

ORIGINAL SCIENTIFIC PAPER

Reducing Packaging Waste – Mechanical Characteristics and Network Parameters of the Gelatin-Based Thin Film for Cruciferous Vegetables Packaging

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Abstract

The role of the materials used for packaging is to protect inner content from external, mostly negative, factors. Besides being safe, and with good mechanical properties, these materials need to align with other consumers' needs and demands, being both attractive and authentic, while at the same time meeting ecological requirements. Gelatin-based thin films are good candidates for production of semi-soft cup-shaped products that could be used for cruciferous vegetables packaging. These films were prepared by a simple solvent evaporation method. Prepared films were characterized by swelling study and their mechanical properties were investigated. The optical characteristics of the films were determined by optical microscopy revealing that these systems possess attractive clear and transparent gold-yellowish appearance with a smooth surface.

1. INTRODUCTION

It is commonly known that synthetic polymeric materials are used for food packaging. Their role is to protect food from external factors' negative influence. These materials show many advantages, such as the ease of manufacturing/handling, attractive appearance, but also possess good mechanical properties as well as the possibility of easy printing (Hopewell et al., 2009). Although they are cheap, the modern era requires reducing the use of synthetic materials (Vinicius Tosati et al., 2017; Tulamandi et al., 2016), banning their use in direct contact with food (migration of chemicals into packed goods) (Schrenk 2014) and forcing the production of materials made from renewable raw materials. These "new-old" materials will cause less (or zero) pollution to the environment after their

disposal, despite the fact that around 40.9% of collected plastic packages is recycled according to the last Plastic Europe report (www.plasticseurope.org) announced in 2017. Nowadays, we are witnessing that the use of natural polymers is gaining importance. Edible and/or biodegradable films derived from natural resources have evoked much interest due to the environmental concerns caused by the vast majority of disposable petroleum-based packaging materials (Wang et al., 2015). Thus, natural films can be made from plant sources (corn, soybean, wheat, cottonseed, peanut, and rice) or even be of animal origin, but consumption of the latter is limited due to its origin, especially for vegans (Mann, 2014). The majority of natural polymers based on polysaccharides is relatively cheap, cost-effective,

highly available, and very well degradable thus representing good alternatives to the use of synthetic materials for the production of packaging. Among the natural polymers, pectin, gelatin, etc. are very often used in the production of these types of packaging, and gelatin has drawn attention due to its biodegradability, biocompatibility, low cost, and excellent film solution properties with good mechanical stability ([Sánchez-Ortega et al., 2014](#)). Gelatin-based polymeric materials/films are nowadays being used in the field of food ([Sánchez-Ortega et al., 2014](#); [Tosati et al., 2017](#); [Wang et al., 2011a](#)) and agricultural industry ([Ramos et al., 2016](#)), pharmaceutical ([Wisotzki et al., 2014](#)) and medical ([Alam et al., 2015](#); [Bokeriya et al., 2016](#); [Wilaiwan et al., 2010](#)) industries. These materials fall into the category of acceptable, renewable and non-reactive raw materials. However, gelatin shows sol-gel transition temperature around 37 °C, and its inherent instability presents an obvious problem for its applications in the pure form ([Ramos et al., 2016](#)) with reduced mechanical strength and elasticity. The problem can be circumvented by combining gelatin with other natural polymers, while tailoring desired properties of the final product. Numerous studies discuss different combinations of polymers that use glycerol as a food approved plasticizer ([Mellinas et al., 2016](#); [Otoni et al., 2017](#)), with the aim at reducing the brittleness of prepared films ([Liu et al., 2017](#); [Wang et al., 2011b](#); [Wang et al., 2015](#)). However, the demand for prolonging the shelf life of food has encouraged the development of polyphenols containing gelatin-based films, with slow and controlled antioxidants release ([Bao et al., 2009](#); [Gomez-Estaca et al., 2014](#)). Additionally, we hypothesized that addition of tea extract compounds could affect the diffusion paths, which has to be further instigated. In this paper we investigate the possibility of making gelatin-based thin films that would be used as a packing bag for cruciferous vegetables.

2. MATERIAL AND METHODS

2.1. Materials

Gelatin Type B (Gel) and Glycerol (Gly) were purchased from Centrohem (Serbia). Chamomile tea extract (C-TE) was prepared by conventional water extraction process, by pouring 50 mL of boiled distilled water over 3 g of ground plant. After sitting at room temperature for 30 min with occasional stirring, the mixture was filtered through medical gauze. All materials were used as received, without further purification.

