FABRICATION AND CHARACTERIZATION OF CHITOSAN-GELATIN COMPOSITE FILM

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ABSTRACT

Recent research is focusing on the development of bio-packaging materials by mixing different polymers to enhance the bonding strength within the matrix and combining favorable properties of the constituent materials. This work developed a biopackaging material by combining chitosan, gelatin, and glycerol. The effect of the molecular weight of chitosan and the composition of the casting solution on the properties of the biopacking material was investigated. Increasing chitosan concentration resulted in an increased mechanical strength and lowered water solubility. Thermogravimetric analysis and UV-vis spectroscopy analysis suggested that increasing gelatin concentration would allow for a more elastic packaging with higher transparency and better thermal stability. From this result, a thin (< 0.05 mm) biopackaging material with a mechanical strength of up to 17 N/mm^2 with an allowed 30% elongation at break was attained. The smoother surface structure under scanning electron microscope and the variations in properties between samples were allocated to the inherent characteristics of chitosan and gelatin and the promotion of intermolecular bonding from the glycerol plasticizer. These results therefore not only develop a functional packaging material with multiple applications in food and healthcare, but also provide a deeper understanding on the linking behavior of biopolymers.

1. INTRODUCTION

Following recent trends in sustainable development, increasing concerns regarding food safety has given rise to research for safe biodegradable packaging. Within this scope, combinations of biopolymer material with favorable properties are on the increase for suitable replacement of the traditional plastics (Wang et al., 2024).

Chitosan is a polymer product of chitin deacetylation, deriving from waste crustacean shells, which is gaining much interest due to favorable properties of low toxicity, high biodegradability, inherent antimicrobial properties and high biocompatibility (Haghighi et al., 2020). The material is widely used in the pharmaceutical and food industry, focusing on the development of self-preserving packaging by utilizing its inherent antibacterial activity and unique characteristics. This gives rise to a wide variety of chitosan-containing products, including hydrogel beads, films, fibers, and biopackaging.

Chitosan can be combined with other polymers to improve its functionality (de Mesquita et al., 2012). Gelatin is a biological polymer prepared by naturally reducing collagen with heat. It comes in the form of a clear, colorless or pale-yellow powder and is almost tasteless. Gelatin can create clear and flexible polymer where, depending on the type of gelatin, it can undergo poly-ionic complexation with positively or negatively charged agents. Gelatin is very hydrophilic and has good gas barrier properties, making it valuable for tissue engineering application. However, its poor mechanical properties have limited its application as a packaging material, leading to promoted research to improve its mechanical properties (Yin et al., 2018).

Previous research has suggested that when combining chitosan and gelatin, an ionic interaction will occur between the $-NH_2$ group of chitosan and the -COO group of gelatin. Furthermore, the addition of plasticizers such as glycerol can allow for the increase in interactions to increase the mechanical strength, flexibility and barrier properties of the bio-packaging material (Sethi et al., 2022). In this research, the interaction between chitosan and gelatin in a polymer system were evaluated for applications in food preservation **(**Figure 1**).**

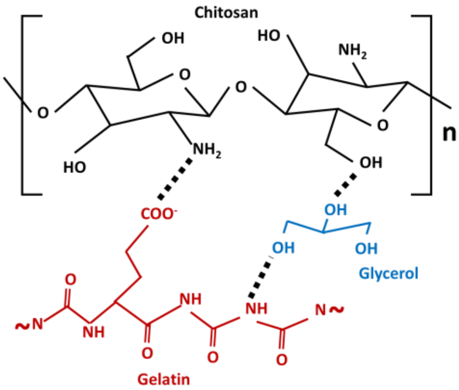

Figure 1. Model of the bonding mechanism between chitosan, gelatin and glycerol.

