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Effect of protein and plasticizer concentrations in film forming solutions on physical properties of edible films based on muscle proteins of a Thai Tilapia

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Abstract

Proteins from fish muscle are biopolymers capable of forming edible films. Thus, the objective of this paper was the study of some physical properties of films produced with the sarcoplasmic and myofibrillar proteins (MP) from Thai Tilapia muscle, as function of the protein and plasticizer concentration in the film-forming solution, thermally treated at 90 °C/30 min. The films were prepared by a casting technique. The filmogenic solutions (FFS) were prepared as follows: 1 or 2 g of protein/100 g of FFS, 15–65 g of glycerin/100 g of protein, pH = 2.7 (acetic acid) and FFS thermal treatment of 90 °C/30 min. These films were characterized for color, opacity, mechanical properties, and viscoelastic properties. Only the films made with 2 g of MP/100 of FFS were characterized for the thermal properties. In general, all properties were affected by the plasticizer concentration. The effect of the protein concentration was observed mainly upon the mechanical properties. The films prepared with 2 g of MP/100 g FFS were more resistant than the others. In a general manner, the films properties were similar of that of myofibrillar fish films excepted the optical properties. The MP films were more colored and opaque than the myofibrillar one.

Keywords: Myofibrillar protein; Sarcoplasmic protein; Mechanical properties; Viscoelastic properties; Color; Opacity

1. Introduction

According to Sothornvit and Krochta (2001), the edible films are interesting because they have the potential to guarantee the food shelf life and quality, when used as food packaging material. This interest is even greater when the biodegradable character of these biomaterials is considered (Gontard & Guilbert, 1996). However, the functional properties of the edible films, in the present state of the art, are still inferior to those of synthetic plastics. This way, more research is necessary in order to improve the characteristics of edible films.

The biopolymer to be used as raw material of the edible films should be capable of forming a continuous matrix and normally are from a renewable and abundant source (Gontard & Guilbert, 1996). The proteins are biopolymers capable of forming edible films, and among them, the myofibrillar and sarcoplasmic proteins of fish muscle have been the reason of innumerous researches in the last 10 years (Cuq, Aymard, Cuq, & Guilbert, 1995; Cuq, Gontard, Cuq, & Guilbert, 1997a; Cuq, Gontard, & Guilbert, 1997b, 1997c; Monterrey-Quintero & Sobral, 1999, 2000; Sobral, 2000; Sobral, Monterrey-Quintero, & Habitante, 2002; Iwata, Ishizaki, Handa, & Tanaka, 2000; Tanaka, Iwata, & Sanguandeekul, 2001).

The myofibrillar proteins are insoluble in water, but can be made soluble adjusting the pH of the solution. These proteins are fully stretched and closely associ-

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ated with each other in parallel structures (Iwata et al., 2000). Probably in function of this, these proteins are capable of forming a continuous matrix during drying of the solution (Cuq et al., 1995; Monterrey-Quintero & Sobral, 2000; Sobral, Ocuno, & Savastano, 1998). On the other side, the sarcoplasmic proteins are globular proteins, which in general have to be thermally denatured to form a continuous matrix (Iwata et al., 2000). Besides, the protein concentration in filmogenic solution (FFS) can also influence the formation of the protein matrix. The production of films with whey protein isolate, for example, requires a relatively high protein concentration (>8%) in FFS so that the formation of S–S bridges occurs (Sothornvit & Krochta, 2001).

The fish muscle proteins, when made soluble by decreasing pH, provide extremely viscous colloidal solutions (Cuq et al., 1995). This way, it is necessary to work with lower protein concentrations in FFS. Cuq et al. (1995) developed films from FFS with 0.5–2.75% of myofibrillar proteins of Sardine and treated at 20–50 °C. Monterrey-Quintero and Sobral (1999) studied the characteristics of myofibrillar protein films of Nile Tilapia from FFS with 0.5–2.0% of proteins and treated at 40 °C/10 min. On the other hand, Iwata et al. (2000) and Tanaka et al. (2001) treated FFS with 2–4% of sarcoplasmic proteins of Blue Merlin at temperatures ranging between 55 and 90 °C for up to 60 min. And more recently, Paschoalick, Garcia, Sobral, and Habitante (2003) treated FFS with 1% of Nile Tilapia muscle proteins (myofibrillar + sarcoplasmic) at 40, 65 and 90 °C/30 min.

