

THE EFFECT OF PLASTICIZERS ON PROPERTIES OF THERMALLY CROSSLINKED GELATIN FILM

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Abstract

The market of edible films and coatings has been growing exponentially and continuously. A gelatin material was selected for the development of edible films in this research. Plasticizers: glycerol, propylene glycol, PEG400 and PEG4000 were chosen for comparison. The non-plasticized and plasticized gelatin films were formed by solution casting process. Dehydrothermal treatment (DHT) crosslink process was used for crosslinking those of gelatin films. The effect of plasticizers on physical characteristics, thermal properties and the moisture absorption of crosslinked and non-crosslinked gelatin films were studied. The results showed that the addition of all plasticizers did not obviously change the color of the gelatin film, except PEG4000. The color of plasticized crosslinked gelatin films became more yellowish compared to those of non-crosslinked plasticized gelatin films. In the case of non-plasticized gelatin film, T_g and moisture absorption of crosslinked gelatin film was lower than non-crosslinked gelatin film. The addition of plasticizers affected the moisture absorption of films.

Keywords: Edible films, gelatin, plasticizers, crosslink, dehydrothermal treatment

Introduction

In recent years, the environmental problem associated with plastic waste has become a worldwide problem (Rigamonti *et al.*, 2014; Khoo, 2019). One of solution to solve this waste problem is the use of biodegradable plastics, which are polymers originating from bio-based renewable resources or petro-based resources. When these polymers undergo composting after use, they are biodegraded into carbon dioxide, and biomass (Havstad, 2020). For food packaging aspect, the market of edible films and coatings has been growing exponentially and continuously. This has resulted in the advancement of work development in materials science and process technology. The term

“edible film” has been in use over the past 50 years and is divided into two aspects: films and coatings. The film is a stand-alone wrapping material while the coating is applied and formed directly on the food surface (Embuscado and Huber, 2015). Edible film can be divided into 3 types as polysaccharide films, protein films, and lipid films (Šuput *et al.*, 2015). They are very interesting films, with many research examples developing them such as gelatin and polysaccharides (Ma *et al.*, 2012; Podshivalov *et al.*, 2017; Soo and Sarbon, 2018; Łupina *et al.*, 2019). In this research, a gelatin material was selected for the development of edible films. Gelatin, a type of protein film, is very

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distinctive because of its α -amino acid group, cheap and biodegradable. This makes a gelatin-protein film has been used to design and develop a wide range of film applications in food packaging (Peña *et al.*, 2008). The gelatin film has an important property which is hydrophilic (oil resistant) and able to prevent the penetration of oxygen and carbon dioxide well. However, there are some limitations on the permeability of water vapor and brittleness (Hui, 2007). Due to the bond firmness resulting from the hydrogen bond in the structure of the gelatin and polar amino acid groups, the gelatin becomes brittle as it dries.

Plasticizers are essential for the production of films made up of proteins and polysaccharides because they tend to be brittle due to extensive interactions between polymer molecules (Baldwin *et al.*, 2002; Hoque, 2011). The addition of plasticizers and absorption of water molecules into hydrophilic polymers increase polymer chain mobility (Bourtoom, 2008), which makes polymers more flexible.

There are many ways to improve and control gelatin film properties. One approach to improve the performance of gelatin film is by introducing some covalent bonds between protein chains of gelatin by cross-linking (Martucci *et al.*, 2012). Gelatin is a denatured product of collagen (Tsujimoto *et al.*, 2014) and dehydrothermal (DHT) treatment is a common technique for stabilizing collagen and its composite materials. It is a physical treatment that involves increasing temperature of film over 90°C under vacuum condition. This process removes water from molecules, resulting in the formation of intermolecular crosslinks through condensation reactions either by esterification or amide formation (Haugh *et al.*, 2009). DHT treatment is a selected method for crosslinking in this research because it does not involve the use of other chemical reagents that maybe not eaten.

The objective of this research is to study the effect of type and amount of plasticizers on physical characteristics, thermal properties and the moisture absorption of the thermally crosslinked and non-crosslinked gelatin films.

