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GLASS TRANSITION STUDY OF NILE TILAPIA MYOFIBRILLAR PROTEIN FILMS PLASTICIZED BY GLYCERIN AND WATER

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Abstract

Studies on glass transition of myofibrillar proteins based edible films are scarce. This work aimed to determine the T_g of edible films from Nile Tilapia myofibrillar proteins as a function of water content. Films with 30 or 70 g of glycerol/100 g of protein and several water content, were analyzed with a DSC TA 2010. Samples conditioned at water activity between 0.11 and 0.75, clearly showed one glass transition at low temperatures (<223 K), and another transition, less visible, above 273 K. DSC curves of samples conditioned at a_w=0.84, also showed an endothermic peak below 273 K. These results rendered evidence of phase separation within edible films.

Keywords: edible film, glass transition, glycerin, myofibrillar protein, water

Introduction

Edible films are continuous flexible thin materials based on biopolymers, such as polysaccharides or proteins. These materials are also interesting because of their biodegradable attribute [1]. Edible films made by casting technique, are according to physical chemistry, a multicomponent dehydrated colloidal solution. The functional properties of these biomaterials depend on intrinsic conditions, such as pH of the filmogenic solution, type and content of plasticizer and moisture content, and on extrinsic conditions, such as temperature and relative humidity (RH).

Usually, biopolymers and plasticizers are hygroscopic and therefore film moisture content is affected by ambient condition [2]. Plasticizers, necessary to avoid film brittleness, reduce the glass transition temperature (T_g) of the system. At temperatures below T_g, the film behaves as a brittle glass, but at temperatures above T_g, the material exists in a soft rubbery state. Water molecules also reduce the glass transition temperature of biomaterials, and thus are considered as plasticizers, too [2].

Glass transition of biopolymers had been studied by food scientists since the eighties, mainly considering the plasticizing effect of water [2]. Studies on T_g of edible films only gained expression in the last five years. Cherian *et al.* [3] studied the

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glass transition of gluten films plasticized by glycerin and saccharose, at several proportions, and conditioned at 298 K and 50% RH. Gontard and Ring [4], also working with gluten films, verified the effect of moisture on T_g with a single formulation (20 g of glycerol/100 g of gluten). Beck *et al.* [5] studied the T_g of zein films with different plasticizers and conditioned at various RH. Other authors [6–8] determined T_g of films made of blends of biopolymers, like starch, gelatin, cellulose derivatives, pullulan and caseinates, plasticized with polyols.

Debeaufort and Voilley [9] and Arvanitoyannis and Biliaderis [10] characterized the state of films of methyl cellulose with polyethylene glycol 400 and of blends of methyl cellulose and starch plasticized with glycerol, sorbitol or xylose, respectively, determining T_g as a function of the moisture of the material. Specifically on myofibrillar protein based films, Cuq *et al.* [11] and Souza *et al.* [12] determined T_g of films of myofibrillar proteins of Atlantic sardines plasticized with 35 g of equal mass blend of sorbitol and sucrose/100 g of dry matter and of bovine meat with 60 g of glycerin/100 g of proteins, respectively, as function of the moisture of the samples. In all these studies, T_g was determined by differential scanning calorimetry (DSC) and/or by dynamic mechanical analysis (DMA).

The objective of this work was the determination of the glass transition of Nile Tilapia myofibrillar protein (NTMP) based films by differential scanning calorimetry as function of moisture, using two concentrations of plasticizer (glycerin).

Materials and methods

Freeze-dried NTMP, previously characterized were used [13]: 93.2% of protein, 2.4% of fat and 1.7% of ash in dry solids. Proteins were composed mainly of high molecular mass myosin fragments, actin and light myosin chains. Proteins were rich in aspartic acid (12.1%), glutamic acid (12.2%), lysine (10.3%) and proline (8.9%) and very poor in cystine (0.7%), what indicates a low density in disulfide bonds.

NTMP films were prepared by casting. The composition of filmogenic solutions was [14]: 1.25 g protein/100 g solution, 30 or 70 g glycerol/100 g protein, pH maintained at 2.5 with acetic acid. The films were dried in a ventilated oven (Marconi, MA037), with PID temperature control (±0.5 K), at 303 K and 55–65% RH. Film thickness was about 45 μm.

To obtain films with different moisture content, samples were conditioned in desiccators containing saturated salt solutions with water activity ranging from 0.11 to 0.84 for at least three weeks, at 298 K. The moisture content of the films was calculated from samples sorption isotherms [15]. For DSC analysis, equilibrated samples of about 10 mg (±0.1 mg) (Scientech, SA210) were placed in hermetically sealed aluminum TA pans and analyzed in a DSC TA2010 (TA Instruments) equipped with a TA4000 controller. The samples were heated at 5 K min^{-1} between 123 and 423 K. Before the second run, the DSC cell was quench cooled with liquid N_2 just 15 K above the observed glycerin T_g in the first DSC trace. The ambient was maintained inert with 100 mL min^{-1} of dry N_2. The reference was an empty pan. The calibration standard used was indium, supplied by TA. The results were analyzed by Universal

Analyze TA software, considering T_g as midpoint of inflexion. All DSC analyses were run in triplicates.