On another hand, the utilization of plasticizers is necessary to reduce brittleness and/or to improve the workability of the film by reducing the intermolecular interactions between adjacent chains of the biopolymer, resulting in an increase of mobility of these chains and consequently, in flexible films (Cuq et al., 1995). As a consequence, at a macroscopic level, a reduction in the mechanical resistance and an increase in the elasticity and water vapor permeability of the films may occur (Cuq et al., 1997a). Thus, the effect of the plasticizer content on the functional properties of edible films may be known.

Tilapia is a very well adapted fish to cultures in various countries, and it has appeared in terms of production in cultures, due to its resistance and productivity (Chimits, 1995). More recently, a Thai Tilapia has shown an even larger productivity, practically resulting in substitution of Nile Tilapia in the Brazilian farms.

This way, the objective of this work was the study of color, opacity, mechanical, viscoelastic and thermal properties of films produced with a Thai Tilapia muscle proteins (sarcoplasmic + myofibrillar) in function of protein concentration (1 and 2 g/100 g of solution) and plasticizer (15–65 g of glycerin/100 g of protein) in the film forming solution, which were thermally treated under a unique condition (90 °C/30 min).

2. Materials and methods

2.1. Protein preparation

Freeze-dried muscle proteins from a Thai variety from the Nile Tilapia (*Oreochromis niloticus*) were used in this work. These proteins were prepared from fish raised at the campus of Pirassununga of the University of São Paulo and slaughtered after insensibilization in cold water. The proteins were prepared such as described in a previous paper (Paschoalick et al., 2003).

2.2. Films production

The muscle protein (MP) films were prepared by the casting technique. The studied film forming solutions (FFS) compositions were as follows (Sobral, 2000; Paschoalick et al., 2003): protein, 1 or 2 g of MP/100 g of FFS; plasticizer, 15–65 g of glycerin/100 g of protein; pH kept at 2.7 using acetic acid, and thermal treatment of 90 °C/30 min. The films were obtained by casting the FFS on previously prepared Plexiglas plates ($12 \times 12\,cm^2$), always with the same dry matter density to guarantee constant thickness, and drying at 30 °C and room relative humidity (55–65%), for 24 h in an oven with air renewal and circulation (Marconi, MA037), with PID control (±0.5 °C) of temperature (Monterrey-Quintero & Sobral, 2000). The weighting (±0.0001 g) of all films components was accomplished using an analytical scale (Scientech, SA210).

The films made with 1 and 2 g of MP/100 of FFS were characterized for the color, opacity, mechanical and viscoelastic properties determinations. For that, these films were previously conditioned at 22–25 °C and 58% of relative humidity, in desiccators with saturated solution of NaBr, for 7 days. All these tests were made in quadruplicate.

Only the films made with 2 g of MP/100 of FFS were characterized for the thermal properties, and in this case, the samples were conditioned at 22–25 °C over silica gel, for 2 weeks before analysis. These tests were made in triplicate.

All characterizations were done in controlled room conditions ($T = 22-25$ °C and relative humidity between 55% and 65%). The thickness of the films was measured averaging nine random positions, using a digital micrometer (±0.001 mm) with a 6.4 mm diameter probe. The sample humidity was determined by drying in an oven at 105 °C for 24 h (Paschoalick et al., 2003).

2.3. Color

The color of the MP 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 (Gennadios, Weller, Hanna, & Froning, 1996). The color of the films was expressed (Eq. (1)) as the difference of color (ΔE^*).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

where ΔL^*, Δa^* and Δb^* are the differentials between the color parameter of the samples and the color parameter of the white standard ($L^* = 94.8$, $a^* = -0.78$, $b^* = 1.43$) used as the film background.

2.4. Opacity

The opacity of the MP films was determined according to a Hunterlab method (Sobral, 2000), with the same equipment used for the color measures, also operating in the reflectance mode. The opacity (Y) of the samples was calculated as the relationship among the opacity of each sample on the black standard (Y_b) and the opacity of each sample on the white standard (Y_w). This calculation ($Y = Y_b/Y_w$) was made automatically by the Universal Software 3.2 (Hunterlab Associates Laboratory).

