



Effects of bovine gelatin viscosity on gelatin-based edible film mechanical, physical and morphological properties

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Abstract

This study was conducted to determine the effect of gelatin reology on mechanical, physical and morphological properties of gelatin-based edible films. The aim of this study was to better understand the variation of viscosity on the structural behaviour of gelatin-based films in the presence of glycerol and sorbitol plasticizers. Gelatin-based films were casted by using gelatins of different viscosities as 2.5, 3.0 and 3.5 centipoise with plasticisers as glycerol and sorbitol. Finally, the physical, mechanical and morphological properties of the films were investigated via pH, thickness, tensile strength and elongation, fourier transform infrared spectroscopy and scanning electron microscopy. As a result of the study, it was observed that a durable film structure could be obtained with gelatin viscosity at 3 centipoise. Furthermore 5.5% gelatin, 0.1% glycerol and 0.4% sorbitol concentration were found as the most suitable formulation for gelatin based film structure with Tukey Test. The results suggest that gelatin-plastisizer combinations can be an excellent source of biobased packaging material with further investigations.

Keywords Gelatin · Plasticizer · Viscosity · Edible film · Food package · Biopolymer

1 Introduction

Plastic packaging materials are commonly used by food and beverage industry [1]. As it is known, the physical structure of the package protects food from external effects with ideal cost and secure food protection. However, as a result of ammesment of tremendous amounts of petroleum-based plastic wastes in the environment. and also rapid dwindling of fossil reserves, food packaging industry is forced the toward the development of eco-friendly materials like bioplastics as a substitute of petroleum-based plastics [2]. Bioplastics can be called as plastics derive from renewable materials that are biocompostable and/or biodegradable materials [3, 4]. Bioplastic resources such as proteins (can be referred as gelatin and collagen) [5], polysaccharides [6], lipids [7], and their combinations

have a huge potential to reduce plastic waste with development on new implementations.

With the use of bio-nanocomposites instead of petroleum-based packaging materials, significant progress can be made in terms of protecting the environment and human health in the future [8]. Gelatin and its composites can be used as a biodegradable material on food packaging due to their good film forming properties [9–12]. It is studied in the literature that; composite films obtained by using potato starch chicken gelatin have rheologically and mechanically stable properties [13], biodegradable film structures produced by using gelatin obtained from fish residues and glycerol were characterized [10], the potential applications of gelatin-based films on food packaging were investigated [14], nano-technological food packaging implementation methods were delevopled for fish gelatin-based

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biomaterials [15], bio-compatibility of the gelatin based bio-polymers were examined [16] and the effect of drying temperatures on gelatin-based composite films were analysed [17].

Although a lot of research carried out on this subject, still not enough information to reveal the unique rheological properties of gelatin, as a result of the use of different plasticizers with varying ratios of the ability to form a gelatin-based film in integrity. In this study, it was aimed to investigate the effects of different gelatin viscosities on physical, mechanical and morphological properties such as thickness, tensile strength, elongation, surface morphology and chemical structure of gelatin based films plasticized with glycerol and sorbitol. With this work carried out; it may be possible to fill the information gap regarding the effect of the rheological properties of gelatin on the film structure, which has not been explained in detail in the literature.

For this purpose, gelatin-based films were casted using film solutions prepared with the RSM experimental modelling method. Then, physical, chemical, mechanical and morphological analyzes were applied to the films obtained. Additionally, the usability of the obtained films as food packaging was evaluated.

2 Materials and methods

2.1 Materials and reagents

Commercially available food-grade type B bovine gelatin was purchased from Halavet Jelatin (Turkey) in granule form, sorbitol (liquid) was provided by Sunar Mısır Entegre Tesisleri San. ve Tic. A.Ş (Turkey) and glycerol (liquid) was purchased from Sigma-Aldrich. Pure water and all chemicals used in this study were at least food grade.

For all gelatin samples, average molecular weight (MW) value is between 100.000–150.000 g/mol. Other technical specifications of gelatin samples are given in Table 1.

Table 1 Technical specifications of gelatin samples

Technical specifications	Gelatin 1	Gelatin 2	Gelatin 3
Jel strength (bloom)	270 ± 10	240 ± 10	210 ± 10
Viscosity (cP)	3.50 ± 0,1	3.00 ± 0,1	2.50 ± 0,1
Transmittance (%)	93.05 ± 0,1	97.37 ± 0,1	98.40 ± 0,1
Moisture (%)	9.35 ± 0,1	9.65 ± 0,1	9.40 ± 0,1
pH	5.72 ± 0,1	5.71 ± 0,1	5.75 ± 0,1
Isoelectric Point	5,50 ± 0,1	5,45 ± 0,1	5,55 ± 0,1

2.2 Determination of gelatin and film solution viscosity

Viscosity of gelatin and film solutions was determined according to the Gelatin manufacturers institute of America (GMIA) standard testing methods for edible gelatin [18]. According to the this method gelatin solution prepared with the solid matter content 6.67% at 60 °C temperature and results was reported in centipoise [19].