Results and discussions

The DSC curves of the edible films of NTMP with 30 and 70% of glycerin, conditioned by absorption or desorption, are presented in Figs 1 and 2. The result at a_w=0.84 in Fig. 2A is missing because of mold growth in the samples. A phase separation appeared as a dual transition [9, 10] and by broadening of the transition [8, 12], in all DSC curves. According to Arvanitoyannis and Biliaderis [10] phase separation in systems composed of biopolymers and polyols, happens when the plasticizer concentration exceeds 20% and/or when the difference between the biopolymer and the plastificizer T_g's is more than 20 K, what seems to be happening with the edible films studied in this work. These phenomena had been reported for edible films of different

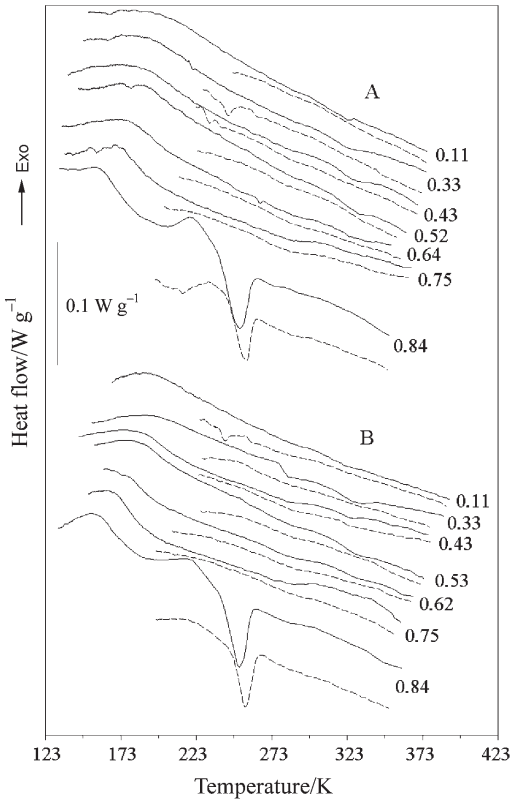

Fig. 1 DSC curves of films of Nile Tilapia myofibrillar proteins, with 30% of glycerin, conditioned from 0.11 to 0.84 of a_w, by A – absorption and B – desorption: — first run, ——second run

biopolymers and plasticizers as gluten and glycerin [3, 4], methylcellulose and poly(ethyleneglycol) 400 [9] and bovine myofibrillar proteins and glycerin [12].

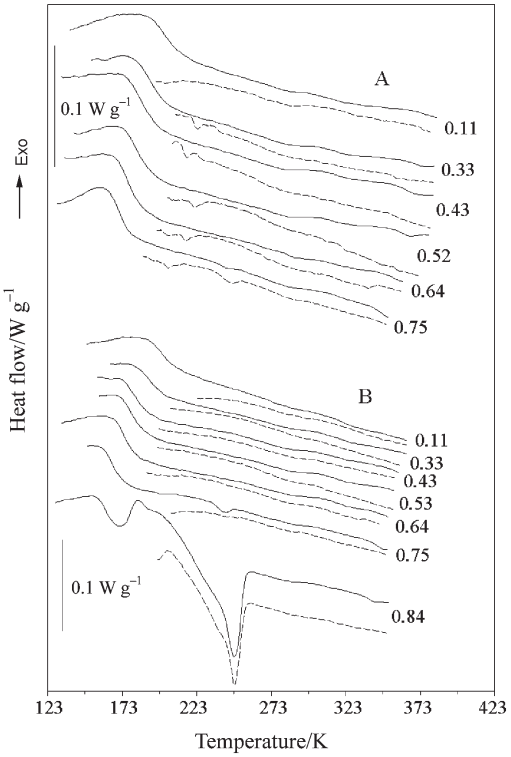

Fig. 2 DSC curves of films of Nile Tilapia myofibrillar proteins, with 70% of glycerin, conditioned from 0.11 to 0.84 of a_w, by A – absorption and B – desorption: — first run, - - - second run

The first and well defined glass transition observed at a very low temperature (<223 K), in DSC curves obtained in the first run for samples conditioned at 0.11 to 0.75 water activity, by absorption and desorption (Figs 1 and 2), is related to a rich glycerin fraction, well hydrated and possibly combined with low molecular mass protein fractions. Another T_g observed above 273 K, in both runs for all the films, is associated with the principal protein fraction maximally hydrated and plasticized. This phenomenon is less visible, i.e., the baseline inflexion was very subtle, because of very small changes in heat capacity across T_g in systems formed by certain proteins [3, 16].