Distilled water was used for all preparations and buffer solutions. Acetic acid, sodium acetate (Zorka Pharma, Serbia), sodium dihydrogen phosphate dihydrate and di-sodium hydrogen phosphate dodecahydrate (Lach-Ner, s.r.o., Czech Republic) were used to prepare buffer solutions of different pH values.

2.2. Thin film preparation

Gelatin solution (10% w/v) was prepared by dissolving 3.00 g of gelatin type B in 30 mL of distilled water. The solution was homogenized by magnetic stirring for 60 minutes while being heated at 50 °C. Then, one-third of the solution, equal to 10 mL, was extracted and left at room temperature to cool down. The remaining volume was used to make glycerol solution (1% v/v), by dissolving 200 µL of glycerol in 20 mL of gelatin solution. The mixture was further homogenized by magnetic stirring for additional 15 minutes at 50 °C. Then, one half of the solution, equal to 10 mL, was left at room temperature to cool down. The remaining volume was used to make the third mixture (10% v/v), by adding 1 mL of Chamomile tea extract to 9 mL of the gelatin/glycerol solution. The prepared solution was additionally homogenized by magnetic stirring for 15 minutes at 50 °C. Then, this mixture was left at room temperature to cool down. The solutions were used to form thin films. A spirit level was used to make a horizontal surface, for solutions to spread evenly in the Petri dishes. Films were made by pouring 5 mL of each solution in a separate polystyrene Petri dish (R = 100 mm), for a total of six films (2 x 5 mL gelatin, 2 x 5 mL gelatin/glycerol, and 2 x 5 mL gelatin/glycerol/tea). The solutions were slowly poured in a circular manner to ensure even distribution. The films were left at room temperature to dry for the next 24 hours.

2.3. Optical characterization/microscopy

The obtained thin film transparency and overlook were obtained using optical microscope Leica FS C Comparison Macroscope, equipped with The Leica IM Matrox Meteor II Driver Software Module. The films were examined immediately after formation, in dry state, and with and without backlight.

2.4. Swelling of the films

The swelling study was monitored gravimetrically at room temperature, by immersing disc-shaped thin film in water, buffer solution of pH 3.50 ± 0.01

(CH₃COOH/ CH₃COONa) and in buffer solution of pH 8.00 ± 0.01 (NaH₂PO₄ × 2H₂O/Na₂HPO₄ × 12H₂O) for the period of 24 h, and the equilibrium swelling degree was calculated according to the following equation:

$$q = w_t / w_0 \quad (1)$$

where w_t corresponds to the weight of swollen disc-shaped film at time t and w_0 to the weight of dried disc-shaped film.

2.5. Network parameters

The molecular weight between two neighboring crosslinking points, in natural hydrogels which are prepared in the presence of water at equilibrium, is calculated by the following equation (Chun-Liang 2010; Peppas et al., 2000):

$$\bar{M}_c = \frac{1}{[\ln(1 - \frac{v_{2r}}{v_{2m}})]} \frac{v_{2r}^3 v_{2m}^{1/2}}{\chi v_{2m}^2} \quad (2)$$

where v_{2r} represents the polymer volume fraction within the gel during relaxation period (defined as the state of the polymer just after synthesis, but before swelling), v_{2m} is the polymer volume fraction within the gel in its swollen state, V is the specific volume of the polymer, V_1 is the molar volume of water or the swelling media, f is the crosslinking agent functionality and χ represents the polymer/solvent interaction parameter.

The mesh size represents a structural parameter defined as the linear distance between two neighboring crosslinks. The mesh sizes were calculated using the following equation (Milašinović et al., 2010):

$$\alpha \bar{r}_0^2 \quad (3)$$

where α represents the fraction of the polymer chain stretching in any direction, while $(\bar{r}_0^2)^{1/2}$ is the average distance between two adjacent crosslinks in the solvent-free state.

The case of isotropic swelling of the hydrogel gives the following equation (Peppas et al., 2000):

$$\alpha \bar{r}_0^2 \quad (4)$$

$(\bar{r}_0^2)^{1/2}$ depends on the molecular weight between the crosslinks by the expression (Bao et al., 2009):

$$\bar{r}_0^2 = \left(\frac{2}{M_r} \bar{M}_c \right)^2 \quad (5)$$

where C_n represents the characteristic Flory ratio ($C_n, \text{gelatin} = 8.26$ (Van Hoorick et al., 2017), l is the length of the C-C bond (1.54×10^{-10} m in case of gelatin (Visakh et al., 2014) and M_r molecular weight of the basic structural unit within the polymer chain, which was adopted to be around 270 g mol⁻¹.