2.3 Preparation of response surface methodology

Response surface method (RSM) is a convenient technique in modelling and optimising a process through statistical and mathematical measures to disclose the correlation between process variables and outputs [20]. RSM experimental design was created by Minitab 17 software and variable used as follows; gelatin viscosity (2.0, 2.5 and 3.0 cP), gelatin concentration (1%, 5.5% and 10%), glycerol and sorbitol concentrations (0.1%, 0.3% and 0.5%).

2.4 Preparation of gelatin films

In order to obtain biodegradable films, 100 ml of pure water was measured using a pipette and placed into a 250-ml flask. Then, pre-weighed bovine gelatin concentrations 1%, 5,5% and %10 was added into the flask respectively. The gelatin mixture was allowed to stand for 30 min. at room temperature to absorb water, swell and settle. The flask was then covered with aluminium foil before placing it into a water bath set at 60 °C. The gelatin was completely dissolved for 30 min by shaking the mixture occasionally. In accordance with the RSM experimental design, glycerol and/or sorbitol (0.1%, 0.3% and 0.5%) was added to the completely dissolved gelatin using a pipette. The solution was shaken in the water bath set at 60 °C. Polystyrene moulds were used for casting. The casted films were dried for 24 h in an oven heated to 45 °C [21]. This temperature value was chosen because film solution maintains its liquid form at temperatures above its melting point, herewith material can reach high concentrations. Gel formation is avoided because the rate of drying is faster than the rate of nucleation. At the end of drying, the film formation is created from a disordered structure, characterised by entangled and closely packed chains [22]. After drying, the prepared films were then removed from the moulds and kept in a desiccator for another 24 h to eliminate moisture [23].

2.5 Determination of pH and thickness

pH values of gelatin, gelatin–glycerol, gelatin–sorbitol and gelatin–glycerol/sorbitol film-forming solutions were measured using a pH metre (Hanna Instruments, China) [24]. Thickness measurements were taken from five different points of the film samples using a digital thickness gauge [25].

2.6 Determination of tensile strength and elongation

For the determination of tensile strength and elongation, test samples were prepared by cutting 2 × 12 cm strips. Thickness measurement was taken from three different points of the strips and the average value was recorded. Tensile strength and elongation were measured using a Shimadzu AG–X tensile tester [14].

2.7 Determination of transmittance

Film transparency was taken using a Konica Minolta colorimetric spectrophotometer [26].

2.8 Fourier transform infrared spectroscopy

The spectra of the gelatin films were taken using a DTGS-KBR detector of the Agilent 600-IR Series Fourier transform infrared spectroscopy–attenuated total reflectance (FTIR–ATR) equipment at room temperature. The films were placed on a crystal cell, and the cell was clamped into a slot of the FTIR spectrometer. FTIR spectra were recorded in the range of 500–4000 cm⁻¹. Automatic signal acquisition was collected in 32 scans with a resolution of 4 cm⁻¹, and these were compared to a background spectrum recorded from a clean blank cell [27].

2.9 Scanning electron microscopy

Scanning electron microscopy (SEM) works on the basis of scanning the surface with high-energy electrons that focalize a very small area. In SEM, the material was examined using a thin electron beam created in a vacuum environment and focused with electromagnetic lenses to create a high-resolution image [28]. In this study, the SEM images of the film samples were taken using a Zeiss Brand MA10 model scanning electron microscope with ×5 magnification.

2.10 Statistical analysis

One-way analysis of variance (One-way ANOVA) following by Tukey's test as post hoc used to compare means at 5% significance level among different samples [20].

3 Results and discussion

3.1 Gelatin viscosity and material profile

Type B bovine gelatin samples with viscosity of 2.5, 3.0 and 3.5 cP and gel strength of 210, 240 and 270 bloom were used. The chemical properties of gelatin used in the study are given in Table 1.

3.2 Viscosity of film solutions

Figure 1 shows the effects of different variables on the film solution viscosity characteristic. The results show that the viscosity of the solution prepared with 3.5 cP viscosity gelatin has the highest film solution viscosity (Fig. 1d). Moreover, as a result of the Tukey test ($p < 0,05$) the effect of gelatin viscosity on film solution viscosity is more effective than other parameters (Fig. 1a, b, c).

The increase in gelatin concentration increases the viscosity of the film solution due to the intermolecular cross-bonding ability of gelatin molecules. Gelatin concentrations of 5.5% and 10% has higher viscosity than the 1% concentration. In addition, it was found that using glycerol and sorbitol together and in equal proportions has a significant positive effect on film solution viscosity (Fig. 1c).

Polyols such as sorbitol and glycerol are potent plasticizers due to their capability to reduce internal hydrogen bonding while improving intermolecular spacing [29]. Depending on the aqueous environmental state and the distribution of the different kinds of functional groups, the overall protein–polysaccharide interaction can be net attractive or net repulsive [30].

