Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films

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Abstract

The objective of this paper was to study the effect of plasticizers and their concentrations on the thermal and functional properties of gelatin-based films. Four polyols (glycerol—GLY, propylene glycol—PPG, di- DTG and ethylene glycol—ETG) were tested in five concentrations: 10, 15, 20, 25, and 30 g plasticizer/100 g of gelatin. For thermal analysis, the films were conditioned in desiccators with silica gel and for functional properties (mechanical properties, water vapor permeability, color and opacity) characterizations, the films were conditioned at 25 °C and 58% relative humidity. The results were discussed in terms of 'plasticizer efficiency' meaning plasticizer content effect and also in terms of 'plasticizer effect' meaning the plasticizer type effect on the properties. In a general manner, the higher plasticizing effect on thermal properties was observed with the DTG, followed by PPG, GLY and ETG, principally with low plasticizer content. However, also in terms of thermal properties, the ETG presented the higher plasticizer efficiency followed by the GLY, DTG and PPG. Concerning the mechanical properties, the GLY showed the greater plasticizing effect and efficiency, but the plasticizing efficiency of DTG on the puncture deformation was also considerable. The mechanical resistance could be related with the glass transition temperature of films. It was not possible to observe the plasticizer effect on the water vapor permeability (WVP), but the DTG had shown greater plasticizer efficiency, followed by GLY and ETG, while a counter effect was observed with PPG. The effect of the type and concentration of plasticizer on the color and opacity of films could be considered as negligible. In conclusion, the more important plasticizer effect and efficiency were observed with DTG and ETG on the thermal properties, and with the GLY in terms of functional properties. The behavior of the mechanical properties of films could be explained by its glass transition temperature.
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1. Introduction

The ability of proteins to form a continuous matrix has been known for a long time. Due to this reason, various proteins of vegetal and animal origin have received attention in the edible film technology (Gennadios, McHugh, Weller, & Krochta, 1994; Torres, 1994). The proteins are interesting as biomaterial because they have a specific structure based on 20 amino acids which confers a wide range of functional and film-forming properties as a function of various extrinsic or intrinsic conditions, such as plasticizer type and concentration (Cuq, Aymard, Cuq, & Guilbert, 1995).

Gelatin was one of the first materials employed in formation of biomaterials (Gennadios et al., 1994), and has been subjected in many patents (Torres, 1994). Gelatin continue to be used in studies on edible films because it is an abundant raw material, produced in the whole world at low cost and has excellent film forming properties. Good revisions on the gelatin structure and its functional properties related to filmogenic abilities have been recently published (Arvanitoyannis, 2002; Gennadios et al., 1994).

Recently, Menegalli, Sobral, Roques, and Laurent (1999), Sobral (1999), and Sobral, Menegalli, Hubinger, and Roques (2001) studied various properties of gelatin-based edible films plasticized by sorbitol as a function of

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plasticizer concentration, drying conditions and/or thickness. More recently, Simon-Lukasik and Ludescher (2004) studied oxygen diffusion in films of gelatin as a function of the relative humidity of conditioning. Also, Arvanitoyannis, Psomiadou, Nakayama, Aiba, and Yamamoto (1997) and Arvanitoyannis, Nakayama, and Aiba (1998) studied the thermal and functional properties of edible films made from blends of gelatin and starches as function of various plasticizers, and Bertan, Tanada-Palmu, Siani, and Grosso (2005) studied microstructural and physical properties of films based on blends of gelatin and two fat acids and plasticized by triacetin. In a general manner, the properties of these materials can be considered as typical of edible films.

To try to reduce the water solubility of gelatin-based films, some authors have tried modifying the gelatin. Sarmento, Sobral, and Menegalli (2000) and Sakanaka, Sobral, and Menegalli (2001) studied some physical properties of films based on gelatin previously cross linked using the glutaraldehyde and plasticized by sorbitol, and Carvalho and Grosso (2004) studied the effect of the cross linking agent (formaldehyde, glyoxal and transglutaminase) on some properties of its films plasticized with the glycerol.

The film solubility in water was reduced, but the other functional properties were not necessarily improved because the amount of plasticizer necessary to maintain these films flexible was increased. These films cannot be considered as edible, but they are always interesting because of their biodegradable characteristics.

Like the collagen, the gelatin chains are macromolecules with a tendency mainly to interchain, rather than intrachain and hydrogen bonding (Arvanitoyannis, 2002). Gelatin forms a three-dimensional network with zones of intermolecular microcrystalline junctions (Slade & Levine, 1987), and the dehydration of this system may produce brittle films. Thus, plasticizers must be added to reduce interchain interactions improving film flexibility (Sothornvit & Krochta, 2001; Torres, 1994).