2.5. Mechanical properties

The mechanical properties of the films were determined by two tests using a texture analyzer TA.XT2i (SMS, Surrey, UK) controlled by the software Texture Expert V.1.15 (SMS).

The puncture tests were run according to Gontard, Guilbert, and Cuq (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. The puncture force (F) and the displacement of the probe (D) at break were determined with the software Texture Expert V.1.15 (SMS) directly from the force × probe displacement curves. The puncture deformation ($\Delta l/l_0$) could be calculated with D considering that stress was perfectly distributed along the film at breaking point (Sobral, Menegalli, Hubinger, & Roques, 2001).

The tensile tests were run using rectangular samples of 100 mm × 16 mm, initial (l_0) grips separation of 80 mm and cross-head speed of 0.9 mm/s (Gennadios, Park, & Weller, 1993). The tensile strength (force/initial cross-sectional area) and elongation at break ($\Delta l/l_0$) were determined with the software Texture Expert V.1.15 (SMS) directly from the stress × strain curves, and the elastic modulus was calculated as the inclination of the linear initial portion of this curve (with deformation in %).

2.6. Viscoelastic properties

The viscoelastic properties of the films were characterized by dynamic mechanical analysis, using an equipment DMA TA2980 controlled by a TA5000 module (TA Instruments, New Castle, DE, USA), operating in uniaxial traction tests. The analysis were carried out in the frequency scanning (0.01–200 Hz) mode, with constant temperature (30 °C), the amplitude of deformation (0.2%) and the flow of N_2 in the measure cell (1180 ml/min) (Paschoalick et al., 2003).

Rectangular samples of about 19 mm × 5 mm, were submitted to oscillatory traction (senoidal stress applied) analysis, obtaining the storage modulus (E'), the loss modulus (E'') and the phase angle ($\mathrm{Tan}\,\delta = E''/E'$) in function of the frequency. For the study of the plasticizing effect of glycerin on viscoelastic properties, E' and $\mathrm{Tan}\,\delta$ were calculated from DMA results at 1 Hz frequency (Lazaridou & Biliaderis, 2002), with the software Universal Analysis V1.7 F (TA Instruments).

2.7. Thermal properties

The thermal properties of the edible films produced only with 2 g of glycerin/100 g FFS were characterized by differential scanning calorimetry, using a DSC TA 2010 controlled by a TA5000 module (TA Instruments, New Castle, DE, USA) and with a quench cooling accessory. The aliquots of the order of 10 mg weighed (±0.01 mg) in a precision balance (Ohaus, Analytical Plus), were conditioned in hermetic aluminum pans, and heated at 5 °C/min, between −120 °C and 150 °C, in inert atmosphere (45 ml/min of N_2) (Sobral et al., 2002). 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 reference was an empty pan. The equipment was calibrated with an indium sample ($T_m = 156.6$ °C, $\Delta H_m = 28.71\ \mathrm{J\,g^{-1}}$) (TA Instruments).

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. All these properties were calculated with help of the software Universal Analysis V1.7 F (TA Instruments).

2.8. Statistical analysis

Duncan's multiple range test was applied to compare means for color, opacity, mechanical and viscoelastic properties muscle proteins films, with a level of significance of $\alpha = 0.05$, using the SAS software (SAS, 1989).

Moreover, Eq. (2), proposed by Ghorpade, Gennadios, Hanna, and Weller (1995), was fitted to data of these properties with the Excel 2002 software (Microsoft, Seattle, WA).

$$P = ae^{bC_g} \quad (2)$$

In these equation, P is the convenient property, C_g is the concentration of glycerin (g/100 g), and a and b are empiric parameters, whose values were calculated by non-linear regression.

3. Results and discussion

The freeze-dried proteins of Thai Tilapia muscle presented an amount of 86% of protein. This value was considered in the calculations of the protein concentration (C_p) in the filmogenic solution (FFS) and as the basis for glycerin concentration (C_g) calculations. The others principal components were humidity (6–7%) and lipids (7–8%) (Paschoalick et al., 2003).