3.3 Thickness, pH and transmittance of films

The thickness of biodegradable films is mostly related to the gelatin/plasticizer concentration and solution's viscosity. As the amount of protein increases, the film thickness is expected to increase with an increase of solid matter [31]. Film thickness has an effect on tensile strength, water vapour permeability and unit density properties [32]. Thickness values of the gelatin–glycerol, gelatin–sorbitol and gelatin–glycerol/sorbitol film-forming solutions were in the range of 0.015–0.19, 0.02–0.23 and 0.13–0.18 mm, respectively. With the use of a constant amount of gelatin and plasticizer, the film thickness of the high-viscosity solution was higher than that of the other samples.

pH measurements were performed on film forming solutions via pH meter. pH values of the gelatin–glycerol, gelatin–sorbitol and gelatin–glycerol/sorbitol film forming

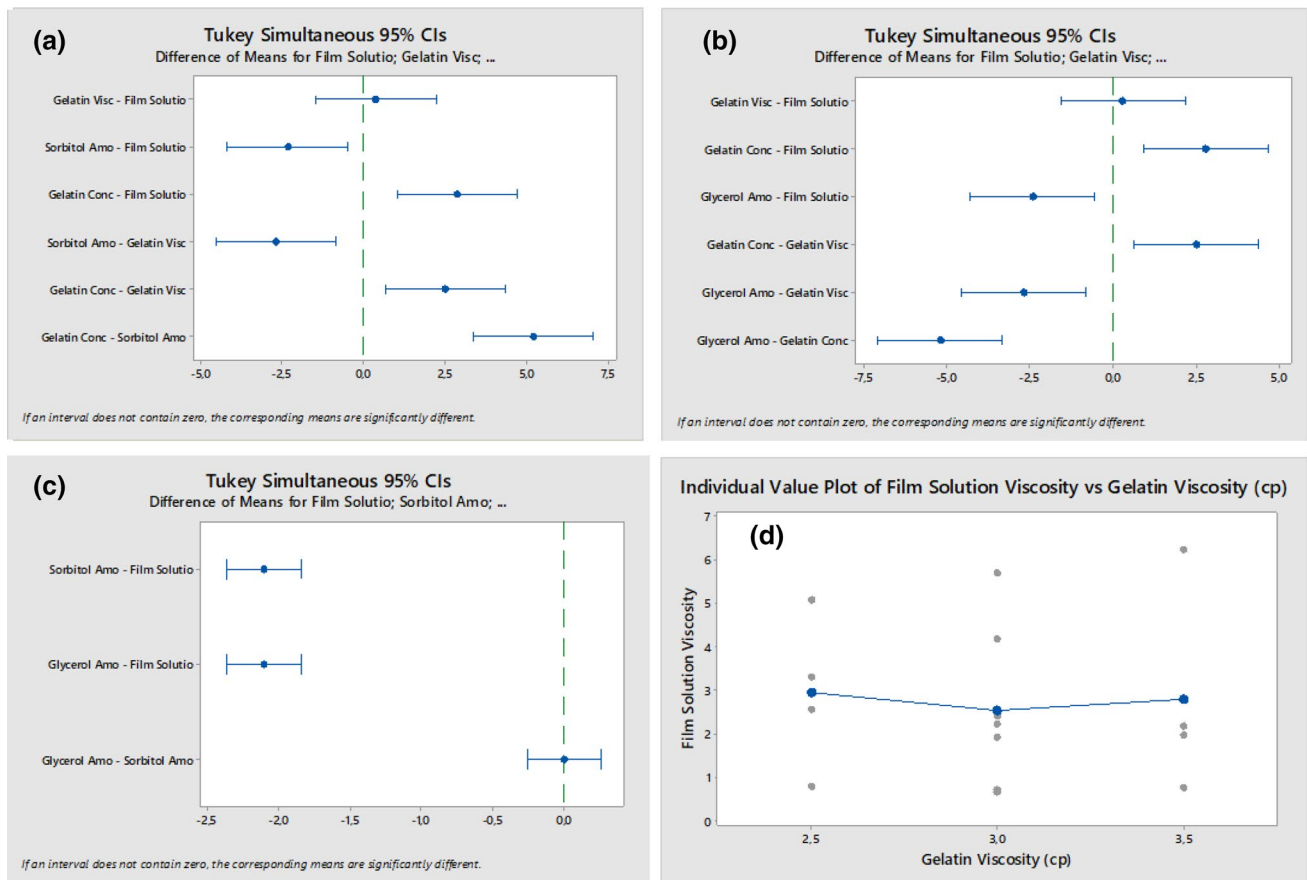


Fig. 1 **a** Tukey pairwise comparisons of gelatin viscosity, gelatin concentration, sorbitol amount on film solution viscosity, **b** Tukey pairwise comparisons of gelatin viscosity, gelatin concentration, glycerol amount on film solution viscosity, **c** Tukey pairwise comparisons of sorbitol and glycerol amounts on film solution viscosity, **d** Individual value plot of film solution vs gelatin viscosity

solutions were in the range of 5.82–5.90, 5.59–5.71 and 5.66–5.84, respectively.

Transmittance measurements were performed using a colorimetric spectrophotometer at 620 nm in response to black and white objects. The results showed that there were no significant differences in the level of transmittance.

3.4 Tensile strength and elongation of films

Figure 2 shows the effects of different variables on the film tensile strength properties. The results showed that tensile strength of films varied between 0 and 25.27 N/mm². Consequently, it was determined that gelatin viscosity was not the main effect on tensile strength. When the Tukey test graphics (Figs. 2a, b, c) were examined, it was determined that the increase in viscosity, increased the tensile strength when the gelatin concentration increased in all glycerol, sorbitol and combined trials.