According to Sothornvit and Krochta (2001), plasticizer selection is normally based on the compatibility between plasticizer and protein, permanence in the film and amount necessary to plasticization. Despite different possibilities of additives that can be used as plasticizer in protein-based films (Table 1), sorbitol and glycerol are more used as plasticizers in gelatin-based films. Besides influencing all the properties of the films, some plasticizers, like sorbitol,

Table 1
Examples of works on plasticizers[a] types in films based on proteins

Biopolymers	Plasticizers	References
Casein	Glycerol, polyethylene glycol 400	Siew, Heilmann, Easteal, and Cooney (1999)
	Glycerol, glucose, sucrose, xylose	Arvanitoyannis, Psomiadou, and Nakayama (1996)[b]
β-Lactoglobulin	Glycerol, sorbitol, sucrose, polyethylene glycol 200 and 400, propylene glycol	Sothornvit and Krochta (2001) and Sothornvit, Reid, and Krochta (2002)
Egg albumen	Glycerol, sorbitol, polyethylene glycol 400	Gennadios, Weller, Hanna, and Froning (1996)
Fish proteins	Glycerol, sorbitol, sucrose	Cuq, Gontard, Cuq, and Guilbert (1997)
	Glycerol, sorbitol, ethylene glycol, polyethylene glycol 400, sucrose	Tanaka, Iwata, Sanguandeekul, Handa, and Ishizaki (2001)
Gelatin	Glycerol, sorbitol, sucrose	Arvanitoyannis, Psomiadou, Nakayama, Aiba, and Yamamoto (1997)[c] and Arvanitoyannis, Nakayama, and Aiba (1998)[c]
Pea protein	Glycerol, di-, tri-, tetra- and ethylene glycol, 1,2- and 1,3-propanediol	Viroben, Barbot, Mouloungui, and Guéguen (2000)
Sunflower protein isolate	Glycerol, di-, tri-, propyl- and ethylene glycol	Orliac, Rouilly, Silvestre, and Rigal (2003)
	Glycerol, 1,3-propanediol, sorbitol, triethylene glycol, tetraethylene glycol	Ayhllon-Meixueiro, Vaca-Garcia, and Silvestre (2000)
Wheat gluten	Glycerol, triethylene glycol	Gennadios, Weller, and Testin (1993)
	Glycerol, sorbitol, sucrose	Cherian, Gennadios, Weller, and Chinachotti (1995)
	Glycerol, polyethylene glycol 200, 400, 1500 and 3400, polypropylene glycol 400, 2000, 3000 and 4000, trimethylolpropane, diethanolamine, dimetylethanolamine, triethanolamine, polyethyleneimine 700, polyvinylalcohol	Irissin-Mangata, Bauduin, Boutevin, and Gontard (2001)
Wheat gliadin	Glycerol, di-, tri-, tetra- and ethylene glycol, diethylene glycol-monomethyl ether	Sánchez, Popineau, Mangavel, Larré, and Guéguen (1998)
Whey protein isolate	Glycerol, sorbitol	Fairley, Monahan, German, and Krochta (1996) and Anker, Stading, and Hermansson (2001)
	Glycerol, xylitol, sorbitol	Shaw, Monohan, O'Riordan, and O'Sullivan (2002)

[a] Excluding the water.
[b] These authors studied blends of casein and starches.
[c] These authors studied blends of gelatin and starch.

can also crystallize inside the film depending on the storage conditions, and the material can completely lose its characteristics (Sakanaka et al., 2001). So, new and old plasticizers should continuously be studied in the edible films technology.

This way, the objective of this work was to study the color, opacity, permeability to water vapor, mechanical and thermal properties of gelatin films produced at various concentrations (10–30 g/100 g gelatin) of four different plasticizers (glycerol, ethylene glycol, diethylene glycol and propylene glycol).

2. Materials and methods

2.1. Films production

The films were obtained by drying film forming solutions (FFS) with the following compositions: 2 g of gelatin/100 mL of water; 10, 15, 20, 25 and 30 g plasticizer/100 g gelatin; and natural pH of the solution These FFS were applied on plexiglass plate (11.8×11.8 cm^2) always with the same dry matter density to guarantee constant thickness. More detail about the pigskin gelatin characteristics and film process conditions may be found on previous works (Sobral, 1999; Sobral et al., 2001). The studied plasticizer were the glycerol ($C_3H_8O_3$, molecular weight, MW = 92 g/gmol), the ethylene glycol ($C_2H_6O_2$, MW = 62 g/gmol), the diethylene glycol ($C_4H_{10}O_3$, MW = 106 g/gmol) and the propylene glycol ($C_3H_8O_2$, MW = 76 g/gmol).

For the study of thermal properties, in triplicate, the edible films were conditioned in desiccators with silica gel, for at least 3 weeks, to obtain the most dehydrated possible material. For functional properties characterization, always in quadruplicate, the films were conditioned at 25 °C and 58% of relative humidity, in desiccators containing saturated solution of NaBr, for 4–7 days. Then, the thickness of films was measured averaging nine different positions, with a digital micrometer (± 0.001 mm) with a 6.4 mm diameter probe. All characterizations were accomplished in climatized room conditions ($T = 22$ °C and relative humidity between 55 and 65%).

2.2. Thermal properties

The determination of the thermal properties of the edible films was accomplished by differential scanning calorimetry, using a DSC TA 2010 controlled by a TA 5000 module (TA Instruments, New Castle, DE, USA), with a quench cooling accessory. The samples were conditioned on hermetic aluminum pans and heated, twice, at 5 °C/min, between −50 and 150 °C in inert atmosphere (45 mL/min of N_2). The glass transition temperature (T_g) was calculated as the inflexion point of the base line, caused by the discontinuity of the specific heat of the sample. The helix–coil transition temperature (T_m) was calculated as the temperature where

the peak of the endotherm occurs, and the enthalpy (ΔH_m) of the sol–gel transition was calculated as the area over the endothermic peak. All these properties were calculated with help of the software Universal Analysis V1.7F (TA Instruments) (Sobral, Monterrey-Quintero, & Habitante, 2002).

