

Mechanical, water vapor barrier and thermal properties of gelatin based edible films

P.J.A. Sobral^{a,*}, F.C. Menegalli^b, M.D. Hubinger^b, M.A. Roques^c

^aZAZ-FZEA-USP, PO Box 23, 13630-000 Pirassununga (SP), Brazil

^bDEA-FEA-UNICAMP, PO Box 6121, 13081-970 Campinas (SP), Brazil

^cLGPP-ENSGTI, Rue Jules-Ferry, 64000 Pau, France

Abstract

Edible films are thin materials based on a biopolymer. The objectives of this work were to determine the water vapor permeability and the mechanical and thermal properties of edible films based on bovine hide and pigskin gelatins. These films were prepared with 1 g of gelatin/100 ml of water; 15–65 g sorbitol/100 g gelatin; and at natural pH. The samples were conditioned at 58% relative humidity and 22°C for 4 days before testing. The mechanical properties were determined by the puncture test and the water vapor permeability by gravimetric method at 22°C. For DSC analysis, samples were conditioned over silica gel for 3 weeks. Samples (~10 mg) were heated at 5°C/min, between –150 and 150°C in a DSC TA 2010. A second scan was run after cell cooling with liquid nitrogen. As expected, the puncture force decreased and the puncture deformation and water vapor permeability increased with the sorbitol content. The origin of gelatin was important only above 25 g sorbitol/100 g gelatin. The DSC traces obtained in the first scan of samples with 15–35 g sorbitol/100 g gelatin, showed a well visible glass transition followed by a sol–gel transition. However, with the increase of sorbitol concentration, the glass transition became broader, typical of the system presenting a phase separation. The model of Couchman and Karasz for ternary system, was used to predict the T_g values as a function of sorbitol concentration. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Edible films; Gelatin; Mechanical property; Water vapor permeability; Glass transition

1. Introduction

Edible films are thin materials based on a biopolymer. Those films have been under the attention of several researchers in the nineties, also because of their biodegradable character (Gontard & Guilbert, 1996). Real or potential applications have been described in several recent works (Gennadios, McHugh, Weller, & Krochta, 1994; Torres, 1994; Chen, 1995; Gontard & Guilbert, 1996).

The main biopolymers used in the edible films elaboration are polysaccharides (Nisperos-Carriedo, 1994) and proteins (Gennadios et al., 1994; Torres, 1994). Among the studied proteins are: wheat gluten (Park & Chinnan, 1995; Gennadios, Park, & Weller, 1993a; Gennadios, Weller, & Testin, 1993b,c; Gontard, Guilbert, & Cuq, 1993), corn zein (Park & Chinnan, 1995; Gennadios et al., 1993a), soy proteins (Ghorpade, Gennadios, Hanna, & Weller, 1995; Kunte, Gennadios, Cuppet, Hanna, & Weller, 1997), rice proteins (Shih, 1996), pistachio proteins (Ayranci & Çetin, 1995), egg albumin (Gennadios, Weller,

Hanna, & Froning, 1996), milk proteins, such as the casein and whey proteins (Avena-Bustillos & Krochta, 1993; Chen, 1995; Maynes & Krochta, 1994; Mahmoud & Savello, 1992), collagen (Gennadios et al., 1994) and fish (Cuq, Aymard, Cuq, & Guilbert, 1995; Cuq, Gontard, Cuq, & Guilbert, 1997a,b,c; Monterrey & Sobral, 1999, 2000) and bovine meat myofibrillar proteins (Souza, Sobral, & Menegalli, 1997; Sobral, Menegalli, & Guilbert, 1999).

The use of gelatin in the elaboration of edible films or coatings was very well studied until the sixties, which resulted in many patents, mainly in the pharmaceutical area (Gennadios et al., 1994; Torres, 1994). Consequently, these materials characteristics are not easily available in the literature. However, gelatin has returned to the attention of several researchers on edible films for food application. Carvalho, Sobral, and Menegalli (1997a) elaborated edible films from bovine hide gelatin and determined their mechanical properties by puncture test, as function of pH and gelatin and sorbitol concentrations in the filmogenic solution, using surface-response methodology. Carvalho, Sobral, Menegalli, and Roques (1997b) and Menegalli, Sobral, Roques, and Laurent (1999) studied the drying of films of gelatin plasticized with sorbitol, with special

* Corresponding author. Fax: +55-19-5611-689.

E-mail address: pjsobral@usp.br (P.J.A. Sobral).

interest in effect of drying conditions on the quality of the formed film. Sobral (1999) verified the influence of film thickness on the mechanical properties, the water vapor permeability and the color of films elaborated with bovine hide and pigskin gelatins, plasticized with 45 g of sorbitol/100 g of gelatin. 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 soluble starch as function of plasticizers (sorbitol, glycerol or sucrose).

Those recent interests are justified by the ready availability and the low cost of gelatin (Bell, 1989). However, there is a lack of more detailed works on the plasticizer effect on the thermal and functional properties of gelatin edible films. That is why the objectives of this work were to study the water vapor permeability and the mechanical and thermal properties of edible films based on bovine hide and pigskin gelatins as functions of sorbitol content.

2. Material and methods

2.1. Gelatins

Gelatins of two different origins donated by Gelatin Davis of Brazil, without any previous preparation, were used: bovine hide gelatin (BHG), of 250 Bloom, molecular weight (Mn) of the order of 4.5×10^5 Da, with 10.5% of moisture content (wet basis) and pigskin gelatin (PSG), of 242–248 Bloom, molecular weight (Mn) of the order of 5.2×10^4 Da and 9.3% of moisture content. The amino acid composition of both gelatins were determined after hydrolysis with HCl 6N to 108°C for 22 h, by ionic exchange chromatography with derivatization post-column with ninhidrin and are shown on Table 1.

2.2. Edible films elaboration

The edible films were prepared according to the casting technique, which consists of dehydrating a filmogenic solution, conveniently applied on a support. The gelatin filmogenic solutions were prepared under the following conditions: protein, 1 g of gelatin/100 ml of water; plasticizer, 15, 25, 35, 45, 55 and 65 g sorbitol/100 g protein; natural pH of the solution. Initially, the gelatin was hydrated with room temperature water in a beaker, and solubilized later in a water bath with digital control ($\pm 0.5^\circ\text{C}$) of temperature (Tecnal, TE184), kept at 55°C. After complete solubilization, the remaining water and sorbitol were added, and the solution was kept in water bath under soft agitation for 30 min. Weighing (± 0.0001 g) of the gelatins and of the sorbitol (Synth) was accomplished using an analytical scale (Scientech, SA210).