The highest tensile strength was obtained with 3.5 cP viscosity and %5.5 gelatin concentration. The increase of

glycerol amount has positive effect on tensile strength while increasing gelatin viscosity. However, the effect of the glycerol amount on tensile strength is negligible.

Figure 3 showed that elongation values varied between 3.21% and 258.41%. When Tukey test graphics (Fig. 3a, b, c) were examined, the results show that the effect of glycerol, sorbitol and gelatin concentrations on the elongation amount was the same as gelatin viscosity. Additionally, the increase in gelatin viscosity negatively affects the elongation percentage of films. Since glycerol are highly hygroscopic, an increase in the plastizer amount used with fixed gelatin concentration increases the plasticising efficiency, which decreases the elongation [33]. Sorbitol also presented an apparent protecting effect against the helix–coil transition, decreasing the formation of junctions responsible for the crystallinity of gelatin observed through the reduction of the enthalpy of the sol–gel transition.

The effects of using glycerol and sorbitol as plasticizers in varying proportions on the mechanical properties of the films can be seen in Fig. 4. The amount of glycerol and sorbitol used was 0.5%, 0.4%, 0.3% and 0.2% while keeping

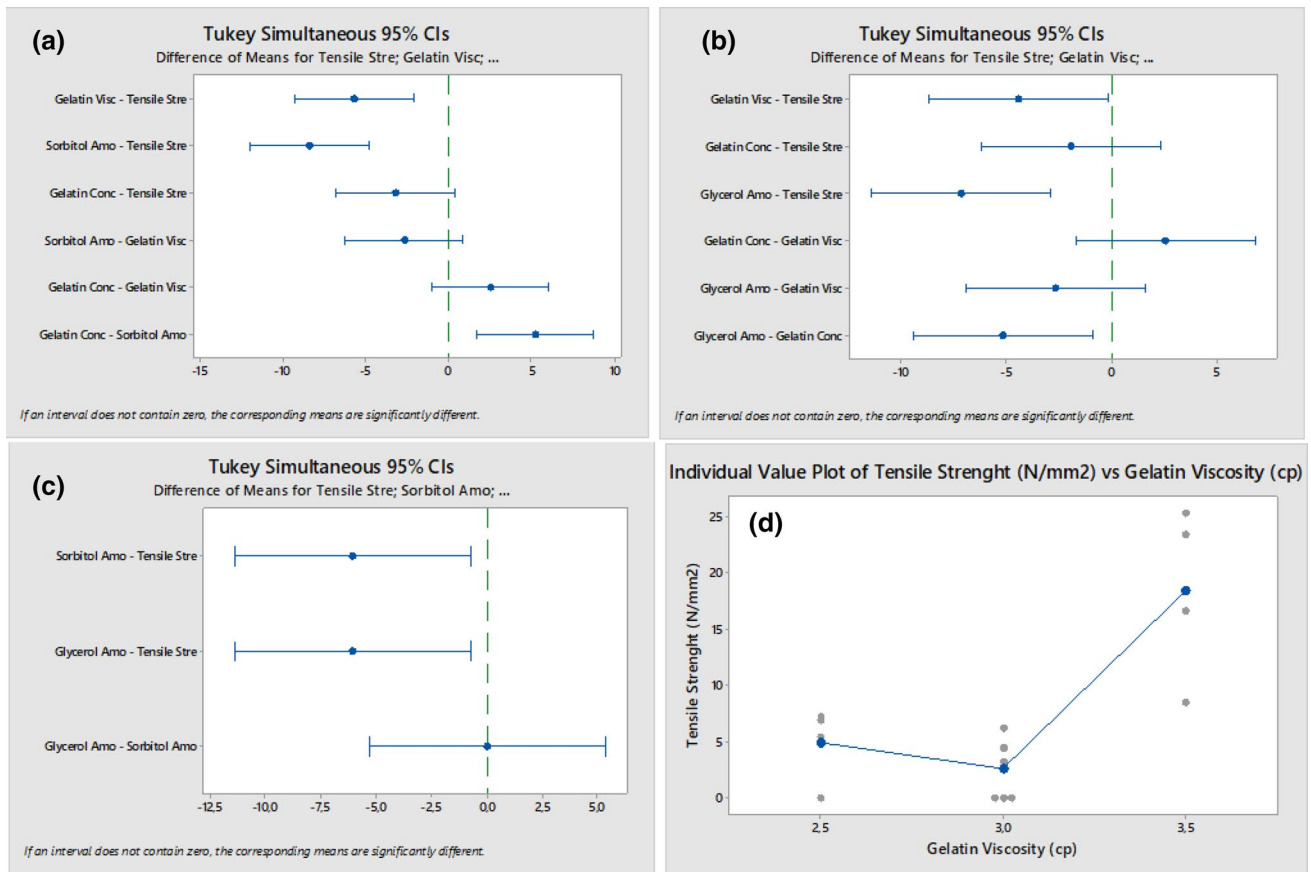


Fig. 2 **a** Tukey pairwise comparisons of gelatin viscosity, gelatin concentration, sorbitol amount on tensile strenght, **b** Tukey pairwise comparisons of gelatin viscosity, gelatin concentration, gly-

erol amount on tensile strenght, **c** Tukey pairwise comparisons of sorbitol and glycerol amounts on on tensile strenght, **d** Individual value plot of tensile strenght vs gelatin viscosity

the total plasticiser concentration of 0.5% and the gelatin concentration of 5.5% (gelatin viscosity was 3 cP) constant. As a result of the use of glycerol and sorbitol in increasing and decreasing amounts, it was found that it shows the opposite effect on tensile strength and elongation percentage. Films obtained when the tensile strength is high as in the case of films with increased sorbitol amount were less flexible. When the amount of glycerol was increased, flexibility was increased while a significant decrease in tensile strength was observed (Fig. 4).