2.3. Mechanical properties

The mechanical properties of films were determined by puncture tests (Gontard, Guilbert, & Cuq, 1993) with an instrument of physical measures TA.XT2i (SMS, Surrey, UK). The films were fixed in a 52.6 mm diameter cell and perforated by a 3 mm diameter probe, moving at 1 mm/s and the puncture force and the puncture deformation were calculated considering that stress was perfectly distributed along the film at breaking point (Sobral et al., 2001).

2.4. Water vapor permeability

The water vapor permeability (WVP) was determined gravimetrically according to a method proposed by Gontard et al. (1993), at 25 °C. The edible films were firmly fixed onto the opening of cells containing silica gel and then, these cells were placed in desiccators with distilled water. These cells were weighed (± 0.01 g) daily, in a semi-analytic balance (Mars, AS2000), during 7–8 days to guarantee the steady state permeation.

2.5. Color and opacity

The color of the films was determined with a colorimeter (HunterLab, model Miniscan XE), working with D_{65} (day light) and a measure cell with opening of 30 mm, using the CIELab color parameters, being expressed as the difference of color (ΔE^*) (Gennadios, Weller, Hanna, & Froning, 1996) The films opacity was determined according to a Hunterlab method, with the same equipment used for the color measures, also operating in the reflectance mode (Paschoalick, Garcia, Sobral, & Habitante, 2003).

2.6. Approaches and statistical analysis

Considering that the domain of plasticizers concentration studied on this work was not large, the plasticizer behavior was represented by a linear equation ($P = k_{ef}C_p$) proposed by Sothornvit and Krochta (2001), where P is the convenient property, C_p is the plasticizer concentration (expressed as g/g gelatin) and k_{ef}, is an empirical parameter.

Thus, the results of this work will be discussed with two approaches:

(i) 'Plasticizer efficiency' meaning plasticizer content effect, i.e. the capacity of plasticizer in reducing the property values, calculated as k_{ef}, in the domain of concentrations studied. This parameter was calculated

Table 2
Plasticizer effectiveness (k_{ef}) on gelatin-based films

	Plasticizer	k_{ef}	R	p
Glass transition temperature (°C)	GLY	-219.0 ± 57.0	-0.912	0.03
	PPG	-43.0 ± 17.4	-0.813	0.09
	ETG	-340.4 ± 85.6	-0.917	0.03
	DTG	-143.4 ± 27.4	-0.949	0.01
Helix–coil transition temperature (°C)	GLY	-144.0 ± 40.4	-0.899	0.04
	PPG	-29.0 ± 8.1	-0.900	0.04
	ETG	-254.8 ± 64.2	-0.916	0.03
	DTG	-80.4 ± 9.9	-0.978	<0.01
Helix–coil transition enthalpy (J/g)	GLY	21.8 ± 11.3	0.745	0.15
	PPG	4.2 ± 3.9	0.530	0.36
	ETG	47.0 ± 8.9	0.950	0.01
	DTG	-17.4 ± 5.8	-0.865	0.06
Puncture force (N)	GLY	-54.8 ± 10.3	-0.951	0.01
	PPG	-23.2 ± 8.1	-0.856	0.06
	ETG	-4.0 ± 5.2	-0.400	0.50
	DTG	-53.2 ± 6.1	-0.981	< 0.01
Puncture deformartion (%)	GLY	25.9 ± 2.0	0.991	<0.01
	PPG	6.0 ± 1.9	0.878	0.05
	ETG	1.6 ± 1.3	0.565	0.32
	DTG	8.9 ± 2.9	0.869	0.10
WVP ($10-8$ g mm h^{-1} cm^{-2}Pa^{-1})	GLY	7.7 ± 2.3	0.889	0.04
	PPG	-5.8 ± 2.1	-0.844	0.07
	ETG	6.3 ± 1.3	0.940	0.02
	DTG	16.1 ± 1.1	0.993	<0.01
Color difference	GLY	-0.8 ± 0.08	-0.985	<0.01
	PPG	0.12 ± 0.16	0.387	0.52
	ETG	0.16 ± 0.29	0.305	0.62
	DTG	-3.98 ± 1.10	-0.902	0.04
Opacity	GLY	-0.12 ± 0.27	-0.247	0.69
	PPG	1.12 ± 0.56	0.759	0.14
	ETG	-0.68 ± 0.28	-0.813	0.09
	DTG	3.50 ± 1.15	0.868	0.06

GLY—glycerol, PPG—propylene glycol, ETG—ethylene glycol, DTG—diethylene glycol.

by linear regression (Table 2) with the Microcal Origin software V.5.0 (Microcal Software INC., Northampton, MA) which allowed the determination of the regression coefficient and the regression probability.

(ii) And 'plasticizer effect' meaning plasticizer type effect, determined by comparison of properties values between different plasticizers at similar concentration. For that, Duncan's multiple range test was applied to compare the means of functional properties with a level of significance of $\alpha = 0.05$, using the SAS software (version 8.2, SAS Inc. Cary, USA).

And, the linear regressions necessary to the calculation of WVP, were accomplished with the Excel 2000 software (Microsoft, Seattle, WA).

3. Results and discussions

In general, the films prepared with all studied plasticizers showed to be easily workable having a good general aspect. This is not necessarily a rule, because for example, according to Tanaka, Iwata, Sanguandeekul, Handa, and Ishizaki (2001), it was impossible to prepare films based on fish sarcoplasmic proteins with the ETG. Thus, it can be considered that, in this study, all the plasticizers presented good compatibility with gelatin.