Afterwards, the filmogenic solution was conveniently applied on plexiglass plates (11.8×11.8 cm²) previously prepared. The weight was controlled (± 0.01 g) with a

Table 1

Amino acid composition (g amino acids/100 g of protein) for gelatins used in this work

	Bovine hide	Pigskin
Alanine	12.92	10.08
Arginine	4.80	4.01
Aspartic acid	7.31	6.76
Glutamic acid	11.98	11.12
Phenylalanine	2.00	1.99
Glycine	22.16	22.60
Histidine	0.73	0.72
Isoleucine	1.82	1.33
Leucine	2.73	2.54
Lysine	3.87	3.50
Methionine	0.66	0.68
Proline	3.29	2.21
Serine	3.26	7.63
Tyrosine	0.40	0.62
Threonine	1.78	1.35
Valine	2.37	4.84
Amonium	7.68	9.60

semi-analytical balance (Marte, AS2000). The filmogenic solutions were dehydrated in an oven with air renewal and circulation (Marconi, MA037), with PI control ($\pm 0.5^\circ\text{C}$) of temperature, at 30°C and room relative humidity (55–65%), for 18–20 h.

For functional properties characterization, the obtained edible films were conditioned at 22°C and 58% of relative humidity, in desiccators with saturated solution of NaBr, for 4 days, then, the thickness of the films was measured averaging nine different positions, with a digital micrometer (± 0.001 mm) with a 6.4 mm diameter probe. All the characterizations were accomplished in climatized room conditions ($T = 22^\circ\text{C}$ and relative humidity between 55 and 65%). Only one sample per film was taken for test, i.e., each film originated only one replicate.

For the study of thermal properties, the edible films were conditioned in desiccators with silica gel, for at least 3 weeks, to obtain the most dehydrated possible material. In this experiment, the pure gelatin, also conditioned over silica gel, was considered as the film with 0% of sorbitol.

2.3. Mechanical properties

The force and the deformation at the breaking point of the film were determined in puncture tests (Cuq et al., 1997a; Gnanasambandam, Hettiarachchy, & Coleman, 1997; Gontard et al., 1993). The films were fixed in a 52.6 mm diameter cell and perforated by a 3 mm diameter probe, moving at 1 mm/s. These tests were accomplished with an instrument of physical measures TA.XT2i (SMS, Surrey, UK). The puncture force (F) and the displacement of the probe (D) in the puncture were determined with the software Texture Expert V.1.15 (SMS). The puncture deformation

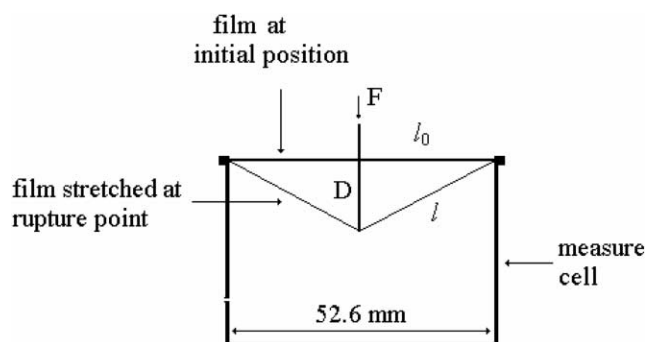


Fig. 1. Diagram to explain the use of Eq. (1).

$(\Delta l/l_0)$ was calculated using Eq. (1) (Gontard et al., 1993).

$$\frac{\Delta l}{l_0} = \frac{\sqrt{D^2 + l_0^2} - l_0}{l_0} \quad (1)$$

To use Eq. (1), we consider that stress is perfectly distributed along the film as shown in Fig. 1, where l_0 is considered the initial length of the film, equal to the radius of the measurement cell (26.3 mm). All tests were made in quadruplicate.

2.4. Water vapor permeability

The water vapor permeability (WVP) was determined according to a method proposed by Gontard et al. (1993), based on the ASTM E96-80 test (ASTM, 1989). 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. The cells were weighed (± 0.01 g) daily, in a semi-analytic balance (Marte, AS2000), during 8–9 days. The water vapor permeability (WVP) was calculated with the Eq. (2) (Gontard et al., 1993):

$$\text{WVP} = \frac{w}{tA} \frac{x}{\Delta P} \quad (2)$$

where x is the average thickness of the edible films, A is the permeation area (12.29 cm^2), ΔP is the difference of partial vapor pressure of the atmosphere with silica gel and pure water (2642 Pa, at 22°C), and the term w/t was calculated by linear regression from the points of weight gain and time, in the constant rate period. All tests were made in quadruplicate.

2.5. 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 4000 module (TA Instruments, New Castle, DE, USA) and with a quench cooling accessory. The aliquots, of the order of 10 mg, weighed (± 0.0001 g) in a precision balance (Scientech, SA210), were conditioned in hermetic aluminum pans, and heated at $5^\circ\text{C}/\text{min}$, between -150 and 150°C , in inert atmosphere (100 ml/min of N_2). The

reference was an empty pan. The equipment was calibrated with an indium sample ($T_m = 156.6^\circ\text{C}$, $\Delta H_m = 28.71 \text{ Jg}^{-1}$) (TA Instruments). The remaining samples were used to determine moisture content, accomplished by vacuum oven drying (95°C , 16.7 kPa) for 48 h. After the first scan, the test cell was cooled quickly with liquid nitrogen, to the temperature below the first observed glass transition, and then a second scan was started.

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. The enthalpy (ΔH_m) of the sol–gel transition, calculated as the area over the endothermic peak observed, was also determined. All these properties were calculated with help of the software Universal Analysis V1.7F (TA Instruments). All the analyses were run in triplicate.

2.6. Statistical analysis

The linear regressions ($R^2 > 0.98$) necessary to the calculation of WVP, were accomplished with Excel 97 software (Microsoft, Seattle, WA). All linear and non-linear regressions with the data of the WVP, the mechanical and the thermal properties were obtained with the Statistica V.5.1 software (StatSoft, Inc., Tulsa, OK).

Duncan's multiple range test was applied to compare means for mechanical properties and WVP of the gelatin films, with a level of significance of $\alpha = 0.05$. These analyses were accomplished using the Statistica V.5.1.

3. Results and discussion

The films of both gelatins were transparent, homogeneous, flexible and easily handled. The average thickness of the films was 0.043 ± 0.009 mm and 0.041 ± 0.010 mm, for BHG and PSG, respectively. The calculated standard deviations of thickness averages are typical for thin edible films. It can be observed in the work of Gennadios et al. (1993c) which measured thickness between 0.064 and 0.101 mm, that the standard deviation of the thickness average varied between 9 and 23%. On the other hand, in a work with less thin edible films (between 0.119 and 0.128 mm), Gennadios et al. (1993b) calculated smaller standard deviations, varying between 6.2 and 8.8%.