Materials and Methods

Materials

Gelatin powder (food grade, limed bone gelatin BP 250 Bloom) was purchased from Chemipan, Thailand (Shipped from Japan). There were 4 types of plasticizers (food grade) used in this research; glycerol (Gly), propylene glycol (PG), polyethylene glycol with molecular weight 400

(PEG400), and 4000 (PEG4000). Gly, PG, and PEG400 were purchased from Krungthepchemi, Thailand, and PEG4000 was purchased from Chemipan, Thailand.

Preparation of Gelatin Films

Plasticized or unplasticized gelatin films were obtained by solution casting. 10 g of gelatin powder was dissolved in 100 mL of distilled water at 50±2°C and stirring for 30 min. Various types of plasticizers were then added into the gelatin solution and the solution was held at 50±2°C under stirring for further 20 min. The concentration of plasticizers was varied as 0, 25, and 50 wt% on gelatin powder basis. The solutions were then subjected to ultrasonic treatment for about 2 min to dispel air bubbles. Finally, the solutions were casted onto a Teflon coated substrate and dried at 30°C for 24 h. The finished intact films were then peeled.

Cross-linking Film

The gelatin films were introduced to crosslink by dehydrothermal treatment (DHT) method with a vacuum oven (Jeio Tech, OV-11) at 140°C for 12 h. The non-crosslink film samples were labeled as GEL-AB where A and B referred to content and type of plasticizer, respectively. In the same way, crosslinked gelatin films were represented as GELX-AB.

Characterization

Film samples were investigated for physical characteristic, thermal properties, and moisture absorption.

For physical characteristic investigation, all gelatin films were photographed in same light and focal length for comparison. Commission International de l'Eclairage (CIE) L^* a^* b^* color space was used for identifying color differences of films.

In thermal properties testing, all film samples were characterized by differential scanning calorimeter (DSC) (Mettler Toledo DSC3+). The measurement was performed in temperature range from -10 to 120°C with the heating rate of 10°C/min under nitrogen atmosphere at a flow rate of 30 ml/min.

The moisture absorption was tested by the following experiments. First, all hydrated films at 80°C for 24 h. Then the films were weighed before testing. The films were incubated in the humidity by T&H-chamber (Jeio Tech, OF-11E) at a temperature of 35°C with relative humidity of 85% for 24 h. Finally, the films were weighed and calculated for the moisture absorption.

Results and Discussion

Physical Characteristic

Physical characteristic photos of all gelatin films were shown in Figure 1 and the CIE L^* a^* b^* color space data identifying color differences of films were illustrated in Table 1. The L^* indicates the lightness of the sample from black to white. The chroma coordinates a^* ranging from green (–) to red (+) and chroma b^* varying blue (–) to yellow (+). The results from L^* , a^* and b^* data were calculated to represent the total color change (dE^*). All crosslinked films were more brittle and stiffer than those non-crosslinked films due to the loss of water, by the condition of DHT, in the gelatin body and the rigid 3D network structure of the crosslinked.