2. METHODOLOGY

2.1. Materials

Chitosan $(C_6H_{11}O_4N)_n$ (CS) was provided by Vietnam Food Joint Stock Company, Ca Mau, Vietnam with deacetylation level >75%. Gelatin (GE) was purchased from a local vendor, which was food-grade gelatin powder used for baking. All other chemicals, including glycerol ($C_3H_5(OH_3)$) and acetic acid (CH_3COOH) were purchased and used directly without any modifications.

2.2. Synthesis of biopackaging material

The chitosan/gelatin biopackaging fabrication process was referenced from a previous process with suitable adjustment. CS was dissolved in 100 mL of acetic acid 1% by stirring at 60 ºC for 1 h to create a homogeneous chitosan solution. Four types of chitosan with different molecular weight were investigated, including low molecular weight (LW, 158 kDa, ≤150 cPs), medium molecular weight 1 (MW1, 272 kDa, 150-500 cPs), 2 (MW2, 290 kDa; 500-1000 cPs), and high molecular weight (HW, 324 kDa, ≥1000 cPs). The solution was filtered through a 100 µm NMO membrane to remove impurities. The GE solution was prepared by dissolving gelatin in 100 mL of water. Different CS and GE solutions were mixed in 1:1 volume ratio for 30 min so that the final concentration of chitosan and gelatin reaches 0.500, 0.625, 0.750, 0.875, and 1.000 % (w/v). Subsequently, 0.3 mL of glycerol was added to 100 mL of the mixture and mixed for another 30 min. Every 50 mL of the resulting mixture was poured into a 12.4 cm × 15.4 cm stainless steel mold and dried at 60 ºC for 24 h to evaporate, creating a complete film. The final film was removed using a surgical blade and stored in a desiccator for further use.

2.3. Characterization methods

The thickness of the film was measured using a Mitutoyo thickness gauge (Japan) with the smallest scale being 0.001 mm. The measurements were performed at 10 random points and presented as mean ± standard deviation.

The opacity of the film was determined by the ratio of the absorbance at 600 nm using a VIS 754 Stech International UV spectrophotometer (China) and the film thickness (Equation (1)).

$$Opacity = \frac{Abs_{600}}{L} \quad (1)$$

where Abs_{600} is the absorbance of the film at 600 nm and L is the thickness of the sample (m)

The tensile strengths were measured at 25 ºC using a Testometric X350 (UK) following the ASTM D882 standard (ASTM International, 2018). The films were cut into 1 cm × 7 cm pieces and stretched at 50 mm/min using a 1 N load cell.

The water vapor permeability (WVP) tests were according to a modified version of the ASTM E96 standard (ASTM International, 2017). Film samples were firmly wrapped onto plastic cups filled with 6 mL of distilled water where the open diameter is 20 mm. The initial mass and difference in mass of each cup was monitored every hour for 8 h.

The water vapor permeability (WVP) was then defined as Equation (2)

$$WVP = \frac{WPTR.L}{\Delta P}.100\% = \frac{G.L}{t.A.s.(R_1 - R_2)} \times 100\% \quad (2)$$

where WVTR is the water vapor transmission rate ($g.h^{-1}.m^{-2}$). G is the change in weight (g), L is the thickness of the sample (m), ΔP is the vapor pressure difference (Pa), t is the time (h), A is the test area (m^2), s is the saturated vapor pressure at test temperature (Pa), R_1 is the relative humidity in the dish, R_2 is the relative humidity at the vapor sink.

The water solubility of the sample was determined using a previously reported method (Bertolo et al., 2022) where film sample was first dried and then weighed. The membrane was then soaked in 10 mL of distilled water for 7 h at room temperature to allow for dissolution. The insoluble membrane fraction was dried at 105 ºC for 15 min and reweighed. Water solubility was calculated using Equation (3):

$$Solubility = \frac{W_1 - W_2}{W_1} \times 100\% \quad (3)$$

where W_1 is the mass of the sample before soaking in water (g) and W_2 is the mass of the sample after drying in the oven (g).

Field emission scattering electron microscopy (FE-SEM) was performed on a Hitachi S-4800 equipment. Fourier transform infrared spectroscopy (FTIR) results were collected on a Bruker Alpha II spectrometer in the 4000–500 cm^{-1} range.