3.1. Mechanical properties

The results of puncture force on the gelatin films are shown on Fig. 2. As expected, the sorbitol addition considerably reduced the puncture force. The increasing of sorbitol content from 15 to 65 g sorbitol/100 g gelatin decreased the puncture force from 16.0 to 8.2 N and from 16.2 to 9.0 N, in the case of the films of BHG and PSG respectively. Comparing the values of the puncture force

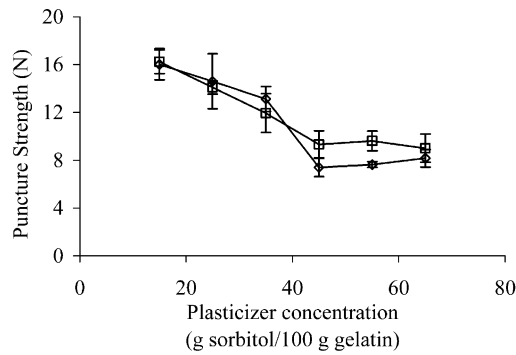


Fig. 2. Puncture force of bovine hide (◇) and pigskin (□) gelatin edible films as a function of sorbitol concentration.

between successive concentrations of sorbitol, significant differences ($p < 0.05$) were noticed only between the average values for 35 and 45 g sorbitol/100 g gelatin, in the BHG films. Comparing the average values between the two gelatins films, significant differences ($p < 0.05$) were observed only for the higher values (45 and 55 g sorbitol/100 g gelatin).

The effect of plasticizer on reduction of the puncture force is well known and its explanation is found in the literature (Cuq et al., 1997a; Gontard et al., 1993; McHugh & Krochta, 1994; Parris, Coffin, Joubran, & Pessen, 1995). It can be observed in the work by Parris et al. (1995), that the tensile strength of alginate edible films decreased exponentially with increasing sodium lactate. Sobral, Ocuno, and Savastano (1998) observed a decrease, shaped as a parabolic segment, in the puncture force on the films of meat myofibrillar proteins, between 25 and 100 g glycerin/100 g protein, acidified with acetic acid or lactic acid.

Gontard et al. (1993) observed a linear reduction of the puncture force in gluten films, from 1.9 to 0.3 N, between 16 and 33 g glycerol/100 g dry matter, that corresponds to 19 and 49% of glycerin. Cuq et al. (1997a) also observed a linear reduction of the puncture force of edible films based on myofibrillar proteins of Atlantic sardine from 5.1 to 2.6 N, between 0 and 40 g of glycerol/100 g of protein. However, these authors worked with low plasticizer concentrations.

The reduction of the puncture force of the films of PSG, in the plasticizer concentration range studied, was exponential. These experimental points can be adjusted (curves not shown) by Eq. (3).

$$F = Ae^{BC} \quad (3)$$

where F is the puncture force (N), C is the sorbitol concentration (g/100 g), and A and B are empiric parameters, whose values were calculated by non-linear regression: $A = 19.46$ N and $B = -0.013$ (100 g/g), with $R^2 = 0.94$. Nevertheless, the adjustment of the Eq. (3) to the experimental points obtained with the films of BHG presented a quality loss ($A = 21.49$ N and $B = -0.017$, $R^2 = 0.86$),

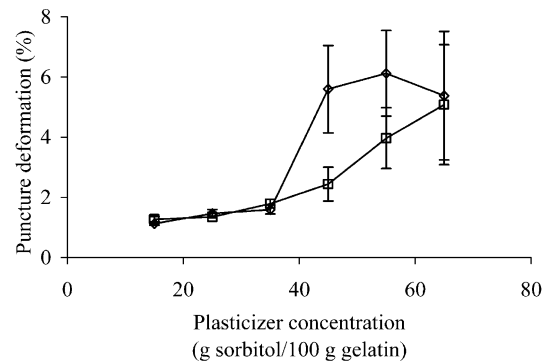


Fig. 3. Puncture deformation of bovine hide (◇) and pigskin (□) gelatin edible films as a function of sorbitol concentration.

possibly due to the difference observed between the points representing the concentrations of 35 and 45 g sorbitol/100 g gelatin. Ghorpade et al. (1995) applied this exponential Eq. (3) to the data obtained by traction test in films of soy protein, and obtained a very good coefficient of correlation (0.97). On the other hand, the increase from 15 to 65 g sorbitol/100 g gelatin content increased the puncture deformation of both gelatin films from 1.2 to 5.3% with different behaviors (Fig. 3). Comparing the values for each film between successive concentrations of sorbitol, significant differences were noticed ($p < 0.05$) only between the average values for 35 and 45 g sorbitol/100 g gelatin, on the film of BHG. And comparing the average values between the two gelatin films, significant differences were observed ($p < 0.05$) just for the values of deformation at 45 and 55 g sorbitol/100 g gelatin. These results are similar to that presented in the study of the puncture force.

Gontard et al. (1993) observed an increase from 6 to 20% in the puncture deformation of edible films of gluten, caused by the increase of glycerol concentration (16–33 g glycerol/100 g dry matter) also following a parabolic behavior as in the case of the films of PSG (Fig. 3). Sobral et al. (1998) observed that the puncture deformation also increased as a parabolic segment, between 25 and 100 g glycerin/100 g protein, only with the films of meat myofibrillar protein acidified with acetic acid. In the case of the films acidified with lactic acid, the puncture deformation remained practically constant. Cuq et al. (1997a), differently, observed a sigmoidal behavior, working with values below 40% of glycerin.

The increase of the puncture deformation of the films of PSG can be easily represented by an exponential equation, similar to the Eq. (3) (curve not shown), with very good coefficient of correlation ($A = 0.62\%$ and $B = 0.033$ (100 g/g), $R^2 = 0.98$). On the other hand, the behavior of the puncture deformation of the films of BHG was not represented satisfactorily by the exponential equation ($R^2 = 0.70$) also because of the jump between the points at 35 and 45 g sorbitol/100 g gelatin.

According to the classic polymer science, the plasticizers weaken the intermolecular forces between the chains of adjacent macromolecules, increasing the free volume and causing a reduction of T_g of the system (Jastrzebski, 1987). Thus, the increase in the plasticizer concentration causes a reduction of the puncture force due to the decrease in the intermolecular interactions and causes an increase of the puncture deformation due to the increase in the mobility of the macromolecules. Besides, the increase in the plasticizer concentration increases the moisture content of the film because of its high hygroscopic character, which also contributes to the reduction of the forces between the adjacent macromolecules (Sobral et al., 1999).

3.2. Water vapor permeability (WVP)

The results of the WVP tests with the films of BHG and PSG are presented in Fig. 4. The WVP of the films of PSG and BHG, respectively, increased linearly from 1.8 to $3.2 \times 10^{-8} \text{ g mm h}^{-1} \text{ cm}^{-2} \text{ Pa}^{-1}$, and from 1.7 to $3.8 \times 10^{-8} \text{ g mm h}^{-1} \text{ cm}^{-2} \text{ Pa}^{-1}$ between 15 and 65 g sorbitol/100 g gelatin. The linear regression coefficient of correlation of the data of BHG films was very good ($Y = 1.24 \times 10^{-8} + 0.043 \times 10^{-8} X$, $R^2 = 0.96$) but the one of the PSG films was less good ($Y = 1.16 \times 10^{-8} + 0.028 \times 10^{-8} X$, $R^2 = 0.83$). McHugh, Aujard, and Krochta (1994) also determined that the WVP of gluten films, plasticized with glycerin, at 25°C , varied linearly with the plasticizer concentration, with very good correlation coefficient ($R^2 = 0.97$). That same behavior can be observed in the work of Gontard et al. (1993).