In general, the films prepared with 1 and 2 g of proteins/100 g of FFS showed to be easy workable and sufficiently resistant (subjective evaluation), after all, with a good general aspect. Choi and Han (2002) and Sothornvit and Krochta (2001) also obtained good workable edible films after thermal treatments at 90 °C of the film forming solutions based on pea protein isolate and β-lactoglobulins, respectively. These proteins, like the sarcoplasmic proteins, are globular proteins and their denaturations improve the formation of a continuous matrix (Iwata et al., 2000).

The average thickness (±standard deviation) calculated with the data of all the films used in this work, for each protein concentration in FFS were 0.081 ± 0.006 mm and 0.078 ± 0.004 mm for 1 and 2 g of proteins/100 g FFS, respectively. This observed difference was considered negligible. But, after conditioning, the humidity of films varied between 6 g water/100 g moist sample in films with 15 g glycerol/100 g of FFS, and 30 g water/100 g of moist sample within films with 65 g of glycerol/100 g FFS.

3.1. Color

The increase of C_g caused reduction in the color difference (ΔE^*) of films (Fig. 1), possibly due to the effect of dilution of glycerin, which is a colorless substance, practically in an independent manner from C_p in FFS. A significant difference ($P < 0.05$) was observed only between values relating to 15% glycerin. Besides, the results of the non-linear regression (Table 1) allowed to suggest that the behaviors of ΔE^* in function of C_g were similar in both the C_p. These behaviors are according to the results of Paschoalick et al. (2003) who, however, observed a linear reduction of color with C_g. The concentration of the proteins in the FFS not affected the color of its films possibly because the relation of dried solids by surface of films was maintained constant after drying.

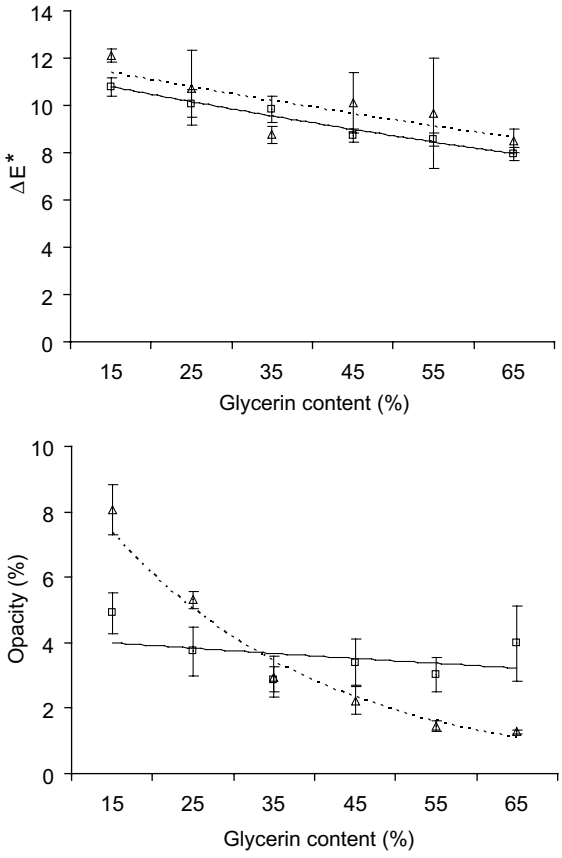

Fig. 1. Color difference (ΔE^*) and opacity of muscle proteins based films as a function of the glycerin concentration: (□, —) 1 and (△, - - -) 2 g of Thai Tilapia proteins/100 g of FFS.

The films obtained in this work presented, in general, color comparable to the films produced with Nile Tilapia muscle protein (Paschoalick et al., 2003) but were more colored than films based on Nile Tilapia myofibrillar proteins (Sobral, 2000), egg albumins (Gennadios et al., 1996), and pigskin gelatin (Sobral, 1999).