3.5 FTIR

In the obtained FTIR plot for composite films of gelatin–glycerol, Amid A and Amid B bands were seen at 3300 and 3100 cm^{-1} , respectively. These bands are related to the stress vibrations of the N–H groups. The Amid I band seen at 1650 cm^{-1} is derived from the tensile vibrations of the C=O peptide [34]. Amid I band seen at 1550 cm^{-1} is caused by N–H bending due to C–N tensile vibrations. Amid III band seen at 1250 cm^{-1} is also attributed to N–H

and C–N stresses as well as the vibrations of CH_2 groups in the glycerol spine and pyrroline side chains [35]. The peak of N, N-disubstituted amides shifts from 1659 cm^{-1} for pure gelatin to a lower wave number with an increase in glycerol content, and it shifts to 1650 cm^{-1} for gelatin–glycerol. The shift of N,N-disubstituted amides to a lower wavenumber suggests an increase in average strength of the hydrogen bond because of the disruption of C=O...H–N hydrogen bonds in gelatin and the formation of new stronger C=O...H–O hydrogen bonds between gelatin and glycerol (Ramos OL et al., 2013). In the glycerol-added gelatin film, the characteristic C–O stretching band of glycerol was clearly observed at 1045–1050 cm^{-1} [36, 37] (Fig. 5).

For gelatin–sorbitol composite films, Amid A and Amid B bands are seen at 3300–3100 cm^{-1} . These bands are related to the stress vibrations of the N–H groups. The Amid I band seen at 1650 cm^{-1} is derived from the tensile vibrations of the C=O peptide [34]. Amid I band seen at 1550 cm^{-1} is caused by N–H bending due to C–N tensile vibrations. The most important peaks for sorbitol are at