Comparing the successive values of the WVP for each gelatin film, a significant difference ($p < 0.05$) was observed just in the case of the BHG films, between the average values corresponding to 25 and 35 g sorbitol/100 g gelatin. On the other hand, comparing the average values between the two gelatin films, significant differences ($p < 0.05$) were observed between all values of WVP for concentration of sorbitol above 25 g sorbitol/100 g gelatin.

The increase in WVP with increasing hygroscopic plasticizer concentration is also common in edible films (Buttler, Vergano, Testin, Bunn, & Wiles, 1996; Cuq et al., 1997a; McHugh et al., 1994). According to Cuq et al. (1997a), the proteins network becomes less dense and, consequently more permeable with the plasticizer incorporation. The increase in free volume of the system also raises the solvent mobility, consequently increasing the water diffusion in the matrix of the film. Another factor that also affects the WVP of edible films is the hygroscopic character of the plasticizers, which increase the water content of the film, consequently increasing the mobility of the molecules. In addition, increasing water content could also affect permeate solubility in the film.

The WVP of BHG and PSG edible films with 45 g sorbitol/100 g gelatin (2.9×10^{-8} and $2.2 \times 10^{-8} \text{ g mm h}^{-1} \text{ cm}^{-2} \text{ Pa}^{-1}$, respectively) are comparable to the values ($2.7 \times$

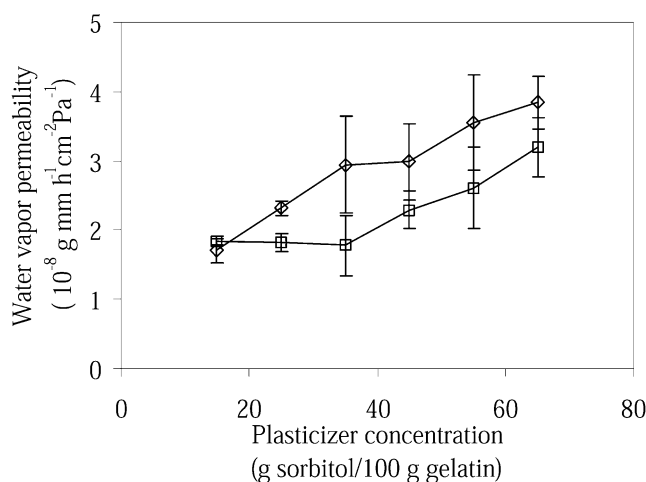


Fig. 4. Water vapor permeability of the bovine hide (\diamond) and pigskin (\square) gelatin edible films as a function of sorbitol concentration.

$10^{-8} \text{ g mm h}^{-1} \text{ cm}^{-2} \text{ Pa}^{-1}$, with 40% of glycerin, $T = 20^\circ\text{C}$, $\text{pH} = 3.0$ and 2.6 mg of proteins/ cm^2) determined by Cuq et al. (1997a) in edible films of Atlantic sardine myofibrillar protein. Psomiadou, Arvanitoyannis, and Yamamoto (1996), working with microcrystalline cellulose films with 0.050 mm of thickness, determined WVP of $5.1 \times 10^{-8} \text{ g mm h}^{-1} \text{ cm}^{-2} \text{ Pa}^{-1}$ in a film with 28% glycerin, at 25°C . This film is therefore, more permeable to water vapor than all the gelatin films produced in this work. Arvanitoyannis et al. (1997) elaborated films with blends of equal parts of gelatin and soluble starch, using sorbitol as plasticizer. They found that the increment from 15 to 30% of sorbitol, increased the WVP from 4.1 to $9.9 \times 10^{-8} \text{ g mm h}^{-1} \text{ cm}^{-2} \text{ Pa}^{-1}$, values higher than those determined for gelatin films ($< 3.9 \times 10^{-8} \text{ g mm h}^{-1} \text{ cm}^{-2} \text{ Pa}^{-1}$) in our work. It can then be suggested that, the inclusion of starch in the gelatin film, makes the material more permeable to water vapor.

3.3. Thermal properties

The DSC traces of samples of BHG and PSG edible films conditioned over silica gel for three weeks are presented in Figs. 5 and 6, respectively. A very visible glass transition (T_g) followed by an endothermic peak due to the helix-coil (sol-gel) transition (T_m) appeared in DSC traces of the first scan of the samples containing from 15 to 35 g sorbitol/100 g gelatin. On the respective DSC traces of the second scan, the glass transition was more pronounced. With the increase of sorbitol content in both films, however, the inflexion of the base line caused by the glass transition becomes broader and more distant from the sol-gel transition. Even in the DSC traces of the second scan of the samples with more than 35 g sorbitol/100 g gelatin, the glass transition broadens equally. The difference between the initial and final temperatures of the glass transition varied from 6.1 to 19.3°C and 6.9 to 18.7°C for the BHG

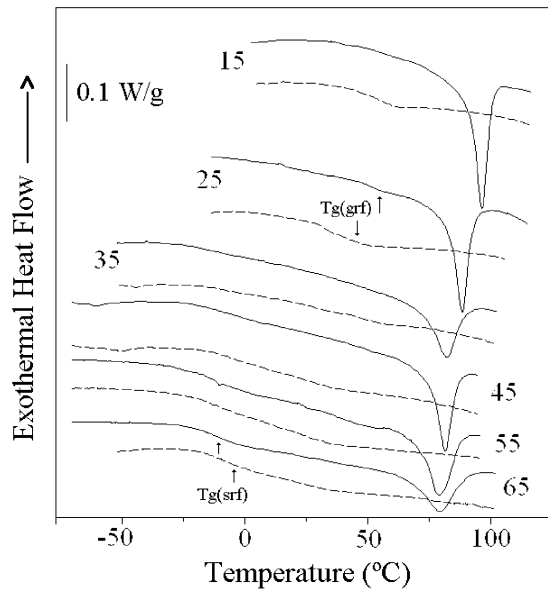


Fig. 5. DSC traces of bovine hide gelatin films at several sorbitol concentrations: — first scan, - - - second scan. Arrows indicate glass transitions of gelatin rich fraction [Tg(grf)] and sorbitol rich fraction [Tg(srf)].