3.2. Opacity

The opacity of films (Fig. 1) with 2 g of proteins/100 g of FFS was greater ($P < 0.05$) in low C_g (<35%), becoming lower ($P < 0.05$) in high plasticizer concentration (>45%). The opacity of the films obtained in this work was comparable to the films based on Nile Tilapia muscle proteins (Paschoalick et al., 2003). However, these films were more opaque than the films based on Nile Tilapia myofibrillar proteins (Sobral, 2000) and pigskin gelatin (Sobral, 1999). In some manner, the denaturation of sarcoplasmic proteins, present in the muscle proteins, caused an increase of opacity of these films in relation to the films made only with myofibrillar proteins (Monterrey-Quintero & Sobral, 1999).

Table 1
Parameters of Eq. (2) calculated by non-linear fitting

Properties	1 g MP/100 g FFS			2 g MP/100 g FFS		
	a	b (10^2)	R^2	a	b (10^2)	R^2
Color difference	11.48	−6.1	0.971	12.05	−5.6	0.628
Opacity (%)	4.17	−4.3	0.168	10.79	−38.1	0.973
Puncture force (N)	7.37	−20.0	0.953	8.10	−18.3	0.962
Puncture deformation (%)	4.89	10.7	0.805	5.98	5.2	0.708
Tensile strength (MPa)	11.19	−34.2	0.976	9.58	−31.8	0.907
Elastic modulus (MPa)	10.82	−37.3	0.905	14.20	−41.3	0.984
Elongation at break (%)	37.57	19.7	0.390	39.13	16.1	0.585
Storage modulus (MPa)	828.05	−2.6	0.795	825.82	−2.9	0.987
Phase angle	14.29	0.8	0.787	13.51	0.8	0.982

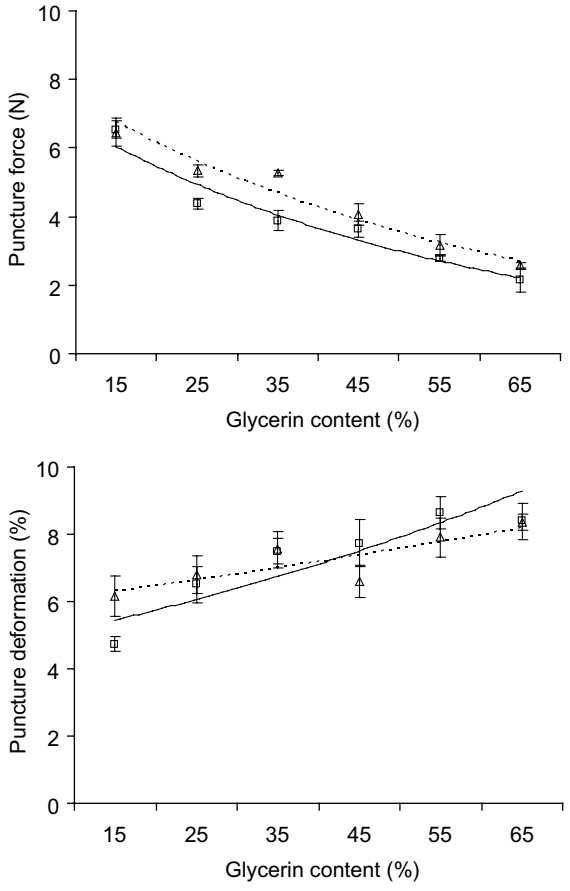

Fig. 2. Puncture force and puncture deformation of muscle proteins based films as a function of the glycerin concentration: (□, —) 1 and (△, ---) 2 g of Thai Tilapia proteins/100 g of FFS.

3.3. Mechanical properties

According to the results of puncture tests (Fig. 2), the films produced with 2 g of proteins/100 g of FFS were more ($P < 0.05$) resistant than the respective films with 1 g of proteins/100 g FFS, except in the case of films with 15% glycerin. This same tendency was observed in the results of tensile strength and elasticity modulus (Fig. 3), however, with significant differences ($P < 0.05$) only in the case of films with 25% and 35% glycerin, in both the properties. It can still be observed in Figs. 2 and 3, that these properties presented an exponential behavior with a high correlation coefficient (Table 1).