The application of a constant dry matter density of FFS on cast support showed to have a good control over the film thickness (Table 2). It can be considered that the overall film thickness remained around 0.060 mm. The effect of the observed thickness differences on functional properties of gelatin-based films could be considered as negligible (Sobral, 1999).

On the other hand, it was observed that the increase of plasticizer concentration on FFS affected the humidity of films after conditioning, with a more important effect in films with GLY and DTG (Table 3) thus that could be considered as the more hygroscopic plasticizers in this work. Shaw, Monahan, O'Riordan, and O'Sullivan (2002) conditioned films based on WPI plasticized with GLY, sorbitol and xylitol and determined the higher moisture in

Table 3
Influence of plasticizer type and concentration on the thickness and humidity of gelatin-based films

Plasticizer content (g/100 g gelatin)	Plasticizer			
	GLY	PPG	ETG	DTG
Thickness (mm)				
10	0.060 ± 0.008^a	0.052 ± 0.006^a	0.052 ± 0.001^a	0.056 ± 0.004^a
15	0.060 ± 0.001^a	0.056 ± 0.005^a	0.062 ± 0.001^a	0.065 ± 0.002^a
20	0.060 ± 0.010^a	0.052 ± 0.006^a	0.057 ± 0.001^a	0.059 ± 0.002^a
25	0.069 ± 0.003^a	0.060 ± 0.006^b	0.058 ± 0.001^b	$0.064 \pm 0.001^{a,b}$
30	0.077 ± 0.004^a	$0.062 \pm 0.003^{b,c}$	0.057 ± 0.004^c	0.067 ± 0.002^b
Humidity (g water/ 100 g moist solids)				
10	14.0 ± 0.9^a	13.5 ± 0.2^a	12.7 ± 0.5^a	15.7 ± 1.9^b
15	14.3 ± 1.1^a	13.3 ± 0.4^a	13.5 ± 0.8^a	18.2 ± 0.4^b
20	16.6 ± 1.5^a	12.9 ± 1.1^b	14.3 ± 0.8^b	18.3 ± 0.4^c
25	21.8 ± 1.5^a	13.2 ± 0.7^b	15.1 ± 1.4^c	19.8 ± 1.1^d
30	21.2 ± 0.7^a	13.7 ± 0.9^b	15.4 ± 1.0^c	22.4 ± 0.7^a

Average $\pm$ standard deviation. Averages with different superscripts at the same row are significantly different ($p < 0.05$); GLY—glycerol, PPG—polypropylene glycol, ETG—ethylene glycol, DTG—diethylene glycol.

films with GLY. On the contrary, Sánchez, Popineau, Mangavel, Larré, and Guéguen (1998) determined the higher residual moisture in films (see Table 1) made using ETG and they also did not observe any difference between the moisture content of the films made with GLY and DTG.

In a general manner, the film humidity was higher than the humidity of the gelatin (without plasticizer, 13 g water/100 g moist solids) equilibrated also at 58% of relative humidity by Sobral and Habitante (2001), due to the hygroscopic character of plasticizers. Similar results can be found in the literature (Gennadios & Weller, 1994; Gontard et al., 1993; Monterrey-Quintero & Sobral, 2000).

3.1. Thermal properties

In these studies, the gelatin films were conditioned over silica gel, which did not guarantee anhydrous samples such as observed previously by Sobral et al. (2001). The residual moisture of samples varied between 4 and 9.5 for films with

GLY; 5 and 7 for PPG; 7 and 10 for ETG; and 12 and 15.5 g water/100 g moist solids for DTG.