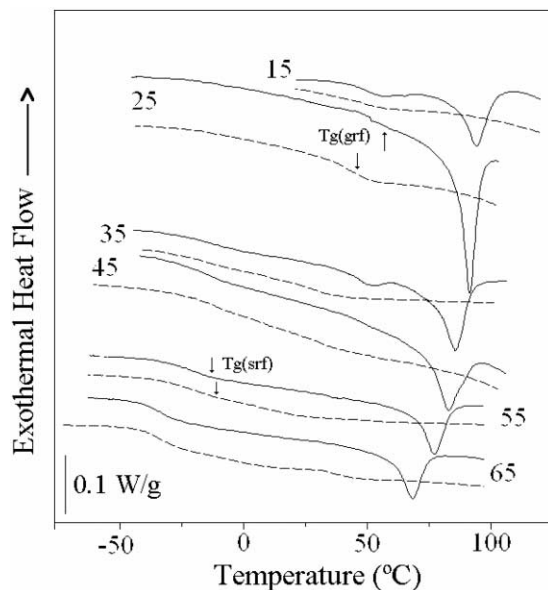


Fig. 6. DSC traces of pigskin gelatin films at several sorbitol concentrations: — first scan, - - - second scan. Arrows indicate glass transitions of gelatin rich fraction [Tg(grf)] and sorbitol rich fraction [Tg(srf)].

and PSG films, respectively. Due to this behavior, it was not possible to calculate Tg in the second scan of the BHG films and in the two scans of the PSG films for sorbitol concentrations of 55 and 65 g sorbitol/100 g gelatin. In fact, we suppose that the inflexion noticed to the left of the DSC traces (therefore more distant of the sol–gel transition) starting from 45 g (Fig. 5) and 35 g (Fig. 6) sorbitol/100 g gelatin, corresponded to the glass transition of the sorbitol rich fraction, consequence of a phase separation between the biopolymer and the plasticizer.

This phase separation phenomenon was observed in edible films of meat myofibrillar protein plasticized with glycerin (Souza, Sobral, & Menegalli, 1999), in films of methylcellulose plasticized with polyethylene glycol 400 (Debeaufort & Voilley, 1997), in films of fish myofibrillar proteins plasticized with glycerin (Cuq et al., 1997b,c), and in gluten films with glycerin (Cherian, Gennadios, Weller, & Chinachoti, 1995; Gontard & Ring, 1996) in DSC and/or DMTA studies. These works have shown typical DSC traces with a clear enlargement of the inflexion zone in the base line. DMTA showed similar behavior (Cuq et al., 1997b,c). Ong, Whitehouse, Abeysekera, Al-Ruqaie, and Kasapis (1998), by dynamical rheometry, also observed phase separation in a system formed by gelatin, oxidized starch and glucose syrup, with 70% of solids. On the other hand, Arvanitoyannis et al. (1997), did not observe a phase separation in films of gelatin/starch blend plasticized with glycerin or sorbitol, but their DSC traces began only at 0°C, therefore, above the Tg of the plasticizer rich fraction.

One of the necessary characteristics for a plasticizer is to be soluble and compatible with the biopolymer. However, phase separation in edible films implies that there is not total mixture between the plasticizer and the biopolymer (Gontard & Ring, 1996). Therefore, the phase separation should imply the loss of film flexibility. Nevertheless, according to Debeaufort and Voilley (1997), the plasticizers can also act as lubricants of the biopolymer chains, guaranteeing the flexibility of the film.

Glass transition temperatures (Tg) could be calculated after an important scale amplification with the help of the Universal Analysis software. It can be observed in Figs. 7 and 8, that the Tg depression caused by the sorbitol in gelatin films is small, which may be due to the phase separation. For the BHG films (Fig. 7), Tg dropped from 56.5°C (329.6 K) at 15 g sorbitol/100 g gelatin, to 37.3°C (310.4 K) at 65 g sorbitol/100 g gelatin, and with the PSG films (Fig. 8), the drop was from 50.3°C (323.4 K), at 15 g sorbitol/100 g gelatin, to 26.3°C (299.4 K), at 45 g sorbitol/100 g gelatin. The standard deviation of Tg increased with the increase of the sorbitol concentration, due to the phase separation.

The conditioning of the gelatin films over silica gel did not guarantee anhydrous samples. The residual moisture content of the samples varied from 7 g water/100 g dry solids in the films with 15 g sorbitol/100 gelatin, to 3.5 g water/100 g dry solids in the films with 65 g sorbitol/100 g gelatin. Evidently this moistness also presents a plasticizer effect and leads to consider the gelatin films as a ternary system, formed of gelatin, sorbitol and water. In that way, the Couchman and Karasz model (Pinhas, Blanshard, Derbyshire, & Mitchell, 1996), for ternary systems (Eq. 4), should be appropriate to represent the experimental points of Tg.

$$T_g = \frac{\omega_1 \Delta C_{p1} T_{g1} + \omega_2 \Delta C_{p2} T_{g2} + \omega_3 \Delta C_{p3} T_{g3}}{\omega_1 \Delta C_{p1} + \omega_2 \Delta C_{p2} + \omega_3 \Delta C_{p3}} \quad (4)$$

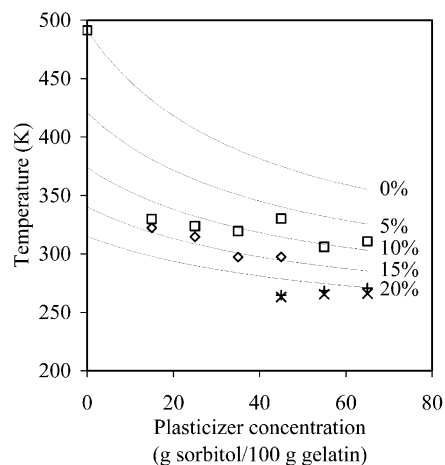


Fig. 7. Glass transition temperature of bovine hide gelatin films: (□) first scan, (◇) second scan; glass transition temperature of the sorbitol rich fraction: (×) first scan, (+) second scan; — calculated values by Eq. (4) for several moisture contents.

Where T_g is the glass transition temperature of the ternary system, T_{g_i} is the glass transition temperature, ω_i is the molar fraction and ΔC_{p_i} is the variation of the specific heat during the glass transition of the constituents: water ($i = 1$), anhydrous sorbitol ($i = 2$) and anhydrous gelatin ($i = 3$).

For the utilization of Eq. (4), the following data was found in the literature (Cuq et al., 1997b,c; Pinhas et al., 1996): $T_{g_1} = 138$ K, $\Delta C_{p_1} = 1.94$ J g⁻¹ K⁻¹ (water); $T_{g_2} = 270$ K, $\Delta C_{p_2} = 0.96$ J g⁻¹ K⁻¹ (sorbitol); and $T_{g_3} = 476$ K, $\Delta C_{p_3} = 0.45$ J g⁻¹ K⁻¹ (gelatin). However, for the gelatin data, it was preferred to use the values determined in another work (Sobral et al., 1999): $T_{g_3} = 491.3$ and 493.4 K, and $\Delta C_{p_3} = 0.39$ and 0.37 J g⁻¹ K⁻¹, for the BHG and PSG, respectively.

The values of T_g of both films, calculated with the Eq. (4), as a function of the sorbitol concentration and for several moisture contents, are plotted in Figs. 7 and 8. The values of T_g of the sorbitol rich fraction are also plotted in these figures, for better visualization of the phase separation phenomenon.