The resistance behavior of the films in function of C_p can be explained by the possible effect of this on the kinetics of reaction of sulphur residues of cystine present in proteins; in other words, the increase of C_p may have favored the formation of sulphite bridges among the

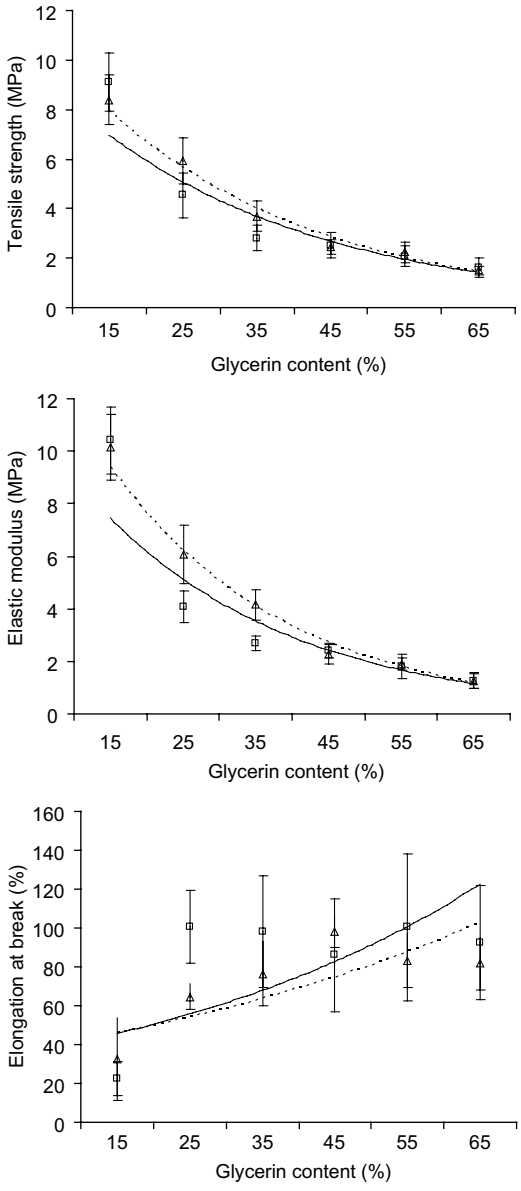

Fig. 3. Tensile strength, elastic modulus and elongation at break of muscle proteins based films as a function of the glycerin concentration: (□, —) 1 and (△, ---) 2 g of Thai Tilapia proteins/100 g of FFS.

protein chains due to the thermal treatment (Choi & Han, 2002; Perez-Gago & Krochta, 2001). However, Iwata et al. (2000) observed that the reduction of sarcoplasmic protein concentration from 4% to 2% in FFS caused a significant increase in the tensile strength of the films.

The films produced in this work presented puncture force values equivalent to the Nile Tilapia muscle protein films (Paschoalick et al., 2003), to the Nile Tilapia myofibrillar protein based films (Monterrey-Quintero & Sobral, 1999) and also to the sarcoplasmic protein based films (Iwata et al., 2000). On the other side, the tensile strength of the films produced in this work was superior to whey protein isolate films (Shaw, Monahan, O'Riordan, & O'Sullivan, 2002). The values of elasticity module of the films produced in this work were of the same order of the results encountered in the works of Shaw et al. (2002) and Perez-Gago and Krochta (2001).

As it was expected, it can be observed in Figs. 2 and 3 that an increment of 15–65% in C_g caused a tendency of increasing in puncture deformation as well as elongation at break, respectively. Unfortunately, the dispersion of data obtained in both the tests prevented to verify a logical behavior in the comparison between the two C_p. Iwata et al. (2000) observed that the increase of sarcoplasmic protein concentration from 2% to 4% increased the elongation at break of films.

The reduction of puncture force, tensile strength and elasticity modulus, as well as the tendency of increasing of puncture deformation and elongation at break with the increase of glycerin concentration are typical behaviors of films based on proteins (Cuq et al., 1995; Gontard et al., 1993; Sobral et al., 1998). The presence of plasticizers and water molecules, considering that the increasing of the glycerin content increased the final humidity of films, decreased the protein–protein interactions increasing the mobility of polypeptide chains allowing the films less resistant and more elastic.