It is also observed, in Figs 1 and 2, that all DSC curves of the samples conditioned at 0.84 of a_w, presented an endothermic peak below 273 K. In the case of films containing 70% glycerin (Fig. 2), this endothermic peak appeared as well, even though very subtle, in samples conditioned at 0.75 of a_w. These endotherms should be associated with the rich glycerin fraction fusion, which occurred at very low temperatures due to glycerin depression capacity on the melting temperature of ice. The DSC

curves obtained in conditioned films with the high water activity, are typical of gel systems [17], but can also be observed in studies about edible films [9, 12].

Several models based on the free volume theory, i.e., considering the additivity of the free volumes, or on the classic thermodynamics, considering for example, the loss of the configurational entropy at T_g, can be used to predict the depressing effect of plasticizer over the T_g [2]. In this study, the film constitute a ternary system, so the Couchman and Karaz (C&K) equation (Eq. (1)) is the most indicated.

$$T_g = \frac{\omega_1 \Delta C_{p_1} T_{g_1} + \omega_2 \Delta C_{p_2} T_{g_2} + \omega_3 \Delta C_{p_3} T_{g_3}}{\omega_1 \Delta C_{p_1} + \omega_2 \Delta C_{p_2} + \omega_3 \Delta C_{p_3}} \quad (1)$$

In Eq. (1), T_g is the glass transition temperature of the ternary system (film), 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: myofibrillar proteins (i=1), glycerin (i=2) and water (i=3). Because of the difficulty in determining T_g of the NTMP by DSC (it was not observed a baseline change in DSC curves – results not shown), it was decided to use the following literature values: T_{g_1}=508 K and ΔC_{p_1} = 0.42 J g^{-1} K^{-1} [11], T_{g_2}=180 K and ΔC_{p_2}=0.88 J g^{-1} K^{-1} [4] and T_{g_3}=134 K and ΔC_{p_3}=1.94 J g^{-1} K^{-1} [11], although there are controversies about these values for water [18].

It can be seen in Fig. 3 that the behavior of the experimental data of this work cannot be represented satisfactorily by the C&K equation, which predicts a monotonic decay of T_g with moisture, for a given concentration of plasticizer. The T_g of the rich protein fraction changed neither with moisture nor with the plasticizer concentration, because proteins were in a maximum plasticized state. This T_g stayed around 318.0±4.6 and 317.0±2.1 K, for films with 30 and 70% of glycerin, respectively. According to Debeaufort and Voilley [9], even that film T_g is higher than usual ambient temperature, the films keep being flexible because of the lubrifying effect of the plasticizers.

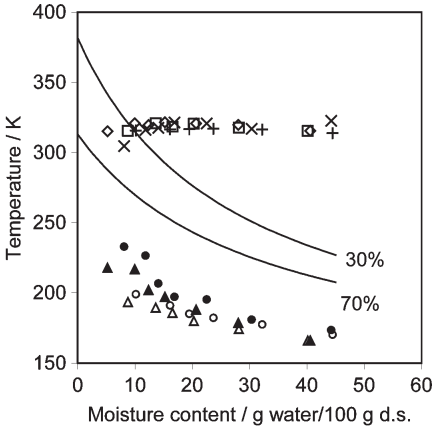

Fig. 3 T_g of the films of Nile Tilapia myofibrillar proteins with 30% (◊, x, o, •) and 70% (□, +, Δ, ▲) of glycerin. T_g of the protein rich fraction: absorption (◊, □) and desorption (×, +). T_g of the glycerin rich fraction: absorption (▲, Δ) and desorption (• , o). — Couchman and Karaz model

Cuq *et al.* [11] observed that the C&K model would describe just partially, the plasticizer effect of water in fish myofibrillar protein based edible films. On the other hand, Gontard and Ring [4] adjusted the model of C&K 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 significance.

It can be observed also in Fig. 3, that T_g of the rich glycerin fraction was well plasticized by the moisture of the sample, as a water-compatible monomer [2]. T_g values obtained with the samples conditioned by desorption were higher than the respective values of the samples conditioned by absorption, for a given moisture.

The microstructure of these films with 30 and 70% of glycerin was studied by scanning electron microscopy after fracture within liquid nitrogen [13]. In spite of the existence of a dense matrix, typical of films of proteins, clusters in an elliptic form were observed in both films. These porous had been probably occupied by the rich fraction in glycerol. The results of this analyses contribute to the confirmation of the occurrence of the separation of phases in both films.

Conclusions

The DSC curves of films of Nile Tilapia myofibrillar proteins, with 30 and 70% of glycerin, were typical of systems that could present phase separations. In this case, due to the low solubility of proteins, T_g of the rich fraction in proteins was practically constant, in both films, but T_g of the rich fraction in glycerol was plasticized by the film moisture.

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