by Eman A. Ali, and all authors offered a scientific explanation for the finished manuscript. All authors reviewed and gave their approval for the final article.

Data availability: The data generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate: The authors claim that no humans or animals were used in the study.

Financial support and sponsorship

Nil.

Conflicts of interest

Competing Interests: The authors have no relevant financial or nonfinancial interests to disclose.

References

- 1 Quirós-Sauceda AE, Ayala-Zavala JF, Olivas GI, González-Aguilar GA. Edible coatings as encapsulating matrices for bioactive compounds: a review. J Food Sci Technol 2014; 51:1674–1685.
- 2 Mansour MF, Arisha AH, Al-Gamal M, Yahia R, Elsayed AA, Saad S, El-Bohi KM. Moringa oleifera leaves extract ameliorates melamine-induced testicular toxicity in rats. Adv Anim Vet Sci 2020; 8:47–54.
- 3 Abdelhamid AE, Yousif EAA, El-Saidi MMT, El-Sayed AA. Polyvinyl Alcohol Food Packaging System Comprising Green Synthesized Silver Nanoparticles. Indones J Chem 2020; 21:350.

- 4 El-Sayed AA, Amr A, Kamel OMHM, El-Saidi MMT, Abdelhamid AE. Ecofriendly fabric modification based on AgNPs@ Moringa for mosquito repellent applications. Cellulose 2020; 27:8429–8442.
- 5 Said NS, Sarbon NM. Physical and Mechanical Characteristics of Gelatin-Based Films as a Potential Food Packaging Material: A Review. Membranes (Basel) 2022; 12:442–468.
- 6 Mohamed SAA, El-Sakhawy M, Nashy ESHA, Othman AM. Novel natural composite films as packaging materials with enhanced properties. Int J Biol Macromol 2019; 136:774–784.
- 7 Lu Y, Luo Q, Chu Y, Tao N, Deng S, Wang L, Li L. Application of Gelatin in Food Packaging: A Review. Polymers (Basel) 2022; 14:436–455.
- 8 Luo Q, Hossen MA, Zeng Y, Dai J, Li S, Qin W, Liu Y. Gelatin-based composite films and their application in food packaging: A review. J Food Eng 2022; 313:110762–110778.
- 9 Irfan NI, Mohd Zubir AZ, Suwandi A, Haris MS, Jaswir I, Lestari W. Gelatinbased hemostatic agents for medical and dental application at a glance: A narrative literature review. Saudi Dent J 2022; 34:699–707.
- 10 Simon M, Maumus M, Legrand B, Sole L, Dufaud M, Mehdi A, et al. Gelatin modified with alkoxysilanes (GelmSi) forms hybrid hydrogels for bioengineering applications. Biomater Adv 2023; 147:213321–213329.
- 11 Nur Hazirah MASP, Isa MIN, Sarbon NM. Effect of xanthan gum on the physical and mechanical properties of gelatin-carboxymethyl cellulose film blends. Food Packag Shelf Life 2016; 9:55–63.
- 12 Shan P, Wang K, Yu F, Yi L, Sun L, Li H. Gelatin/sodium alginate multilayer composite film crosslinked with green tea extract for active food packaging application. Colloids Surfaces A Physicochem Eng Asp 2023; 662:131013–131027.
- 13 Yavari Maroufi L, Norouzi R, Ramezani S, Ghorbani M. Novel electrospun nanofibers based on gelatin/oxidized xanthan gum containing propolis reinforced by Schiff base cross-linking for food packaging. Food Chem 2023; 416:135806–135816.
- 14 Mohsin A, Zaman WQ, Guo M, Ahmed W, Khan IM, Niazi S, et al. Xanthan-Curdlan nexus for synthesizing edible food packaging films. Int J Biol Macromol 2020; 162:43–49.
- 15 Shibaev A.V., Muravlev DA, Muravleva AK, Matveev V.V., Chalykh AE, Philippova OE. pH-Dependent gelation of a stiffanionic polysaccharide in the presence of metal ions. Polymers (Basel) 2020; 12:1–15.
- 16 Raschip IE, Fifere N, Varganici CD, Dinu MV. Development of antioxidant and antimicrobial xanthan-based cryogels with tuned porous morphology and controlled swelling features. Int J Biol Macromol 2020; 156:608–620.
- 17 Kurt A, Toker OS, Tornuk F. Effect of xanthan and locust bean gum synergistic interaction on characteristics of biodegradable edible film. Int J Biol Macromol 2017; 102:1035–1044.
- 18 Wu Y, Ding W, He Q. The gelation properties of tara gum blended with \$k \$-carrageenan or xanthan. Food Hydrocoll 2018: 77:764–771.
- 19 Zhu Y. Fabrication and characterization of the enhanced tensile strength xanthan/curdlan/gelatin blend films for food-packaging applications. IOP Conf Ser Earth Environ Sci 2021; 657:012078–0120785.
- 20 Ban SJ, Rico CW, Um IC, Kang MY. Antihyperlipidemic effects of hydroxyethyl methylcellulose with varying viscosity in mice fed with high fat diet. Food Res Int 2012; 48:1–6.
- 21 Ban SJ, Rico CW, Um IC, Kang MY. Comparative evaluation of the hypolipidemic effects of hydroxyethyl methylcellulose (HEMC) and hydroxypropyl methylcellulose (HPMC) in high fat-fed mice. Food Chem Toxicol 2012; 50:130–134.
- 22 Bilbao-Sainz C, Avena-Bustillos RJ, Wood DF, Williams TG, McHugh TH. Composite edible films based on hydroxypropyl methylcellulose reinforced with microcrystalline cellulose nanoparticles. J Agric Food Chem 2010; 58:3753–3760.
- 23 Malik GK, Mitra J, Kaushal M. Rheology of nano ZnO Hydroxypropyl Methylcellulose (HPMC) based suspensions and structural properties of resulting films. J Food Eng 2023; 337:111187–111198.