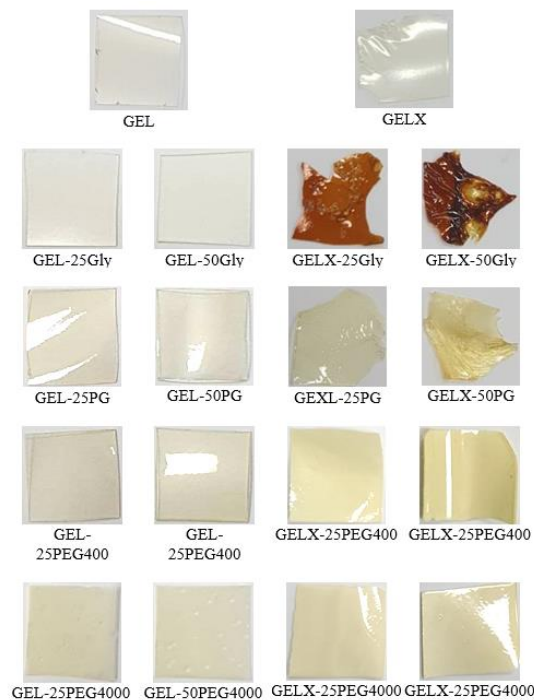


Figure 1. Physical characteristic of films

From Figure 1, the difference in the color of the GEL and GELX cannot be noticed, indicating that the DHT crosslinking process did not affect the color of the non-plasticized gelatin film. This investigation was confirmed by dL^* , da^* , db^* and dE^* values that showed a little different L^* a^* b^* value and dE^* between GEL and GELX. The dE^* value greater than 5 indicates a change of color perceptible by the human eye and values greater than 12 indicates an absolute color difference (Moreira *et al.*, 2018). However, L^* a^* b^* color different of GEL and GELX indicated that the

crosslink process made that non plasticized gelatin film slightly lighter, toward green and yellow. In the plasticized non-crosslinked gelatin films, the addition of all plasticizers did not change the color of the gelatin film, except PEG4000. The CIE investigation showed dE^* value of 7.1 to 10.8 confirming that the slightly different color of those films. The color of GEL-25PEG4000 and GEL-50PEG4000 were off white, cream, and opaque with dE^* value of 20.9 and 19.2, respectively, because of the distribution of off-white particle of high Mw PEG plasticizer. The L^* value correlates with the level of whiteness of the film. If the L^* value of the film is close to 0 (Blank in Table 1), it will indicate the whiter color of film. The CIE investigation revealed that adding higher amount of PEG4000 made non-crosslink gelatin film slightly lighter (whiter), redder and bluer.

Table 1. CIE L^* a^* b^* color space of films

Film	dE^*	dL^*	da^*	db^*
GEL	8.6	-2.1	-1.9	8.1
GELX	8.6	-1.8	-2.0	8.2
GEL-25Gly	8.6	-2.1	-2.2	8.1
GEL-50Gly	7.7	-1.5	-2.1	7.3
GELX-25Gly	62.5	-33.4	18.3	48.7
GELX-50Gly	62.5	-52.6	17.9	28.4
GEL-25PG	9.0	-2.4	-2.1	8.4
GEL-50PG	8.4	-1.7	-2.1	8.0
GELX-25PG	21.1	-4.4	-4.4	20.1
GELX-50PG	24.0	-4.1	-4.7	23.1
GEL-25PEG400	10.8	-2.5	-2.4	10.2
GEL-50PEG400	7.1	-2.0	-1.7	6.6
GELX-25PEG400	25.1	-5.3	-3.9	24.2
GELX-50PEG400	29.4	-7.9	-3.2	28.1
GEL-25PEG4000	20.9	-3.8	-4.5	20.0
GEL-50PEG4000	19.2	-1.9	-4.0	18.7
GELX-25PEG4000	31.9	-5.3	-3.4	31.2
GELX-50PEG4000	36.6	-5.5	-2.1	36.1
Blank (whitepaper)	0.0	0.0	0.0	0.0

Considering the effect of crosslinking by DHT process on the color of the plasticized gelatin film, it was found that the film color became more yellowish, which may be due to the loss of hydroxyl group as a result of this process. The confirmation data was shown as the higher db^* value of crosslinked film comparing to those non-crosslink films. In addition, the more plasticizer content, the color change is more noticeable, as seen in the case of addition of PG and PEG4000. For the addition of glycerol plasticizer, GELX-25Gly and GELX-50Gly became darker than other film samples as seen in very negative dL^* values of -33.4 and -52.6, respectively. This was caused by the thermal degradation of glycerol in DHT process. Moreover, it was observed that the more glycerol content, the

darker the color. In the case of adding PEG400, the film was changed from transparent film to yellowish white opaque film due to the loss of hydroxyl group in plasticizer molecule.

Thermal Properties

The thermal properties of the film were shown through the glass transition temperature (T_g) and melting temperature (T_m). The data were summarized in Table 2. First heating scan of all gelatin films showed T_g , except the case of PEG4000 plasticized gelatin films. However, their T_m were not observed but it could be seen only in the case of PEG4000 plasticized gelatin films.