The DSC curves of these films are presented in Fig. 1. In all cases, only a glass transition (T_g) followed by an endothermic peak due to the helix–coil transition (T_m) appeared in DSC curves of the first scan, and only the glass transition appeared and more noticeably on the respective DSC curve of the second scan, shifted to lower temperature with the increase of plasticizer concentration. These behaviors indicated that no phase separation occurred between plasticizer and gelatin (Sobral et al., 2001), and that all films produced in this works were partially crystalline; being that the second scan DSC curves corresponded to the completely amorphous films (Slade & Levine, 1987; Sobral & Habitante, 2001). Similar DSC curves were obtained by Arvanitoyannis et al. (1997) with films of gelatin/starch blends plasticized with glycerol or sorbitol, and by Carvalho and Grosso (2004) in films based on cross-linked gelatin plasticized with glycerol.
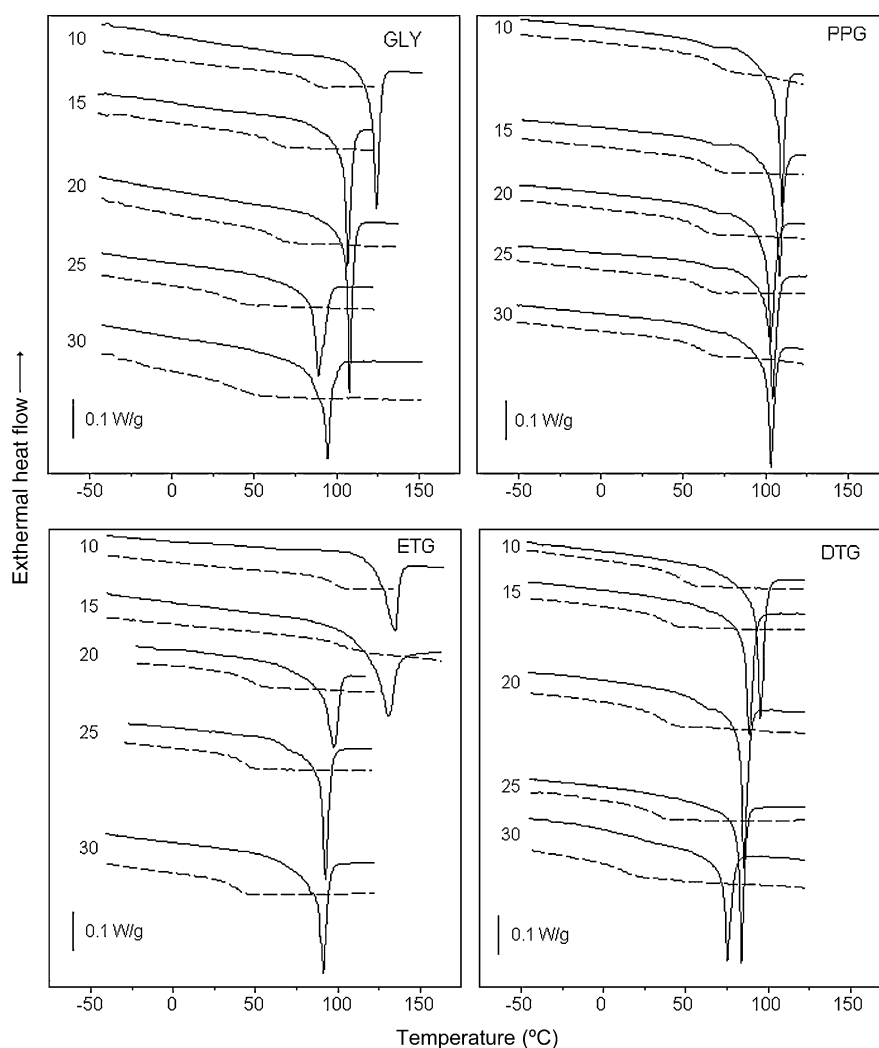

Fig. 1. Representatives examples of DSC curves of gelatin-based films plasticized with glycerol (GLY), propylene glycol (PPG), ethylene glycol (ETG), and diethylene glycol (DTG): — first scan, - - - second scan.

Table 4
Influence of plasticizer type on the concentration on the thermal properties of gelatin-based films

Plasticizer content (g/100 g gelatin)	Plasticizer			
	GLY	PPG	ETG	DTG
Glass transition temperature (°C)				
10	82.5 ± 7.9^a	69.9 ± 4.1^b	98.5 ± 6.4^c	45.8 ± 5.4^d
15	62.8 ± 4.8^a	66.3 ± 2.5^a	95.8 ± 8.4^b	37.7 ± 7.7^b
20	64.3 ± 3.0^a	60.2 ± 2.3^a	49.1 ± 2.4^b	31.5 ± 13.8^c
25	35.5 ± 14.5^a	60.8 ± 2.9^b	42.8 ± 7.0^a	30.8 ± 4.8^a
30	41.4 ± 6.5^a	61.9 ± 1.5^b	39.9 ± 2.9^a	13.4 ± 4.1^c
Helix–coil transition temperature (°C)				
10	121.1 ± 3.5^a	109.4 ± 2.9^b	136.7 ± 6.0^c	93.3 ± 3.8^d
15	106.3 ± 4.1^a	107.3 ± 1.6^a	130.2 ± 6.9^b	88.1 ± 3.9^c
20	107.5 ± 1.8^a	103.8 ± 0.9^a	96.9 ± 0.9^b	83.3 ± 6.3^c
25	$88.9 \pm 8.4^{a,b}$	104.4 ± 1.1^c	92.2 ± 3.0^a	82.7 ± 3.3^b
30	93.8 ± 3.7^a	103.6 ± 0.7^b	92.1 ± 1.1^a	75.9 ± 1.4^c
Helix–coil enthalpy (J/g)				
10	18.0 ± 7.4^a	$20.2 \pm 1.7^{a,b}$	16.9 ± 0.5^a	24.1 ± 2.1^b
15	23.1 ± 0.9^a	$21.3 \pm 1.7^{a,b}$	18.2 ± 1.4^b	23.9 ± 0.4^a
20	23.0 ± 0.4^a	21.6 ± 0.6^a	22.5 ± 2.0^a	24.3 ± 2.3^a
25	23.0 ± 0.9^a	21.8 ± 1.7^a	25.5 ± 0.9^a	22.2 ± 1.1^a
30	23.5 ± 0.6^a	21.0 ± 1.6^a	25.0 ± 1.6^a	20.6 ± 1.5^b

Average $\pm$ standard deviation. Averages with different superscripts at the same row are significantly different ($p < 0.05$); GLY—glycerol, PPG—polypropylene glycol, ETG—ethylene glycol, DTG—diethylene glycol.