3.4. Viscoelastic properties

It can be observed in Fig. 4, that E' calculated at 1 Hz decreased considerably with the increase of C_g, without any significant effect ($P > 0.05$) of C_p in FFS, and that in both the cases, this behavior was exponential (Table 1). This same behavior was observed in the E'' (results not shown), however less evident, in which the phase angle (Tan δ) increased with C_g (Fig. 4), without a clear effect ($P > 0.05$) of C_p on FFS. These behaviors agree with those observed in the work of Paschoalick et al. (2003), with Nile Tilapia muscle protein films, and can be explained by the fact that the solvent (glycerin) as well as the solute (proteins) contribute with E'', while only the solute contributes with E' (Ferry, 1980). This way, a greater influence of glycerin on E'' could have caused the increase of Tan δ.

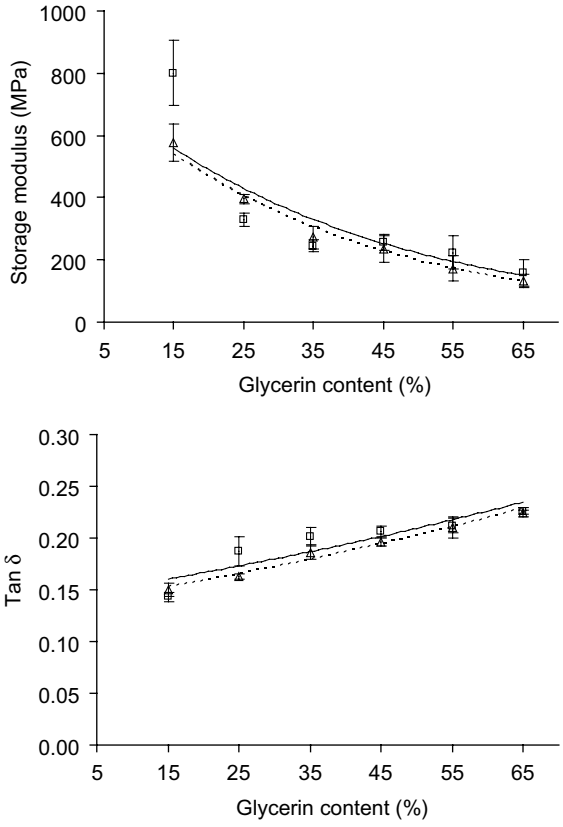

Fig. 4. Storage modulus and phase angle at 1 Hz of muscle proteins based films as a function of the glycerin concentration: (□, —) 1 and (△, ---) 2 g of Thai Tilapia proteins/100 g of FFS.

The values of the viscoelastic properties (E', E'' and Tan δ) determined in this work are of the same order of values observed in the papers of Cuq, Gontard, and Guilbert (1997c), who worked with Atlantic Sardine myofibrillar protein films, and Gontard and Ring (1996) and Cherian, Gennadios, Weller, and Chinachoti (1995), who worked with gluten films containing various plasticizers. In general, it was very difficult to compare these types of results, because most of the authors worked in the temperature scanning mode and mainly verified the plasticizer effect of the sample moisture content and not necessarily of the added plasticizer, as in the present work. In addition, Cuq, Gontard, Cuq, and Guilbert (1996) and Chandra and Sobral (2000) worked with viscoelastic properties of edible films determined however by static methods (stress relaxation tests).

3.5. Thermal properties

The DSC traces of muscle protein based films made with 2 g of proteins/100 g of FFS and conditioned over silica gel for two weeks are presented in Fig. 5. A very visible glass transition (*GT*) appeared in all the DSC curves below 0 °C, and another less visible *GT* appeared around 50 °C, mainly in the first scan results. This

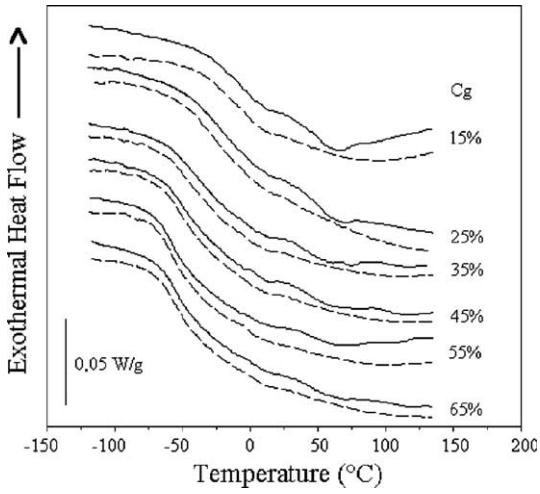

Fig. 5. DSC curves of muscle proteins based films (2 g of Thai Tilapia proteins/100 g of FFS) as a function of the glycerin concentration: (—) first and (---) second scan.