Thermogravimetry analysis was performed on a Mettler Toledo 3+ (Switzerland) at a heating rate of 10 ºC/min, from 30 to 500 ºC under N_2 (50 mL/min).

3. FINDINGS AND DISCUSSION

3.1. Properties of biofilm

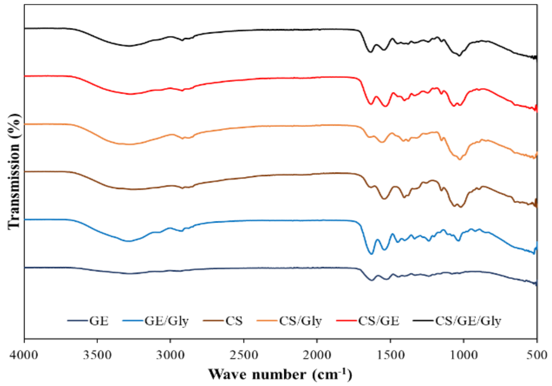

Figure 2. FTIR results of the samples

FTIR results of samples **(Figure 2)** containing chitosan all experience large vibrational bands located at around 3100–3500 cm^{-1} corresponding to the stretching vibrations of -NH and -OH (Karydis-Messinis et al., 2023). The smaller peaks observed at around 2900 cm^{-1} are representative of the stretching vibrations of $-CH_2$ and -CH (Karydis-Messinis et al., 2023). The characteristic peaks at 1635 cm^{-1}, 1555 cm^{-1}, 1065 cm^{-1} and 1020 cm^{-1} are the C=O stretching vibration, -NH bending vibration and C-O stretching vibration, respectively (Hosseini et al., 2013). The carbonyl groups of GE interact ionically with the oppositely charged amino groups (NH_3^+) of CS under acidic conditions. The addition of glycerol to CS and GE is presented as a characteristic peak at 1031 cm^{-1}. Chitosan and gelatin interact with glycerol through the formation of hydrogen bonds between the -OH groups of glycerol and carbonyl and amino groups present in gelatin and chitosan (Sethi et al., 2022).

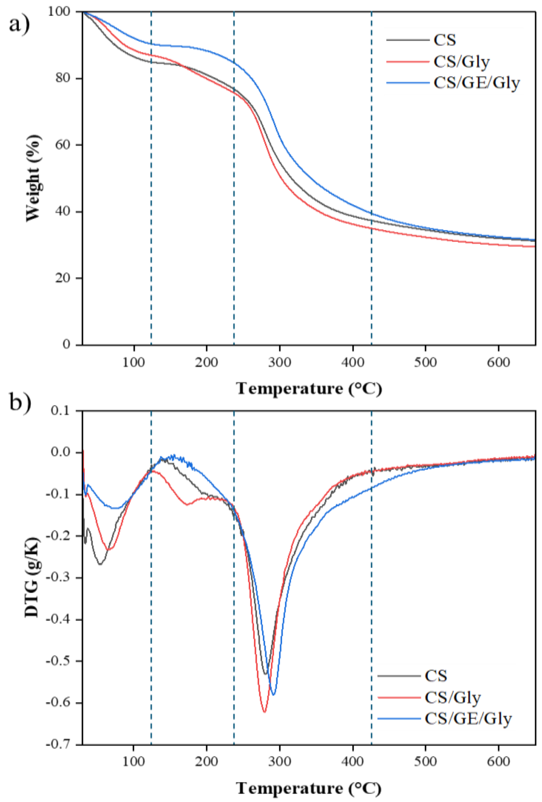

Figure 3. TGA (a) and DTG (b) results of attained biofilm.