In a general manner, the higher plasticizing effect ($p < 0.05$) in terms of T_g values, or in other words, the lower values of T_g and T_m were observed with DTG, followed by PPG and GLY (Table 4), principally in the low domain of plasticizer content ($<20\%$). This behavior is coherent with Sothornvit, Reid, and Krochta (2002), who observed that the type of plasticizer (see Table 1) produced a significant effect on the T_g of β-lactoglobulin films although they not observed difference among the values of T_g in films with PPG and GLY. In a similar work, Shaw et al. (2002) observed that the values of T_g in WPI-based films increased in the following order of plasticizers: GLY, xylitol and sorbitol, while Arvanitoyannis, Psomiadou, and Nakayama (1996) classified GLY < sorbitol < xylose < sucrose in relation to the effect of T_g of casein/starch blends based films containing 5% of humidity. On another side, Arvanitoyannis et al. (1997) did not observe differences between the effect of the GLY, sorbitol and sucrose on the thermal properties of gelatin/starch blends based films with 5% of humidity.

On the other side, Sothornvit et al. (2002) observed that the concentration of plasticizer did not influence the T_g of the films, contrary to that observed in this work. In terms of plasticizer efficiency, it was observed that the most efficient was ETG ($p < 0.05$), followed by GLY ($p < 0.05$), DTG ($p < 0.05$) and finally PPG ($p < 0.10$) (Table 2). This behavior repeated in the case of plasticizer efficiency on T_m (Table 2). The decrease of T_g and T_m with the increasing of plasticizer content was expected and had been observed

by several works (Gontard et al., 1993; Sobral et al., 2001, 2002).

The values of T_g determined in this work were above the room temperature due to the low humidity of the films after conditioning in silica gel, i.e. certainly, the T_g of films conditioned at least 58% relative humidity may be T_g under room temperature because water is a very efficient plasticizer (Cherian, Gennadios, Weller, & Chinachoti, 1995; Sobral et al., 2002). Sothornvit et al. (2002) determined sub-zero values of T_g of films plasticized with GLY, PPG and sorbitol among others, although, they used films conditioned at 50% of relative humidity.

In addition, it was observed in a general manner that the type of plasticizer did not influence the enthalpy of the helix–coil transition (Table 4). This result was also observed by Sobral et al. (2001) in plasticized gelatin films with sorbitol.

The plasticizing effect of polyols on T_g, in particular, can be attributed to their ability to locate between the chains of proteins, bind water, and disrupt intermolecular polymer interactions (Gontard et al., 1993). Thus, Shaw et al. (2002) tried to explain the difference between plasticizer effects by the different solubility of GLY, xylitol and sorbitol. But under the present study, all the plasticizers were highly soluble in water, even though their hygroscopicity were different (Table 3).

Another possible parameter, which could explain the different effects of the studied plasticizers, would be the number of oxygen atoms in the molecule, but Sothornvit et al. (2002) could not explain their results with this theory. This way, an attempt was made to explain the behavior of T_g in the films in terms of plasticizer molecule size associated with its bind water capacity, i.e. in terms of the amount of all molecules of the plasticizers and the water within films. As it could be observed in Fig. 2, the reduction of T_g of the films could be considered as a consequence of the increase of the number of moles of all plasticizers (polyols and water) present in the films. These results were in according to Cuq, Gontard, Cuq, and Guilbert (1997).
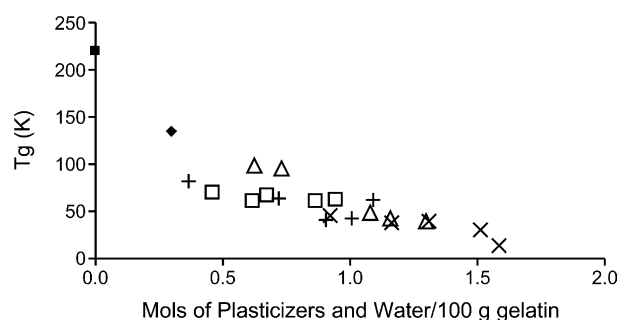
Fig. 2. Glass transition temperature of gelatin-based films as a function of total molar amount of water molecules plus plasticizers: glycerol ($+$), propylene glycol ($\square$), ethylene glycol ($\triangle$), and diethylene glycol ($\times$). The symbols $\blacksquare$ and $\blacklozenge$ correspond to the T_g of the anhydrous gelatin and the gelatin conditioned over silica gel, respectively (Sobral & Habitante, 2001).

3.2. Mechanical properties

Among all the plasticizers studied, glycerol presented the highest ($p < 0.05$) plasticizing effect on the mechanical properties of films causing the lower values of puncture force (PF) when compared to other plasticizers with similar concentrations, and higher values of puncture deformation (PD), mainly in the domain of higher concentrations ($p < 0.05$) (Table 5). The differences among the values of film PD containing PPG, ETG and DTG were negligible, and in a general manner, lower than the values of films with glycerol.

In terms of plasticizer efficiency, or in other words, the capacity of alteration of property due to the increment of plasticizer, it was observed that glycerol and DTG showed higher ($p < 0.05$) efficiency in terms of PF, followed by PPG ($p < 0.10$) (Table 2), while in the case of PD, the efficiency of GLY in increasing this property was higher ($p < 0.05$) than other plasticizers. The dispersion of the results of ETG did not allow the determination ($p > 0.10$) of efficiency of

Table 5
Influence of plasticizer type and concentration on the functional properties of gelatin-based films