2.6. Mechanical testing

Mechanical characteristics of the prepared thin films were conducted using *Universal Testing Machine* (AG x-plus, Shimadzu, Japan) equipped with a 100 kN force load cell. The tensile strength and the elongation at break of the films were determined according to ASTM standard method. The films were cut into standard dumbbell-shapes, 63.5 mm in length and then used for measurement. All tests were performed at least four times.

3. RESULTS AND DISCUSSION

The prepared films were first submerged into distilled water in order to remove all unreacted chemicals. Then, in hydrated state, the films were cut into discs around 7 mm in diameter, and the obtained disc-shaped thin films were left to dry to xerofilms for further experiments. Upon drying, the films were cut into standard shaped samples in order to prepare samples for the extension experiments. Pieces of thin films of around 3 cm in diameter were used for optical experiments measurements (smoothness and transparency).

3.1. Optical microscopy

Dry thin films were used for the optical measurements, and the results of the optical spectroscopy are shown in Figure 1.



Figure 1. Optical images of the prepared films (magnification ×70): a) Pure gelatin film (left), Gelatin-Glycerol film (middle) and Gelatin-Glycerol-Tea (right)

It is evident that all prepared films showed well homogenized structure of slightly yellowish color. This was assumed to be a consumer-acceptable package appearance. The smoothness and transparency of the prepared films were presented in Figure 2.



Figure 2. The smoothness and transparency (top right inserts) of the prepared films (magnification $\times 20$)

Table 1. Equilibrium swelling degree, network parameters, interaction parameter for the investigated Gelatin-based polymer films swollen at 25 °C.

Sample	Water				pH 8.0			
	q_e	M_c ($g\ mol^{-1}$)	ξ , nm	\bar{V}	q_e	M_c ($g\ mol^{-1}$)	ξ , nm	\bar{V}
Gel	9.7006	107.4051	1.008	0.537	14.7394	72.2773	0.791	0.524
Gel/Gly	18.4977	147.4369	1.198	0.519	17.0625	134.9889	1.118	0.520
Gel/Gly/C-TE	12.2122	169.2224	1.117	0.530	16.5056	92.4628	0.904	0.521

The prepared systems have very smooth surfaces, but also that the smoothness of the films was affected by the addition of both plasticizer and tea extract. Chamomile contains terpenoids, flavonoids, and lactones, including matricin and apigenin. Normally standardized extracts contain 1.2% of apigenin which is one of the most effective bioactive agents, showing positive effect to humans fighting against cancer (Yan et al., 2017), but also help calm nerves and provide antioxidant effects, when released (Srivastava et al., 2010). Furthermore, Chamomile tea contains Chamazulene, an aromatic chemical compound that possesses anti-inflammatory, analgesic and antispasmodic properties. The additional effect of plasticizer was discussed in detail in Section 2.6. The transparency of the film was important from the application point of view, since it was very important to produce thin film dishes to satisfy consumers demands.

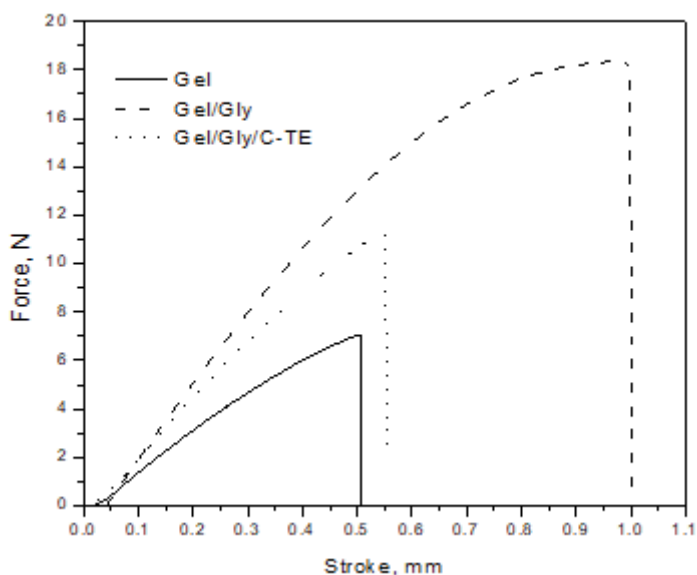
3.2. Swelling of the prepared films and network parameters