Thermogravimetric analyses (TGA) and derivative thermogravimetry (DTG) of CS, CS/Gly and CS/GE/Gly are shown in Figure 3. From DTG plot, 3 distinct peaks corresponding to 3 distinct weight loss regions can be seen at 30–120 °C, 120–240 °C, and 240–420 °C. These weight loss regions were attributed to the bound moisture, unbound moisture, and structural degradation of the materials (Zakuwan et al., 2021). For samples containing GE, the DTG peak intensity and the mass loss due to moisture decreases, meaning that the bonding between CS and GE polymer matrices condenses the structure, reducing the free space that can allow for moisture penetration. In the 30 °C to 100 °C region, all samples showed a slight weight loss due to water removal associated with the hydrophilic groups of the polymer. The volume decreased in the first stage by about 10–15%. The second mass reduction stage occurred in the temperature range from 250 °C to 300 °C corresponding to the decomposition process of chitosan and gelatin.

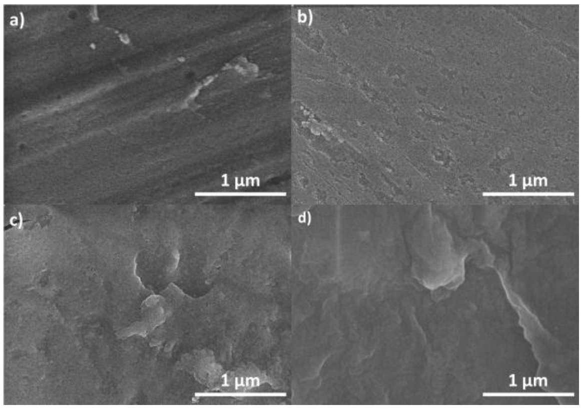

Figure 4. SEM image of CS (a), GE (b), CS/GE (c) and CS/GE/Gly biofilm (d).

FE-SEM image of chitosan/gelatin/glycerol film is presented in Figure 4. Pure CS and pure GE samples showed a relatively simple and flat surface, but with some minor surface pores or defects due to the porous polymer structure. Upon combination, CS/GE no longer exhibited the surface pores but the surface was not as smooth as in the previous case. The addition of Gly to the biocomposite material resulted in a smoother with less accent surface.

3.1. Evaluation of the molecular mass of chitosan

Table 1. Thickness of film samples with different chitosan molecular weight

CS molecular weight (kDa)	Thickness (mm)
158	0.044 ± 0.010
272	0.044 ± 0.008
290	0.045 ± 0.004
324	0.045 ± 0.005

The thickness of four biofilm samples ranged from 0.034 mm to 0.052 mm (Table 1). The results showed that changing the CS molecular weight did not significantly affect the film thickness. Film thickness is mainly affected by the solid content in the solution and the volume of film pouring solution.

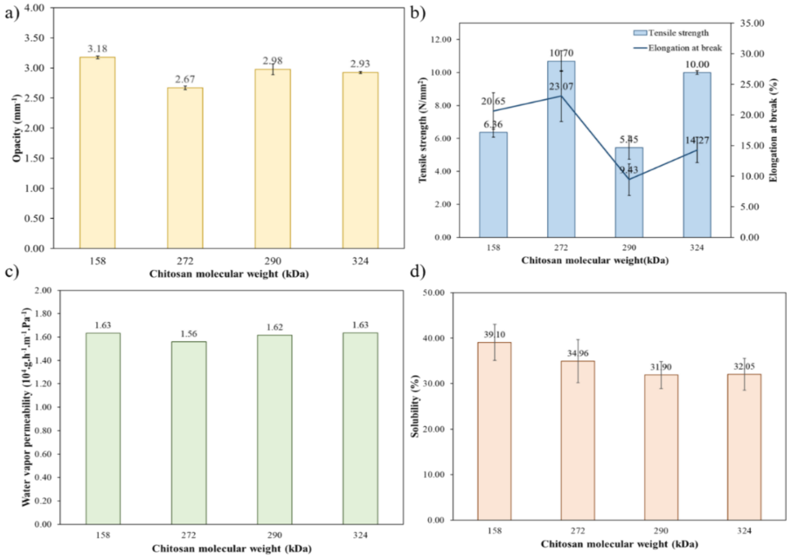

Figure 5. Effect of chitosan molecular weight on the opacity (a), tensile strength (b), water vapor opacity (c), and solubility in water (d) of the biofilm.