The Couchman and Karasz model does not predict satisfactorily the experimental values of T_g . The moisture content of the samples is around 5 g water/100 g dry solids. However, it is observed in Figs. 7 and 8 that the calculated values for a fixed moisture content of 5 g water/100 g dry solids are higher than the experimental points. For example, at 35 g sorbitol/100 g gelatin, the T_g of the BHG and PSG films were 46.3 (319.5 K) and 40.0°C (313.1 K), respectively, and the calculated values (Eq. 4) were 77.4 (350.5 K) and 75.0°C (348.1 K), therefore, more than 30°C above the experimental values.

Gontard and Ring (1996) and Cuq et al. (1997b,c) used the model of Couchman and Karasz to represent the behavior of the experimental values of T_g as a function of moisture content of the sample, for a given film composition. Cuq et al. (1997b) observed that this model described just

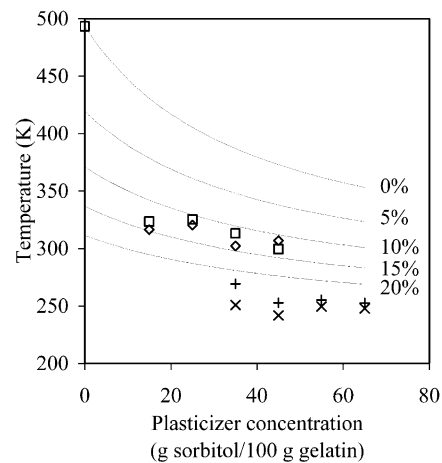


Fig. 8. Glass transition temperature of pigskin gelatin films: (□) first scan, (◇) second scan; glass transition temperature of the sorbitol rich fraction: (×) first scan, (+) second scan; — calculated values by Eq. (4) for several moisture contents.

partially the plasticizer effect of water in fish myofibrillar protein based edible films. On the other hand, Gontard and Ring (1996) adjusted the model of Couchman and Karasz to the experimental data of T_g of gluten film as a function of moisture content, with satisfactory results, but the parameters calculated through the adjustment had no physical sense. In the present study, the difficulty for representation of the glass transition curve of both gelatins films is also explained by the phase separation. In this case, the experimental points (Figs. 7 and 8) form a practical curve, as defined by Slade and Levine (1991), with T_g values tending for the glass transition temperature of gelatins plasticized to the maximum extent, at high sorbitol concentration. Contrarily, the model of Couchman and Karasz predicts a theoretical curve.

The experimental points of the glass transition of the sorbitol rich fraction are lower than the values predicted for the film T_g with 20% of moisture content (Figs. 7 and 8). Considering that the moisture content of the films is less than 7%, these T_g values are effectively a consequence of a phase separation phenomenon. This could be the explanation for the observed difference between the values of the puncture force (Fig. 2), as well as the increase in the puncture deformation (Fig. 3) that occurs between 35 and 45 g sorbitol/100 g gelatin, exactly where the phase separation begins to appear in the case of the BHG films.

Sorbitol also caused a smaller depression on the T_m values, that dropped from about 93°C (366 K) at 15 g sorbitol/100 g gelatin in both films, to about 81 (354 K) and 74°C (347 K) at 65 g sorbitol/100 g gelatin in the BHG and PSG films respectively (Fig. 9). The plasticizing effect of sorbitol can be well represented (empirically) by the Flory-Huggins model (Eq. 5), not considering the moisture content effect.

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \frac{R}{\Delta H_u} \frac{V_u}{V_1} (\nu_1 - \chi_1 \nu_1^2) \quad (5)$$

T_m and T_m^0 are the temperatures of the helix–coil transition as a function of the solvent and in equilibrium, respectively. R is the gas constant. ΔH_u is the fusion latent heat by monomeric unit and V_u/V_1 is the relation between the molar volume of the monomeric unit and the solvent. ν_1 is the volumetric fraction of the solvent and χ_1 the interaction polymeric-diluent parameter, also known as the Flory-Huggins parameter. For application of Eq. (5), the relationship $RV_u/\Delta H_u V_1$ was considered as a constant and the volume fraction equal to the mass fraction. The values of the Flory-Huggins parameter calculated by non-linear regression were 1.61 ($R^2 = 0.99$) and 1.33 ($R^2 = 0.99$) for the BHG and PSG films, respectively.

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. According to Arvanitoyannis et al. (1997), at the molecular level, the gelatin gelification involves the renaturation of the gelatin chains in triple helix structure, just as in the native collagen. In this way, it is possible to quantify the crystallinity of the gelatin in the films, relating the measured enthalpy by DSC with the denaturation enthalpy of the native collagen, which is 62.05 J g^{-1} (Arvanitoyannis et al., 1997). The crystallinity of both gelatin films was calculated in this way and the results are plotted on Fig. 10.

The crystallinity of both films increased in relation to pure gelatin (that would be considered the film with 0% of plasticizer), reaching values between 30 and 40%. These results do not agree with the data presented by Arvanitoyannis et al. (1997), in relation to the general behavior, but they agree if one considers the absolute values. It can be observed in this work that, in a general manner, the increase in plasticizer (glycerin, sorbitol or sucrose) decreased the crystallinity of the gelatin/starch films. But, in the case of the films elaborated according to the high temperature technique and plasticized with 15 and 30 g sorbitol/100 g gelatin and with 5% of moisture content, the observed crystallinity was 35.2 and 32.0%, very close to the values calculated in this work.

The difference in behavior between this work and the work of Arvanitoyannis et al. (1997) can be explained by two facts. These authors elaborated films with a mixture of gelatin and starch so there could have been a protection of starch at the structural level. Another important fact is the phase separation observed in this study with films of both gelatins, then, in this situation, the sorbitol amount interacting with the gelatin can indeed be smaller than expected but practically constant.

4. Conclusions

Sorbitol shows a considerable plasticizing effect on the puncture force, as well as on the puncture deformation of the BHG and PSG films. The origin of the two gelatin samples does not seem to have an influence the effect of

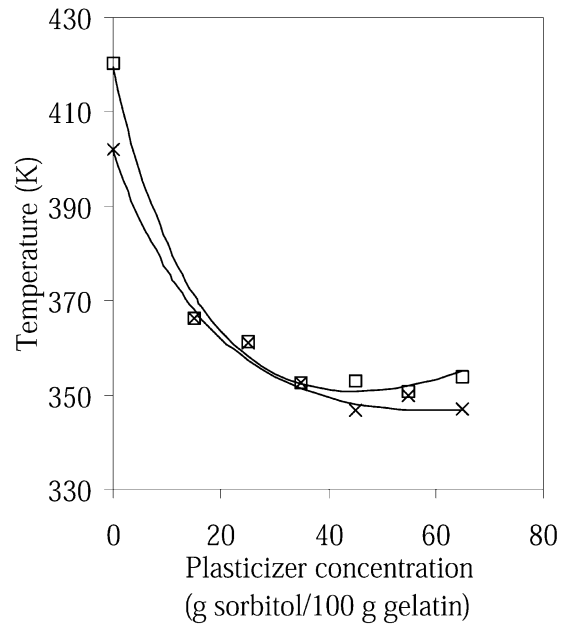


Fig. 9. Helix–coil transition temperature of bovine hide gelatin films (experimental: □, Eq. (5): —) and of pigskin gelatin films (experimental: ×, Eq. (5): —) in function of sorbitol concentration.

the plasticizer on the puncture force, however, the puncture deformation of the BHG films presents a sharp increase, between 35 and 45 g sorbitol/100 g gelatin, contrarily to the case of the PSG films, where a monotonic increase is observed.