Equilibrium swelling degree and network parameters, the volume fraction of the polymer in the swollen state, $v_{2,s}$, the molecular weight between two neighboring crosslinking points M_c , the mesh size, ξ , and the polymer/solvent interaction parameter, \bar{V} , were calculated according to the equilibrium swelling theory and the results were presented in Table 1. As expected, the prepared films dissolve in buffer solution of pH 3.50, hence no results were given in Table 1. Based on the results shown in Table 1, it is clearly noticed that

there was a big difference in the network parameters with the change in the composition of the hydrogels and the pH value of the surrounding media. Glycerol probably contributes to the additional formation of hydrogen bonding (Dirama et al., 2005), therefore allowing gels/film to swell more in both water and buffer solution. Additionally, we hypothesized that besides physical limitation due to the molecular sizes, incorporation of complex structure of tea extract occupies some hydroxyl groups of already incorporated glycerol, hence diminishing the overall swelling degree of the prepared films. The values of the polymer/solvent interaction parameter were low and similar which was quite expected since gelatin-based polymeric films were defined as hydrophilic systems, and their hydrophilicity could be controlled by external factors, as described. The lower the polymer/solvent interaction parameter values the higher the swelling degree of the prepared films was obtained. The incorporation of the tea extract affects the prepared systems in a sense that these molecules physically impeded the regular network formation but also physically occupied the inner network structure, hence reducing the pore volume and both, water sorption and retention, which was in accordance with M_c parameter values. By comparing the network parameters obtained by swelling of the films in water and pH 8.0, it was evident that all parameters had slightly higher values in water than in buffer solution. This pointed out that the composition of the systems, as well as the number of available functional groups affect M_c values and result in the mesh size ξ values.

Table 2. Mechanical characteristics of the investigated films

Sample	Tensile strength (N mm ⁻²)	Elastic modulus (N mm ⁻²)	Elongation to break (mm), (%)
Gel	46.174	3163.2	0.5054, (1.75)
Gel/Gly	112.21	5612.2	0.9980, (3.45)
Gel/Gly/C-TE	69.785	4265.2	0.5514, (1.91)

**Figure 3.** Mechanical tensile testing of the prepared thin films

3.3. Mechanical characterization of the samples

Tensile strength, elongation to break and elastic modulus of the films were measured, and tensile properties were calculated from the plot of stress (tensile force/initial cross-sectional area) versus strain (extension as a fraction of the original length) (Cao et al., 2007), and the results were given in Table 2. Figure 3 shows mechanical testing of the prepared film performed according to the described procedure. As realized from Table 2 and seen from the Figure 3, gelatin film possessed some brittle characteristics, having very little deformation capacity. Upon addition of glycerol the prepared film became twice as stretchable as the film consisting only of gelatin. However, the elasticity and mechanical strength of the prepared films used for cup-shaped packages must be strong enough to sustain some stress (hence being slightly elastic) while at the same time balancing some force. Addition of tea extract compounds (as described in Section 3.2.) could be one of the possible solutions to meet these requirements. Although not fully investigated, this might be a good starting point for our future experiments; hence additional

experiments were a necessity in order to investigate the release of active compounds from the prepared systems. Considering swelling study, mechanical testing of the prepared systems fully complied with each other. Very elastic Gel/Gly films exhibited the highest swelling degree in both investigated media, showing that the distance between the crosslink points was high enough to allow swelling medium to penetrate the film surface and occupy inner polymer volume. However, it was obvious that mechanical strength was also pH dependent, since swelling measurements were hindered by the solubility of the films as they began to dissolve in slightly acidic solution.

4. CONCLUSIONS

This study showed that gelatin-based thin films could be used to produce mechanically strong cup-shaped packaging films for cruciferous vegetables, while at the same time being an eco-friendly material. The hygroscopic properties of gelatin allowed the addition of plasticizers or complex tea extract compounds with antioxidant activity to gelatin-based products in order to improve the

functional properties of biodegradable films that could potentially affect the shelf life of packed food products. Although with promising results, additional experiments, such as release properties and antioxidant activity, must be conducted in order to fully investigate the potential of these gelatin-based thin films and their likely application.

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