The opacity of the four samples ranged from 2.67–3.18 mm^{-1} (Figure 5a). At a medium chitosan molecular weight (272 kDa), the sample exhibited the lowest opacity (2.67 mm^{-1}), suggesting the most appealing to be applied in food preservation.

It has been suggested that polymer samples with higher molecular weight can experience better mechanical strength (Rong Huei, 1996). However, following Figure 5b, the sample with an average chitosan molecular weight of 272 kDa exhibited the highest elongation at break (23.07%), and highest tensile strength (10.70 N/mm^2) because samples at higher molecular weight would be harder and lacks the flexibility during stretching.

The water vapor permeability (WVP) of food packaging films is of high importance and should be as low as possible, since one of the main functions of food packaging films is usually to hinder the transfer of moisture between foods. and ambient air. Figure 5c illustrates the WVP of chitosan/gelatin films with different chitosan molecular weights. In general, chitosan molecular weight has a slight effect on the water vapor permeability of the membrane, WVP was in the range of $1.56–1.63 \times 10^{-4}$ $g.h^{-1}.m^{-1}.Pa^{-1}$.

The water solubility of samples with different molecular weights was relatively equal (31.90–39.10%) and decreased with increasing molecular weight of chitosan (Figure 5d). Medium molecular weight chitosan had high solubility which might be due to reduced intermolecular interactions like Van der Waals forces and hydrogen bonding (Panda et al., 2019).

3.1. Evaluation of chitosan concentration

When increasing the chitosan concentration from 0.5% to 1.0%, the film thickness ranged from 0.030 to 0.052 mm (Table 2).

Figure 6a shows the influence of CS concentration on the opacity of the CS/GE/Gly film. The opacity of the film slightly fluctuated from 2.30 mm^{-1} to 3.00 mm^{-1} thanks to the clearer nature of GE in comparison to CS.

Table 2. Thickness of film samples with different chitosan molecular weight

CS concentration (%)	Thickness (mm)
0.500	0.035 ± 0.005
0.625	0.044 ± 0.007
0.750	0.044 ± 0.008
0.875	0.044 ± 0.005
1.000	0.043 ± 0.005

The tensile strength of the film samples increased from 9.35 N/mm^2 to 16.43 N/mm^2 when increasing the CS concentration in the film pouring solution. The film containing 1.0% chitosan had the highest tensile strength value. The elongation of the film presents its ability to resist shape changes without forming cracks, which ranged from 21.91 to 38.02 % and increased with increasing chitosan concentration.

When increasing the CS concentration from 0.5% to 1.0%, WVP fluctuated slightly in the range of $1.35–1.65 \times 10^{-4}$ $g.h^{-1}.m^{-1}.Pa^{-1}$ (Figure 6c). In general, the water vapor transmission rate depends on both the diffusivity and the solubility of water molecules in the membrane matrix.

When increasing the chitosan concentration, the CS/GE/Gly film significantly decreased its solubility by 1.88 times from 42.39% to 22.46%. The values obtained are the result of interactions between both biopolymers, such as electrostatic forces and hydrogen bonding.