Gelatin film is an amorphous material, while glycerol, propylene glycol, PEG400 are liquid at room temperature, therefore, T_m of gelatin film and their plasticized films cannot be detected with the testing condition in this work. T_m of PEG4000 was observed in the range of 50-60°C, approximately (Fadly *et al.*, 2016; Hempel *et al.*, 2020). It was expected that the T_m of PEG4000 obscures the vision of T_g of gelatin film. The chart for T_g comparison was shown in Figure 2. It could be noticed that the addition of plasticizers is a major cause of reduced T_g . However, adding more plasticizers makes the T_g even higher in the case of PG. This was due to PG having a short branched molecular structure. By increasing the amount of plasticizer, these short branches could hinder the movement of the gelatin polymer chains.

In the case of non-plasticized gelatin film, T_g of GELX was lower than GEL because the loss of some OH-groups and some non-crosslinked polymer chains of GELX could move easier than those full OH-group of GEL. For addition of Gly and PEG400, T_g of GELX-25Gly and GELX-25PEG400 was clearly higher than GELX because the OH-group of plasticizers was lost via the DHT crosslinked process, resulting to decreased plasticization effect. However, the T_g of those films was lower again when the concentration of plasticizer was increased. This was due to the proportion of the plasticizers in the gelatin matrix being greater and it was sufficient to see the plasticized effect on T_g . It was found that T_g of GELX-50Gly was close to T_g of GELX, while T_g of GELX-50PEG400 was slightly higher than T_g of GELX.

In addition, increasing the PG content makes T_g even higher because of the OH-group of PG via the DHT crosslinked process as well as the case of Gly and PEG400. However, the short branching molecular structure of PG hinders the movement of gelatin molecule, resulting to increased PG content making T_g higher. With all of the above, plasticizers

with a low molecular weight linear structure are suitable to lower the T_g of gelatin.

Table 2. T_g and T_m values of all films

Films	T_g (°C)	T_m (°C)
GEL	68.45	n/a
GELX	49.89	n/a
GEL-25Gly	53.52	n/a
GEL-50Gly	51.27	n/a
GELX-25Gly	55.9	n/a
GELX-50Gly	50.19	n/a
GEL-25PG	62.97	n/a
GEL-50PG	65.10	n/a
GELX-25PG	53.38	n/a
GELX-50PG	58.03	n/a
GEL-25PEG400	59.85	n/a
GEL-50PEG400	58.68	n/a
GELX-25PEG400	60.23	n/a
GELX-50PEG400	52.08	n/a
GEL-25PEG4000	n/a	57.05
GEL-50PEG4000	n/a	58.00
GELX-25PEG4000	n/a	55.96
GELX-50PEG4000	n/a	54.36

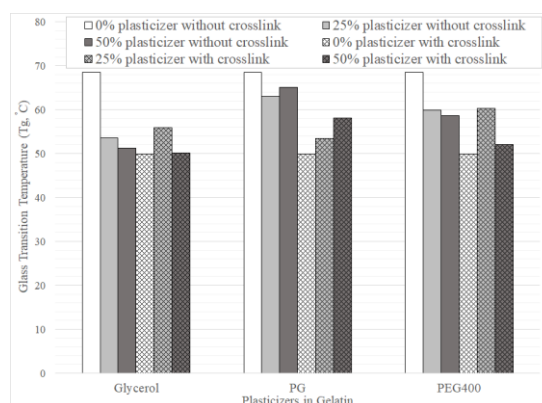


Figure 2. T_g comparison chart of all films

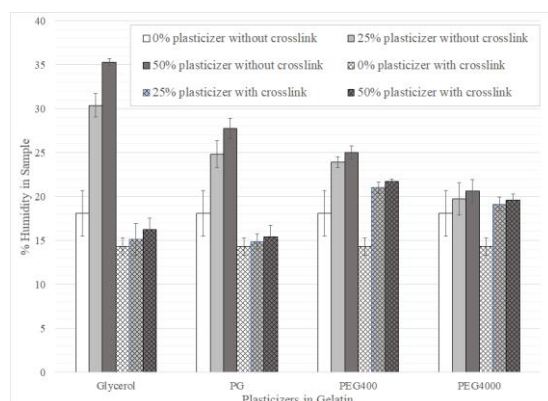
Moisture Absorption

The result of moisture absorption experiment and the comparison chart were shown in Table 3 and Figure 3, respectively.