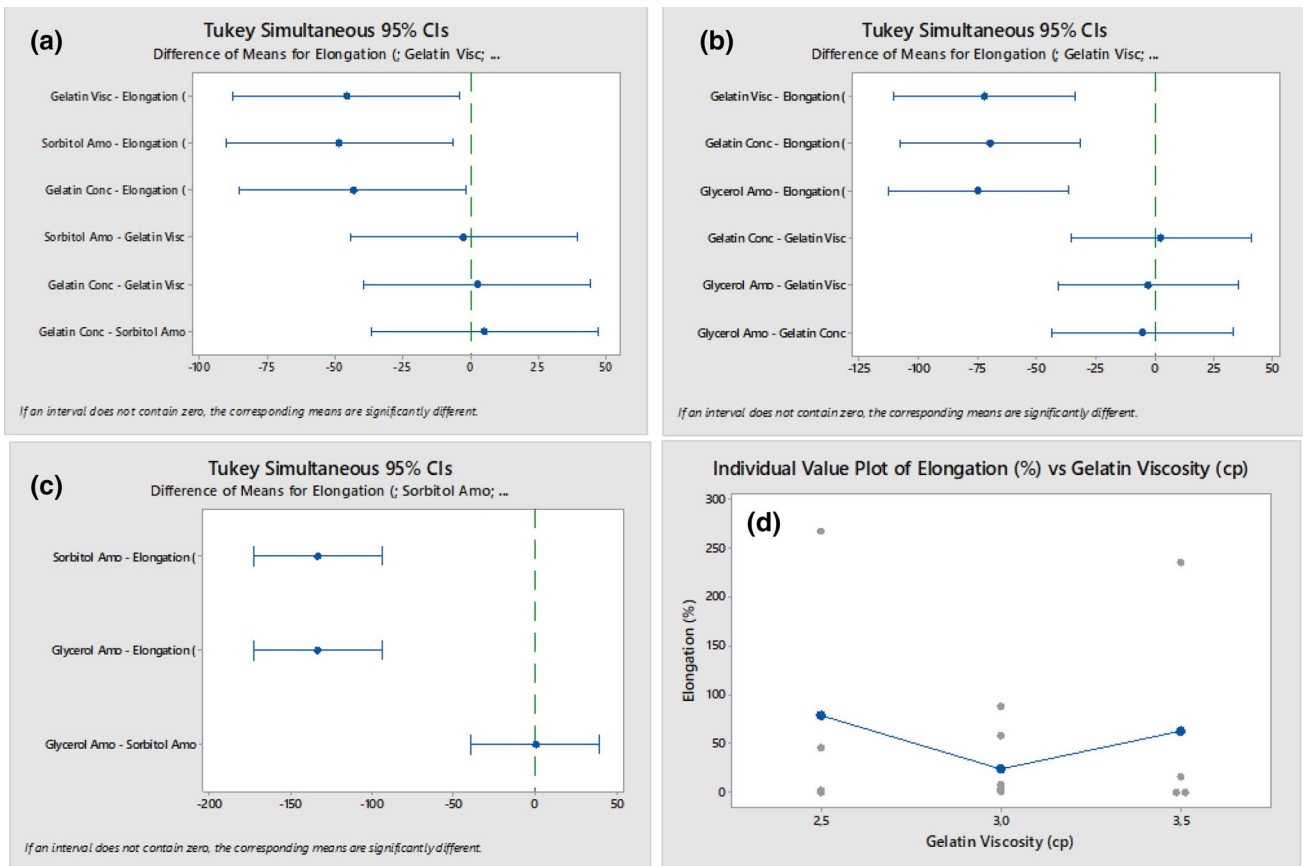


Fig. 3 **a** Tukey pairwise comparisons of gelatin viscosity, gelatin concentration, sorbitol amount on elongation, **b** Tukey pairwise comparisons of gelatin viscosity, gelatin concentration, glycerol

amount on elongation, **c** Tukey pairwise comparisons of sorbitol and glycerol amounts on on elongation, **d** Individual value plot of elongation vs gelatin viscosity

Fig. 4 Tensile strength and elongations of gelatin-based film plasticised with glycerol–sorbitol combination