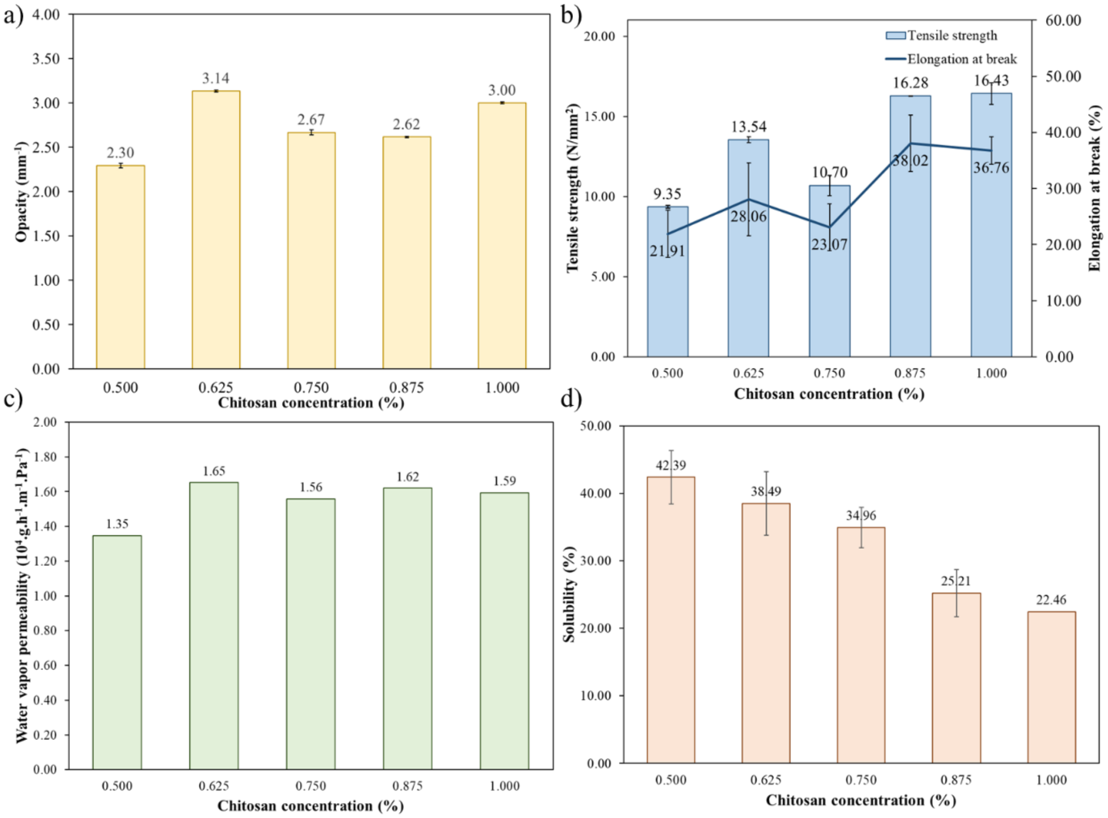

Figure 6. Effect of chitosan concentration on the opacity (a), tensile strength (b), water vapor opacity (c), and solubility in water (d) of the biofilm.

3.2. Evaluation of gelatin concentration

Table 3. Thickness of film samples with different gelatin molecular weight

GE concentration (%)	Thickness (mm)
0.500	0.035 ± 0.007
0.625	0.039 ± 0.007
0.750	0.044 ± 0.008
0.875	0.051 ± 0.007
1.000	0.051 ± 0.005

The film thickness ranged from 0.028 to 0.058 mm when increasing the gelatin concentration from 0.5% (w/v) up to 1.0% (w/v). (Table 3).

The opacity of the CS/GE/Gly film fluctuated slightly in the range of 2.24 – 2.83 mm^{-1} **(**Figure 7a), in contrast to the case of increasing chitosan concentration.

Figure 7b shows the significant influence of GE concentration on the tensile strength and elongation of CS/GE/Gly films. When increasing the gelatin concentration from

0.625% to 0.750%, the tensile strength decreased 1.58 times (from 16.90 N/mm^2 to 10.70 N/mm^2) and the elongation at break also decreased sharply by 1.41 times (from 32.52% to 23.07%). In general, tensile strength and elongation decreased as gelatin concentration increased, which is due to the presence of gelatin reducing the crystallization ability of chitosan in the film.

When increasing the gelatin concentration to 1.0%, the WVP of the CS/GE/Gly film increased significantly by 1.52 times compared to the film with a gelatin concentration of 0.5% (Figure 7c). Membrane WVP changes are associated with the presence of functional groups, such as hydroxyl and amino groups in the structural framework, which are often binding sites for water molecules.