It was found that the addition of plasticizers resulted in a higher hygroscopicity of films. This was due to the fact that plasticizers had hydroxyl groups that can absorb moisture well. In the case of non-crosslinked gelatin films, it was clearly seen that the hygroscopicity of film that was filled by plasticizers with high OH-group numbers per molecular weight is high. The addition of glycerol resulted in the highest hygroscopicity, followed by the PG, PEG400 and the PEG4000, respectively. The higher the amount of plasticizer, the higher the moisture absorption volume as well.

Table 3. The percentage moisture absorption values of all films

Film	Moisture Absorption (%, Wet Basis)	S.D.
GEL	18.09	2.62
GELX	14.28	1.02
GEL-25Gly	30.38	1.34
GEL-50Gly	35.29	0.38
GELX-25Gly	15.13	1.82
GELX-50Gly	16.25	1.27
GEL-25PG	24.82	1.53
GEL-50PG	27.76	1.16
GELX-25PG	14.86	0.89
GELX-50PG	15.43	1.27
GEL-25PEG400	23.93	0.61
GEL-50PEG400	25.00	0.75
GELX-25PEG400	21.00	0.66
GELX-50PEG400	21.75	0.26
GEL-25PEG4000	19.73	1.86
GEL-50PEG4000	20.60	1.33
GELX-25PEG4000	19.13	0.77
GELX-50PEG4000	19.61	0.67

**Figure 3. The percentage moisture absorption comparison chart of films**

For the crosslink gelatin films, GELX is less hygroscopic than GEL because DHT made crosslinking and decreased the OH-group content of the gelatin. The addition of plasticizers made the crosslinked gelatin films more hygroscopic than those of the non-crosslinked films without the addition of plasticizer. The higher the plasticizer content, the higher the moisture absorption as well as the non-crosslinked case. It was observed that the hygroscopicity of the crosslinked gelatin film with the addition of Gly and PG was significantly reduced from the non-crosslinked films. These reductions in moisture absorption are clearly more than those of films with PEG400 and PEG4000.

Since Gly and PG are small molecule (monomers) with a high number of OH-groups, DHT leads to a significant reduction in OH content. On the other hand, PEG400 and PEG4000 are large molecule (polymers) with OH-groups just at the end

of the molecular chain, therefore DHT resulted in a decrease in the amount of OH-group less than Gly and PG cases.

When comparing the moisture absorption of crosslinked gelatin films with the addition of PEG400 and PEG4000, it was found that the addition of PEG400 was more hygroscopic than the addition of PEG4000. This was due to the number of polar ether linkages in the PEG400, the smaller molecules, had better moisture-holding ability than the ether linkage in PEG4000, the larger molecule.

Conclusions

The crosslinked and non-crosslinked gelatin film with and without plasticizers were prepared in this research. The crosslinked films were developed by DHT. The addition of all plasticizers did not change the color of the non-crosslinked gelatin films, except PEG4000. The color of plasticized gelatin films became more yellowish by the DHT. In the case of non-plasticized gelatin film, T_g and hygroscopicity of crosslinked gelatin film was lower than non-crosslinked gelatin film. The addition of plasticizers resulted in a higher hygroscopicity of films. It was expected that plasticizers with a low molecular weight linear structure can lower the T_g of gelatin. The crosslinking is an important step in developing a gelatin film that is stable in ambient conditions and soluble in hot water (cooking conditions) for use in edible food packaging. Propylene glycol plasticized crosslinked gelatin film is still transparent and suitable for developing into packaging that needs to show the product inside. However, crosslinking results in greatly reduced film hygroscopicity, thus reducing the softness of the film. On the other hand, PEG400 provides an opaque film that cannot display the product inside and crosslinking has less of an impact on the film's hygroscopicity. The hygroscopicity of the crosslinked gelatin film was changed insignificantly when the content of plasticizers was increased. In the future, for the development of edible films, it is required to further explore the solubility of the film in water at various temperatures, as well as the mechanical and seal properties.

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