Plasticizer content (g/100 g gelatin)	Plasticizer			
	GLY	PPG	ETG	DTG
Puncture force (N)				
10	18.28 ± 0.91^a	20.93 ± 0.77^b	18.03 ± 1.15^a	27.28 ± 3.19^c
15	17.97 ± 1.21^a	21.40 ± 1.45^b	17.22 ± 0.22^c	25.84 ± 0.40^d
20	15.06 ± 1.95^a	17.56 ± 1.80^b	18.91 ± 1.40^b	23.95 ± 1.53^c
25	9.32 ± 1.36^a	18.86 ± 1.16^b	17.38 ± 1.07^b	19.43 ± 1.58^b
30	8.90 ± 0.87^a	16.41 ± 1.03^b	16.96 ± 0.77^b	17.18 ± 0.43^b
Puncture deformation (%)				
10	1.77 ± 0.24^a	1.52 ± 0.29^a	1.25 ± 0.09^a	1.95 ± 0.57^a
15	2.33 ± 0.14^a	$1.67 \pm 0.31^{a,b}$	1.08 ± 0.02^b	$1.87 \pm 0.08^{a,b}$
20	4.11 ± 0.46^a	1.85 ± 0.31^b	1.62 ± 0.30^b	2.01 ± 0.14^b
25	5.45 ± 1.82^a	1.92 ± 0.37^{bc}	1.29 ± 0.15^b	2.63 ± 0.15^c
30	6.69 ± 0.75^a	2.90 ± 0.22^b	1.54 ± 0.27^c	3.79 ± 0.36^d
Water vapor permeability (10^8) ($g\ mm\ h^{-1}\ cm^{-2}\ Pa^{-1}$)				
10	$2.96 \pm 0.94^{a,b}$	4.43 ± 0.72^a	$3.12 \pm 0.45^{a,b}$	2.68 ± 0.07^b
15	3.71 ± 0.21^a	3.99 ± 0.38^a	3.71 ± 0.54^a	3.46 ± 0.11^a
20	3.57 ± 0.40^a	4.23 ± 2.17^a	3.74 ± 0.34^a	3.94 ± 0.39^a
25	3.77 ± 0.39^a	3.17 ± 0.63^a	3.90 ± 0.71^a	5.11 ± 1.09^a
30	$4.85 \pm 0.17^{a,b}$	3.40 ± 1.11^a	$4.60 \pm 0.99^{a,b}$	5.87 ± 1.77^b
Color difference				
10	3.09 ± 0.08^a	2.95 ± 0.05^b	3.01 ± 0.03^b	3.58 ± 0.01^c
15	3.03 ± 0.02^a	$2.95 \pm 0.02^{a,b}$	2.95 ± 0.02^b	3.53 ± 0.03^c
20	3.01 ± 0.03^a	2.93 ± 0.02^b	2.93 ± 0.01^b	$2.95 \pm 0.05^{a,b}$
25	2.95 ± 0.08^a	2.93 ± 0.03^a	2.99 ± 0.02^a	2.92 ± 0.02^a
30	$2.93 \pm 0.03^{a,c}$	$2.99 \pm 0.06^{a,b}$	3.03 ± 0.09^b	2.89 ± 0.08^c
Opacity				
10	0.45 ± 0.04^a	0.43 ± 0.13^a	0.36 ± 0.03^a	0.20 ± 0.01^b
15	0.49 ± 0.10^a	0.30 ± 0.03^b	0.22 ± 0.04^b	0.21 ± 0.04^b
20	0.43 ± 0.16^a	0.54 ± 0.08^a	0.25 ± 0.04^b	0.79 ± 0.05^c
25	0.39 ± 0.08^a	0.52 ± 0.10^b	0.22 ± 0.02^c	0.78 ± 0.04^d
30	0.47 ± 0.11^a	0.60 ± 0.07^b	0.19 ± 0.02^c	0.79 ± 0.02^d

Average ± standard deviation. Averages with different superscripts at the same row are significantly different ($p < 0.05$); GLY—glycerol, PPG—polypropylene glycol, ETG—ethylene glycol, DTG—diethylene glycol.

this plasticizer. Similar behaviors can be found in the work of Orliac, Rouilly, Silvestre, and Rigal (2003) that tried these same plasticizers in thermo-molded films based on sunflowers proteins and observed that GLY presented the higher plasticizer effect and efficiency, and that the addition of PPG or ETG did not present major effect on shear strength of films, although affected the elongation at break. According to these same authors (Orliac et al., 2003), the observed effect of PPG was probably due to the presence of a hydrophobic group on the plasticizer, resulting in a specific orientation of the molecules within the protein structure.

Cuq et al. (1997) also observed that GLY showed greater effect than sorbitol and saccharose on the mechanical properties of fish myofibrillar protein films, determined by the puncture test. A high plasticizing effect of GLY on the mechanical resistance, in tensile tests, was observed in various works on films based on sodium caseinate (Siew, Heilmann, Easteal, & Cooney, 1999), WPI (Shaw et al., 2002), egg albumen (Gennadios et al., 1996), fish sarcoplasmic proteins (Tanaka et al., 2001), and wheat gliadin films (Sánchez et al., 1998) among others, while Arvanitoyannis et al. (1996, 1997) observed similar effects between GLY and sorbitol in films based on blends (see Table 1).

In a well-detailed work, Ayhllon-Meixueiro, Vaca-Garcia, and Silvestre (2000) compared various plasticizers, which were added on the same molar basis, in films based on sunflower isolated protein and did not verify any significant difference between the tensile strength of these films plasticized with GLY and ETG. However, higher values of elongation at break of the films containing GLY were observed. Cuq et al. (1997) also observed that when the plasticizers are compared in molar concentration, the differences among them were negligible. Due to this reason, various authors (Cuq et al., 1997; Sothornvit & Krochta, 2001) explained the increasing of plasticizing efficiency by the decrease of the molecular weight of the additives.