The solubility of the membrane when increasing the gelatin concentration is shown in Figure 7d. Increasing the gelatin concentration increases its solubility in water. Films with high gelatin concentrations have high solubility due to the hydrophilic nature of gelatin or due to the solubility of gelatin.

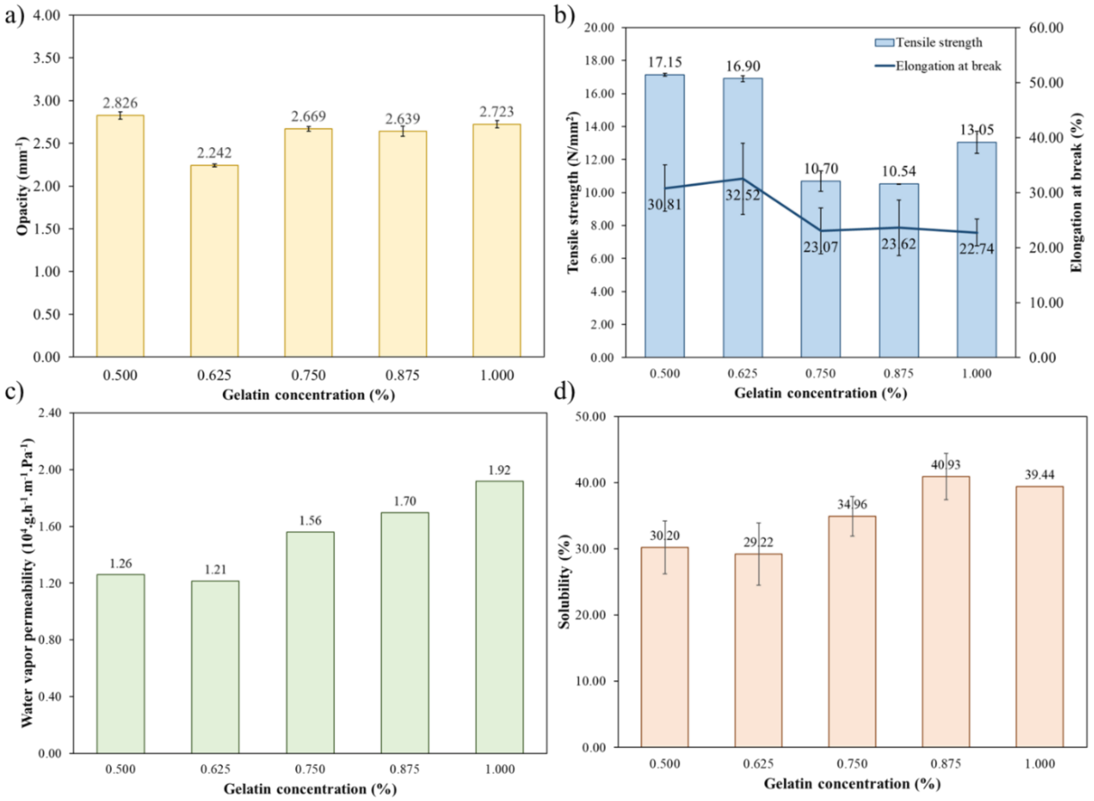

Figure 7. Effect of gelatin concentration on the opacity (a), tensile strength (b), water vapor opacity (c) and solubility in water (d) of the biofilm.

4. CONCLUSION

In this study, thin (<0.05 mm) biopackaging materials with high tensile strength (> 15 N/mm^2), low opacity (< 3mm^{-1}), suitable water vapor permeability properties with low solubility were developed by combining chitosan, gelatin and glycerol as a plasticizer. The molecular weight of chitosan can majorly affect the mechanical strength of the material whereas the change in polymer content can result in changes across mechanical strength, water vapor permeability and water solubility. The attained packaging material can then propose a wide range of applications in the food and medical industries in the process of converting to greener plastic alternatives.

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