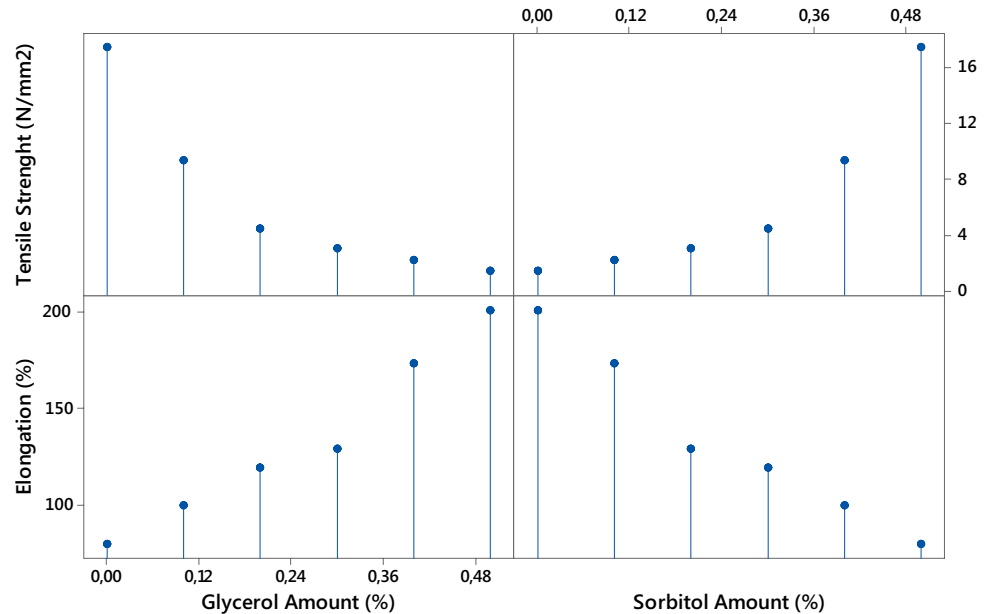
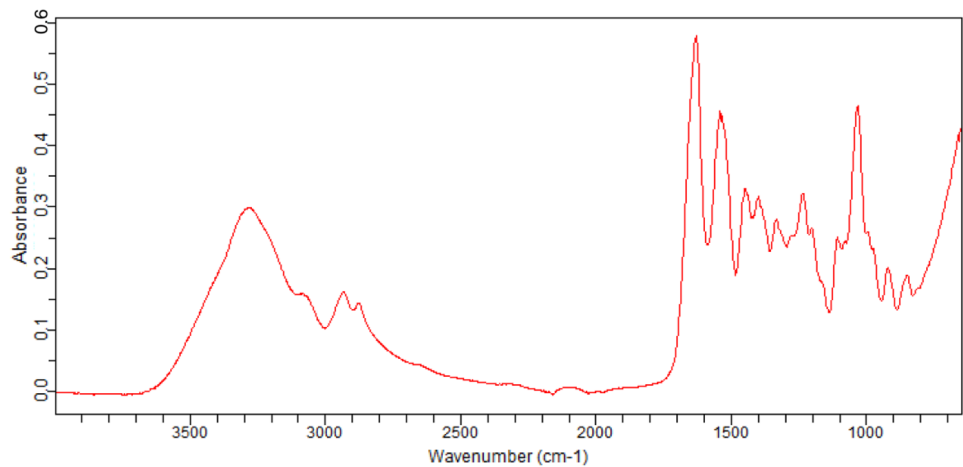
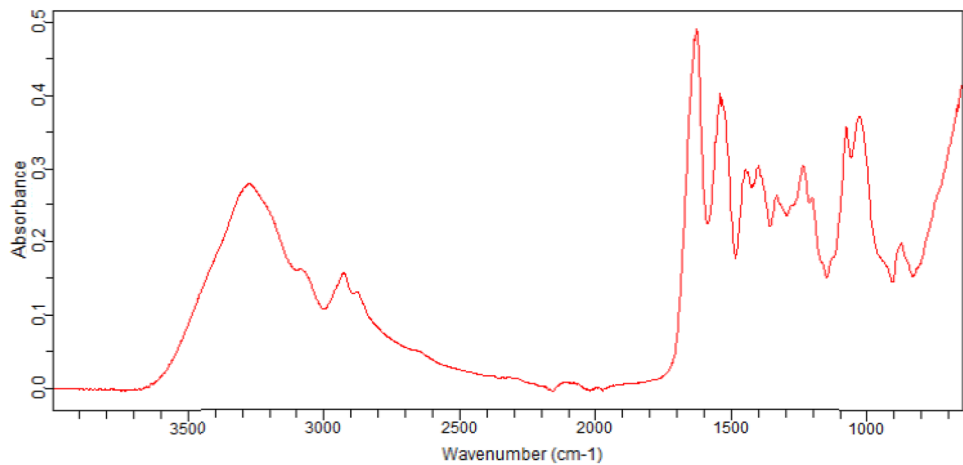
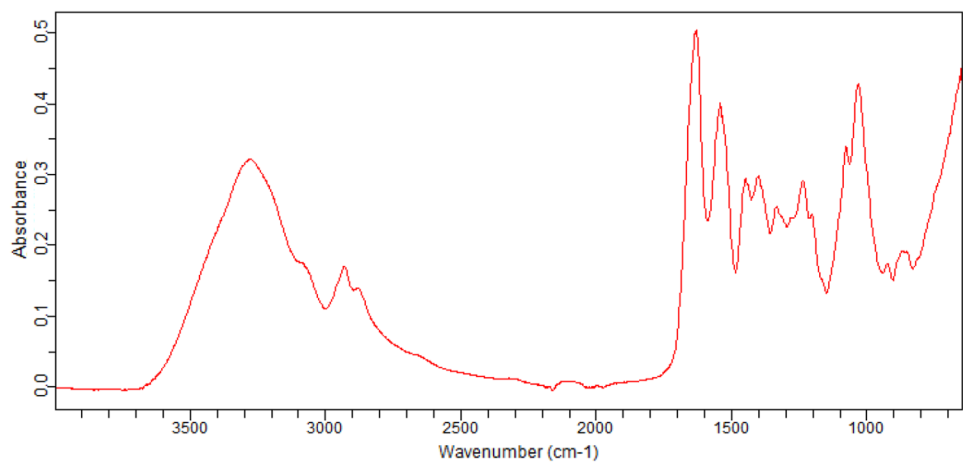
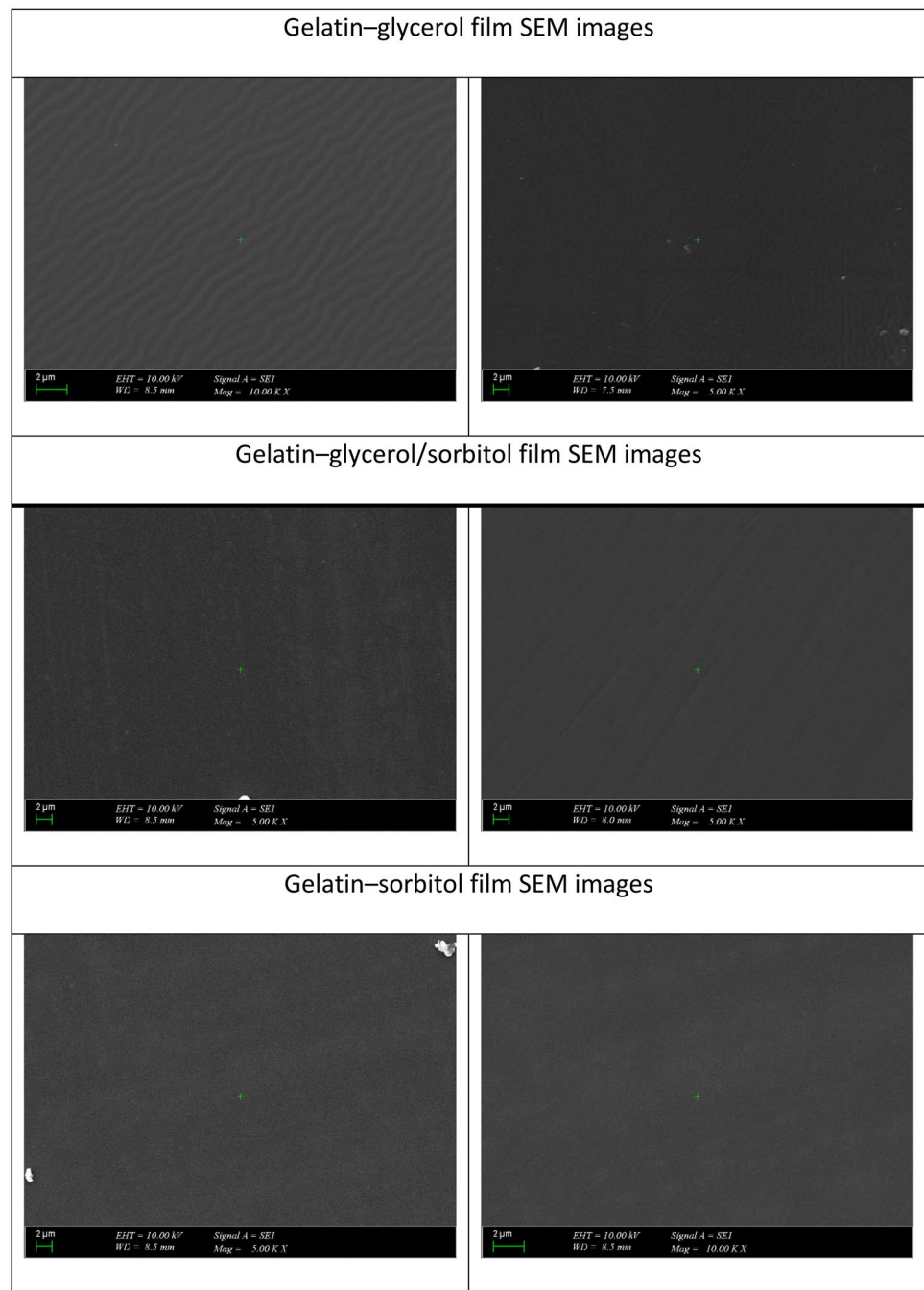