The WVP of gelatin films increased linearly with sorbitol concentration. The origin of the gelatin has an effect on the WVP values, above 25 g sorbitol/100 g gelatin: the BHG films are more permeable to water vapor than the PSG films.

A phase separation between gelatin and sorbitol was

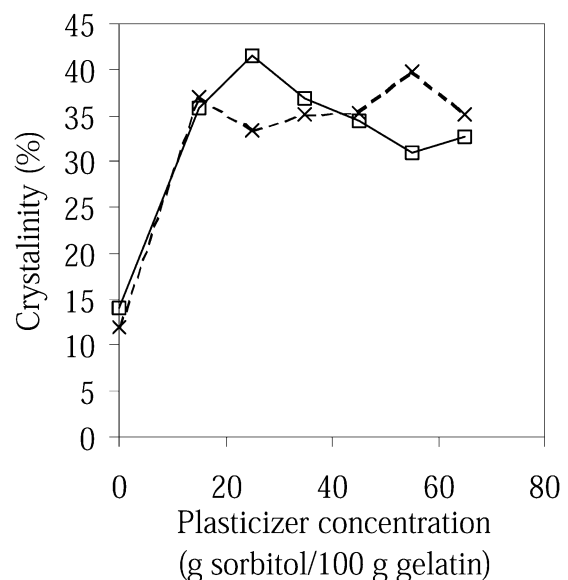


Fig. 10. Crystallinity variation of the bovine hide (×) and pigskin (□) gelatin films as a function of sorbitol concentration.

observed in the BHG and PSG films. This phenomenon increased with sorbitol content. Because of this fact, the Tg of the gelatin rich fraction varied very subtly with the sorbitol concentration of the film. The model of Couchman and Karasz, for ternary systems, did not represent satisfactorily the experimental points of Tg of the film.

The sorbitol content increase did not reduce the formation of junctions in the film, very possibly because of a phase separation. A well visible endothermic peak was observed after the Tg in all films. The reduction of Tm with the sorbitol content followed a monotonic behavior and it could be represented by the Flory-Huggins model, for binary systems, not considering the moisture content of the samples.

Acknowledgements

The authors would like to acknowledge the financial support of FAPESP (95/9315-4) and CAPES/COFECUB (205/97), and to CNPq for the research fellowship (PJAS).

References

- Arvanitoyannis, I., Psomiadou, E., Nakayama, A., Aiba, S., & Yamamoto, N. (1997). Edible film made from gelatin, soluble starch and polyols, part 3. *Food Chemistry*, *60*, 593–604.
- Arvanitoyannis, I., Nakayama, A., & Aiba, S. (1998). Edible films made from hydroxypropyl starch and gelatin and plasticized by polyols and water. *Carbohydrate Polymers*, *36*, 105–119.
- ASTM Standard (1989). *Standard test methods for water vapor transmission of materials. Annual book of ASTM standards. Designation E96–E80* (pp. 730–739). Philadelphia: ASTM.
- Avena-Bustillos, R. J., & Krochta, J. M. (1993). WVP of caseinate-based edible films as affected by pH, calcium crosslinking and lipid content. *Journal of Food Science*, *58*, 904–907.
- Ayranci, E., & Çetin, E. (1995). The effect of protein isolate of Pistacia terebinthus L. on moisture transfer properties of cellulose-based edible films. *Lebensmittel-Wissenschaft und-Technologie*, *28*, 241–244.
- Bell, A. E. (1989). Gel structure and food biopolymers. In T. M. Hardman, *Water and food quality* (pp. 251–275). London: Elsevier Applied Science.
- Buttler, B. L., Vergano, P. J., Testin, R. F., Bunn, J. M., & Wiles, J. L. (1996). Mechanical and barrier properties of edible chitosan films as affected by composition and storage. *Journal of Food Science*, *61*, 953–955 + 961.
- Carvalho, R. A., Sobral, P. J. A., & Menegalli, F. (1997a). Elaboração de biofilmes à base de gelatina. In: Proceedings of 'Workshop sobre Biopolímeros'. (pp. 94–97). Pirassununga (SP), April, 15–17.
- Carvalho, R. A., Sobral, P. J. A., Menegalli, F. C., & Roques, M. A. (1997b). Drying of thermosensitive biofilms. Proceedings of Inter-American Drying Conference. (pp. 302–309). Itú (SP), July, 15–18.
- Chen, H. (1995). Functional properties and applications of edible films made of milk proteins. *Journal of Dairy Science*, *78*, 2563–2583.
- Cherian, G., Gennadios, A., Weller, C., & Chinachoti, P. (1995). Thermo-mechanical behavior of wheat gluten films: effect of sucrose, glycerol and sorbitol. *Cereal Chemistry*, *72*, 1–6.
- Cuq, B., Aymard, C., Cuq, J.-L., & Guilbert, S. (1995). Edible packaging films based on fish myofibrillar proteins: Formulation and functional properties. *Journal of Food Science*, *60*, 1369–1374.
- Cuq, B., Gontard, N., Cuq, J. L., & Guilbert, S. (1997a). Selected functional properties of fish myofibrillar protein-based films as affected by hydrophilic plasticizers. *Journal of Agricultural and Food Chemistry*, *45*, 622–626.
- Cuq, B., Gontard, N., & Guilbert, S. (1997b). Thermal properties of fish myofibrillar protein-based films as affected by moisture content. *Polymer*, *38*, 2399–2405.
- Cuq, B., Gontard, N., & Guilbert, S. (1997c). Thermoplastic properties of fish myofibrillar proteins: application to biopackaging fabrication. *Polymer*, *38*, 4071–4078.
- Debeaufort, F., & Voilley, A. (1997). Methylcellulose-based edible films and coatings: 2. Mechanical and thermal properties as a function of plasticizer content. *Journal of Agricultural and Food Chemistry*, *45*, 685–689.
- Gennadios, A., Park, H. J., & Weller, C. L. (1993a). Relative humidity and temperature effects on tensile strength of edible protein and cellulose ether films. *Transactions of the ASAE*, *36*, 1867–1872.
- Gennadios, A., Weller, C. L., & Testin, R. F. (1993b). Modification of physical and barrier properties of edible wheat gluten-based films. *Cereal Chemistry*, *70*, 426–429.
- Gennadios, A., Weller, C. L., & Testin, R. F. (1993c). Property modification of edible wheat, gluten-based films. *Transactions of the ASAE*, *36*, 465–470.
- Gennadios, A., McHugh, T. H., Weller, C. L., & Krochta, J. M. (1994). Edible coatings and films based on proteins. In J. M. Krochta, E. A. Baldwin & M. Nisperos-Carriedo, *Edible coatings and films to improve food quality* (pp. 210–278). Lancaster, NC: Technomic Pub. Co., Inc.
- Gennadios, A., Weller, C. L., Hanna, M. A., & Froning, G. W. (1996). Mechanical and barrier properties of egg albumen films. *Journal of Food Science*, *61*, 585–589.
- Ghorpade, V. M., Gennadios, A., Hanna, M. A., & Weller, C. L. (1995). Soy protein isolate/poly(ethylene oxide) films. *Cereal Chemistry*, *72*, 559–563.
- Gnanasambandam, R., Hettiarachchy, N. S., & Coleman, M. (1997). Mechanical and barrier properties of rice bran films. *Journal of Food Science*, *62*, 395–398.
- Gontard, N., Guilbert, S., & Cuq, J. -L. (1993). Water and glycerol as plasticizer affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of Food Science*, *58*, 206–211.
- Gontard, N., & Guilbert, S. (1996). Bio-packaging: technology and properties of edible and/or biodegradable material of agricultural origin. *Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos*, *30*, 3–15.
- Gontard, N., & Ring, S. (1996). Edible wheat gluten film: influence of water content on glass transition temperature. *Journal of Agricultural and Food Chemistry*, *44*, 3474–3478.
- Jastrzebski, Z. D. (1987). *The nature and properties of engineering materials*. (3rd) New York: John Wiley & Sons.
- Kunte, L. A., Gennadios, A., Cuppet, S. L., Hanna, M. A., & Weller, C. L. (1997). Cast films from soy protein isolates and fractions. *Cereal Chemistry*, *74*, 115–118.
- Mahmoud, R., & Savello, P. A. (1992). Mechanical properties of and water vapor transferability through whey protein films. *Journal of Dairy Science*, *75*, 942–946.
- Maynes, J. R., & Krochta, J. M. (1994). Properties of edible films from total milk protein. *Journal of Food Science*, *59*, 909–911.
- McHugh, T. H., & Krochta, J. M. (1994). Sorbitol- vs glycerol-plasticized whey protein edible films: integrated oxygen permeability and tensile property evaluation. *Journal of Agricultural and Food Chemistry*, *42*, 841–845.
- McHugh, T. H., Aujard, J. F., & Krochta, J. M. (1994). Plasticized whey protein edible films: water vapor permeability properties. *Journal of Food Science*, *59*, 416–419 + 423.
- Menegalli, F. C., Sobral, P. J. A., Roques, M., & Laurent, S. (1999). Characteristics of gelatin biofilms in relation to drying process conditions near melting. *Drying Technology*, *17*, 1697–1706.
- Monterrey-Q, E. S., & Sobral, P. J. A. (1999). Caracterização de propriedades mecânicas e óticas de biofilmes à base de proteínas miofibrilares