- 24 Zheng M, Chen J, Tan KB, Chen M, Zhu Y. Development of hydroxypropyl methylcellulose film with xanthan gum and its application as an excellent food packaging bio-material in enhancing the shelf life of banana. Food Chem 2022; 374:131794–131802.
- 25 Gómez-Aldapa CA, Velazquez G, Gutierrez MC, Rangel-Vargas E, Castro-Rosas J, Aguirre-Loredo RY. Effect of polyvinyl alcohol on the physicochemical properties of biodegradable starch films. Mater Chem Phys 2020; 239:122027–122034.
- 26 ASTM E 95-96. Standard Test Methods for Water Vapor Transmission of Materials (E96-E95). West Conshohocken, United States: Annual Books of ASTM Standards, 552, 785-792; 2013.
- 27 Xu YX, Kim KM, Hanna MA, Nag D. Chitosan-starch composite film: preparation and characterization. Ind Crops Prod 2005; 21:185– 192.
- 28 Abouelnaga AM, Meaz TM, Othman AM, Ghazy RA, El Nahrawy AM. Probing the Structural and Antimicrobial Study on a Sol-Gel Derived Velosef-Loaded Bioactive Calcium Magneso-Silicate Xerogel. Silicon 2020; 13:623–631.
- 29 Sroor FM, Othman AM, Tantawy MA, Mahrous KF, El-Naggar ME. Synthesis, antimicrobial, anti-cancer and in silico studies of new urea derivatives. Bioorg Chem 2021; 112:104953–104968.
- 30 Kamel MG, Sroor FM, Othman AM, Mahrous KF, Saleh FM. Structurebased design of novel pyrazolyl-chalcones as anti-cancer and antimicrobial agents: synthesis and in vitro studies. Monatshefte für Chemie – Chem Mon 2022; 153:211–221.
- 31 Mohamed SAA, Salama A, El-Sakhawy M, Othman AM. Sustainable cellulose nanocrystal reinforced chitosan/HPMC bio-nanocomposite films containing menthol oil as packaging materials. Cellul Chem Technol 2021; 55:649–658.
- 32 Sroor FM, Othman AM, Aboelenin MM, Mahrous KF. Anticancer and antimicrobial activities of new thiazolyl-urea derivatives: gene expression, DNA damage, DNA fragmentation and SAR studies. Med Chem Res 2022; 31:400–415.
- 33 Hassan N, Ahmad T, Zain NM, Awang SR. Identification of bovine, porcine and fish gelatin signatures using chemometrics fuzzy graph method. Sci Rep 2021; 11:1–10.
- 34 Zheng M, Lian F, Xiong Y, Liu B, Zhu Y, Miao S, et al. The synthesis and characterization of a xanthan gum-acrylamide-trimethylolpropane triglycidyl ether hydrogel. Food Chem 2019; 272:574–579.
- 35 Borredon C, Miccio LA, Cerveny S, Schwartz GA. Characterising the glass transition temperature-structure relationship through a recurrent neural network. J Non-Crystalline Solids X 2023; 18:100185–100193.
- 36 Shehap AM, Mahmoud K, El-Kader M, El-Basheer T. Preparation and Thermal Properties of Gelatin/TGS Composite Films. Middle East J Appl Sci 2015; 5:157–170.
- 37 Nasr RA, Abobakr E. Polyethersulfone / gelatin nano-membranes for the Rhodamine B dye removal and textile industry effluents treatment under cost effective condition. J Environ Chem Eng 2022; 10:107250–107260.
- 38 Ali EA, Nada AA, Al-Moghazy M. Self-stick membrane based on grafted gum Arabic as active food packaging for cheese using cinnamon extract. Int J Biol Macromol 2021; 189:114–123.
- 39 Janjarasskul T, Krochta JM. Edible packaging materials. Annu Rev Food Sci Technol 2010; 1:415–448.
- 40 Mirzadeh M, Arianejad MR, Khedmat L. Antioxidant, antiradical, and antimicrobial activities of polysaccharides obtained by microwaveassisted extraction method: A review. Carbohydr Polym 2020; 229:115421–115433.
- 41 Wang Z, Yang Q, Wang X, Li R, Qiao H, Ma P, et al. Antibacterial activity of xanthan-oligosaccharide against Staphylococcus aureus via targeting biofilm and cell membrane. Int J Biol Macromol 2020; 153:539–544.