However, this theory of chain length does not explain the behaviors obtained in this work because the higher effect and plasticizing effect belongs to GLY, which has a molecular weight larger than ETG and PPG. Viroben, Barbot, Mouloungui, and Guéguen (2000) also observed behaviors which cannot be explained by this theory, with higher effect of GLY compared to ETG in films based on isolate pea protein. According to Donhowe and Fenema (1993), this theory could be used when the molecular weight of plasticizers was quite different, which was not the case of their study.

However, it is well known that the T_g of materials can also affect its mechanical properties (Cherian et al., 1995). Excepted the films plasticized with the ETG, the reduction of the film resistance could be explained by the decrease of the respective T_g values (Fig. 3) provoked by plasticizers. This behavior was also observed by Honary and Orafai (2002) studying the effect of different molecular weight
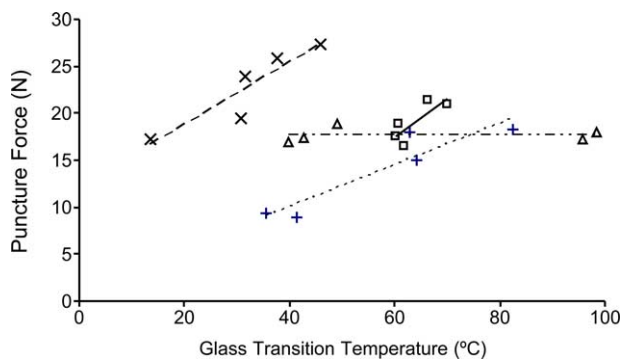
Fig. 3. Puncture force as a function of glass transition temperature of gelatin-based films: glycerol (+), propylene glycol (□), ethylene glycol (△), and diethylene glycol (×).

polyethylene glycols on mechanical properties of hydroxypropylmethylcellulose films. But, contrarily, Sothornvit et al. (2002) did not observe a direct relationship between the thermal and functional properties of films based on β-lactoglobulin (see Table 1).

Only the values of PF of the films plasticized with glycerol were comparable to the values of gelatin films plasticized with sorbitol determined by Sobral et al. (2001), which was 16–14 N between 15 and 25 g sorbitol/100 g gelatin. The other plasticizers produced more resistant films, but with PD very similar to the films with sorbitol (~1.5%).

3.3. Water vapor permeability

In general, no significant difference between the values of WVP relative to similar concentrations of plasticizers was observed (Table 5), thus no plasticizer effect could be evaluated. On another hand, DTG showed more prominent plasticizer efficiency ($p < 0.01$), followed by glycerol ($p < 0.05$) and ETG ($p < 0.010$) in terms of WVP, while a counter effect was observed with PPG (Table 2). Similar behaviors, including the reduction of WVP with the increase of plasticizer concentration, could be observed in the work of Viroben et al. (2000), on films based on pea protein isolate. However, it should be noted that Orliac et al. (2003) observed that the PPG resulted in lower WVP values, and that the WVP decreased with increasing molecular weight of the ethylene glycol family studied by these authors (see Table 1).

Usually, the increase of plasticizer concentration causes an increase in WVP of hygroscopic films, due to the reorganization of the proteinic network, with consequent increase of free volume (Cuq et al., 1997). Various authors had observed this behavior (Cuq et al., 1997; Paschoalick et al., 2003; Sobral et al., 2001). In addition, Cuq et al. (1997) observed higher plasticizing effect of GLY when compared in weight basis with sorbitol and saccharose in fish myofibrillar protein films. Similar results can be observed in other works (Gennadios et al., 1996; Shaw et al., 2002).

The films produced in this work could be considered more permeable to water vapor than films produced by the same type of gelatin, but plasticized with 45 g sorbitol/100 g gelatin, in which WVP was 3.2×10^{-8} g mm h^{-1} cm^{-2} Pa^{-1}, for films with thickness of 0.060 mm (Sobral, 1999).

3.4. Color and opacity

The effect of type and concentration of plasticizer on the color and opacity of films could be considered as negligible (Tables 2 and 5) The overall values including all data remained around 3 and 0.4 for the color difference and opacity, respectively. These results agreed with those in films produced with this same gelatin but plasticized with 45 g sorbitol/100 g gelatin, with equivalent thickness (Sobral, 1999): 2.9 and 0.40 for color difference and opacity, respectively. Gennadios et al. (1996) also did not observe the effect of plasticizer type (GLY, polyethylene glycol, sorbitol) neither concentration on color difference of films based on egg albumen. On the other hand, Paschoalick et al. (2003) observed that the increase of glycerol concentration (15–65 g/100 g proteins) reduced the color and opacity of films based on Tilapia muscle proteins. These authors suggested that this behavior might have been a simple effect of dilution without any probable association with the plasticizing effect of glycerol.

4. Conclusions

All the plasticizers studied were compatible with gelatin. They produced flexible and easy handling films in the range of concentration studied. No typical results of phase separation during the thermal analyses were observed. The more important plasticizer effect and efficiency on the thermal properties were observed when the DTG and ETG were used as plasticizer of the gelatin. But, in a general manner, in terms of functional properties, the GLY presented higher plasticizing effect and efficiency. The behavior of the mechanical properties of films could be explained by its glass transition temperature. By this way, it can be concluded that the plasticizers studied in this work could be perfectly chosen for the production of gelatin-based films depending on desired properties.

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