- de tilápia do nilo usando uma metodologia de superfície-resposta. *Ciência e Tecnologia de Alimentos*, 19, 294–301.
- Monterrey-Q, E. S., & Sobral, P. J. A. (2000). Preparo e caracterização de proteínas miofibrilares de tilápia do nilo (*Oreochromis niloticus*) para elaboração de biofilmes. *Pesquisa Agropecuária Brasileira*, 35, 179–190.
- Nisperos-Carriedo, M. O. (1994). Edible coatings and films based on polysaccharides. In J. M. Krochta, E. A. Baldwin & M. Nisperos-Carriedo, *Edible coatings and films to improve food quality* (pp. 305–336). Lancaster, NC: Technomic Pub. Co., Inc.
- Ong, M. H., Whitehouse, S., Abeyssekera, R., Al-Ruqaie, I. M., & Kasapis, S. (1998). Glass transitions-related or crystalline forms in the structural properties of gelatin/oxidised starch/glucose syrup mixtures. *Food Hydrocolloids*, 12, 273–281.
- Park, H. J., & Chinnan, M. S. (1995). Gas and water vapor barrier properties of edible films from protein and cellulosic materials. *Journal of Food Engineering*, 25, 497–507.
- Parris, N., Coffin, D. R., Joubran, R. F., & Pessen, H. (1995). Composition factors affecting the water vapor permeability and tensile properties of hydrophilic films. *Journal of Agricultural and Food Chemistry*, 43, 1432–1435.
- Pinhas, M. F., Blanshard, J. M. V., Derbyshire, W., & Mitchell, J. R. (1996). The effect of water on the physicochemical and mechanical properties of gelatin. *Journal of Thermal Analysis*, 47, 1499–1511.
- Psomiadou, E., Arvanitoyannis, I., & Yamamoto, N. (1996). Edible films made from natural resources; microcrystalline cellulose (MCC), methylcellulose (MC) and corn starch and polyols—Part 2. *Carbohydrates Polymers*, 31, 193–204.
- Shih, F. F. (1996). Edible films from rice protein concentrate and pullulan. *Cereal Chemistry*, 73, 406–409.
- Slade, L., & Levine, H. (1991). Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. *Critical Reviews in Food Science and Nutrition*, 30, 115–360.
- Sobral, P. J. A. (1999). Propriedades funcionais de biofilmes de gelatina em função da espessura. *Ciência & Engenharia*, 8, 60–67.
- Sobral, P. J. A., Ocuno, D., & Savastano Jr., H. (1998). Preparo de proteínas miofibrilares de carne e elaboração de biofilmes com dois tipos de ácidos: propriedades mecânicas. *Brazilian Journal of Food Technology*, 1, 44–52.
- Sobral, P. J. A., Menegalli, F. C., & Guilbert, S. (1999). Phase transitions of bovine hide gelatin plasticized by water. In P. Colonna & S. Guilbert, *Biopolymer science, food and non food applications* (pp. 111–123). Paris: Editions INRA, Les Colloques no. 91.
- Souza, S. M. A., Sobral, P. J. A. & Menegalli, F. C. (1997). Desenvolvimento de filmes comestíveis à base de proteínas miofibrilares extraídas de carne bovina. Proceedings of 'Workshop sobre Biopolímeros', (pp. 102–106). Pirassununga (SP), April, 15–17.
- Souza, S. M. A., Sobral, P. J. A., & Menegalli, F. C. (1999). Glass transition of a meat myofibrillar protein based edible film. In P. Colonna & S. Guilbert, *Biopolymer science, food and non food applications* (pp. 183–188). Paris: Editions INRA, Les Colloques no 91.
- Torres, J. A. (1994). Edible films and coatings from proteins. In N. S. Hettiarachchy & G. R. Ziegler, *Protein functionality in food systems* (pp. 467–507). New York: Marcel Dekker, Inc.