behavior was a consequence of a phase separation between the biopolymer and the plasticizer: the *GT* occurred at a very low temperature (T_g) has been associated with the glycerin-rich fraction, and the other T_g with the protein-rich fraction (Cuq et al., 1997b, 1997c; Cherian et al., 1995; Gontard & Ring, 1996; Sobral et al., 2001, 2002), nevertheless this phenomenon can be associated with thermal relaxation of native proteins.

Only the first (lower) T_g was well plasticized by glycerin. It can be noted in Fig. 6 that the increase in C_g caused a depression in T_g of around 60 °C. The curve presented in this figure was obtained using the Kwei Equation (Ross, 1995), for binary systems (Eq. (3)).

$$T_g = \frac{\omega_1 T_{g1} + \kappa\omega_2 T_{g2}}{\omega_1 + \kappa\omega_2} + q\omega_1\omega_2 \quad (3)$$

In this equation, ω_i and Tg_i represents the mass fraction and glass transition temperature, respectively, of the two components: proteins ($i = 1$) and glycerin ($i = 2$). The T_g values of films were calculated by non-linear fitting using the Statistica Software (StatSoft, 1995), considering $T_{g2} = 180$ K (Gontard & Ring, 1996). The Kwei Equation was capable to predict the behavior of T_g as a function of C_g in all domain of studied C_p, but the calculated parameters, $T_{g1} = 464.6$ K (191.5 °C), $k = 15.4$ and $q = 93.0$ K, has no physical sense (Roos, 1995).

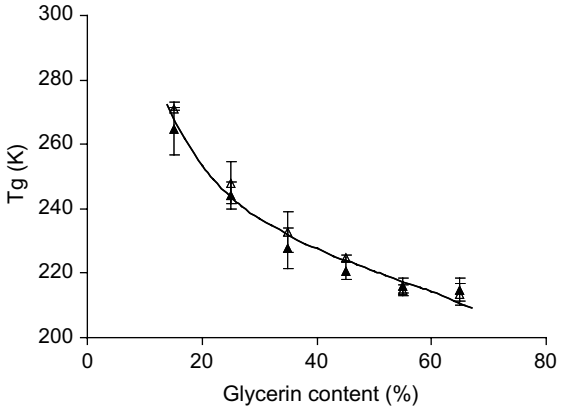

Fig. 6. Glass transition temperature (T_g) of muscle proteins based films (2 g of Thai Tilapia proteins/100 g of FFS) as a function of the glycerin concentration: (△) first and (▲) second scan. The curve was generated by fitting the Eq. (3).

It is not easy to explain the mechanical and viscoelastic properties in terms of GT films, because the observed phases separation. But, despite the limited interaction between the plasticizer and the biopolymer, the films remain flexible even at temperatures below the protein-rich fraction T_g, due to the lubricating effect of the plasticizers (Debeaufort & Voilley, 1997).

4. Conclusions

Practically, all studied properties were affected by glycerin concentration; nevertheless the effect of the protein concentration was not evident in all properties. The increase of glycerin concentration caused reduction in the color difference of films due to the dilution effect of glycerin, independently of C_p in FFS. Moreover, the opacity of the films with higher protein concentration was greater in the low domain of glycerin concentration, becoming lower for high plasticizer concentration.

It can be also concluded that the films made from the more protein concentrated filmogenic solutions were more resistant than the films with less protein in the filmogenic solutions. But, this behavior can be affected by the plasticizer. Contrarily, not all viscoelastic properties were affected by the protein concentration in filmogenic solutions.

In relation to thermal properties, a phase separation was observed. The glass transition temperature of the protein-rich fraction was practically not sensitive to the plasticizer, but the glass transition occurring in lower temperature, credited to the glycerin rich fraction, was well affected by the plasticizer.

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