Fig. 5 FTIR plot of gelatin–glycerol film**Fig. 6** FTIR plot of gelatin–sorbitol film**Fig. 7** FTIR plot of gelatin–glycerol/sorbitol film

890, 1046, 1084 and 1411 cm^{-1} . The characteristic peak for sorbitol is the peak seen at 890 cm^{-1} . In addition, a flat peak appears at 3300 cm^{-1} due to the OH groups in the structure of sorbitol and the OH peak from gelatin [38] (Fig. 6).

For gelatin–glycerol/sorbitol composite films, Amid A and Amid B bands are seen at $3300\text{--}3100\text{ cm}^{-1}$. These bands are related to the stress vibrations of the N–H groups. The Amid I band seen at 1650 cm^{-1} is derived from the tensile vibrations of the C=O peptide [34]. Amid I band seen at 1550 cm^{-1} is

Fig. 8 Gelatin-based films SEM images

caused by N–H bending due to C–N tensile vibrations. Amid III band seen at 1250 cm^{-1} is also attributed to N–H and C–N stresses as well as the vibrations of CH_2 groups in the glycerol spine and pyrroline side chains. The most important peaks for sorbitol are at 890 , 1046 , 1084 and 1411 cm^{-1} . The characteristic peak for sorbitol is the peak seen at 890 cm^{-1} . In addition, because of the OH groups in the structure of sorbitol and the OH peak from gelatin, a flat peak appears at 3300 cm^{-1} (Fig. 7).

When the gelatin–glycerol/sorbitol composite structure was examined by FTIR spectroscopy, gelatin–glycerol and gelatin–sorbitol have similar FTIR structures. However, a double peak is observed for gelatin–glycerol at $800\text{--}1000\text{ cm}^{-1}$ while a single peak is observed for gelatin–sorbitol. In the gelatin–glycerol/sorbitol composite FTIR plot, there are double peaks at $800\text{--}1000\text{ cm}^{-1}$ that reveal the structure difference, but the peak at 890 cm^{-1} , which is the characteristic peak for sorbitol, is more common. These results indicate that the gelatin–sorbitol/glycerol composite was obtained.

Even though glycerol and sorbitol are different types of plasticizers, both classified as polyols. Furthermore, the existence of aliphatic of alcohol in the plasticized film were shown defining due to the availability of the plasticizer [39].

The displacements were arised with the increased in entirety and sharper peaks in comparing the control film with the film incorporated with the plasticizers due to the contribution of–OH group from the plasticizers. The peaks generally correspond to the–NH stretching coupled with hydrogen bond which responsible with the width [40].

3.6 SEM

SEM images were taken by selecting three samples from the gelatin–glycerol, gelatin–sorbitol and gelatin–glycerol/sorbitol films and the surface appearance was successful. All film samples were transparent, but some have a slightly yellow-to-brown appearance, although they were quite translucent. In terms of transparency, no significant difference was observed among the samples. SEM micrographs of films using sorbitol, glycerol and combined plasticisers is shown in Fig. 8. With the images obtained, the films look clean and smooth.

The excellent structural entirety of the film is similar to the currently used transparent packaging products. This result showed that a successive matrix structure of the film component was formed [41].

4 Conclusion

In the presence of sorbitol and glycerol as plasticisers, it was investigated how varying gelatin viscosities affects the chemical, morphological and mechanical properties of gelatin-based films. As a result of the gelatin–plasticiser combination studies, a durable film structure can be obtained with gelatin viscosity 3 cP, 5.5% gelatin, 0.1% glycerol and 0.4% sorbitol formulation. The results obtained in the studies conducted show that gelatin viscosity affects the film structures. SEM results shows that gelatin and glycerol concentrations affect the surface morphology of the film. Increasing or decreasing both variables causes the surface to shrink with temperature. Apart from this, it is possible to obtain acceptable smooth and flat surfaces by using glycerol and sorbitol under optimal conditions. It has been concluded that gelatin films can be a good and sustainable alternative to meet the increasing need for bio packaging. However, advanced studies should be developed to improve the shelf life and degradability of gelatin-based films.

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Data Availability The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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