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# Thermal properties of fish myofibrillar protein-based films as affected by moisture content

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Myofibrilar protein-based films were developed from a film-forming solution based on fish mince. The thermal properties of these films were characterized by dynamical mechanical thermal analysis and by differential scanning calorimetry as a function of their water content. During a temperature increase, the films produced sudden changes in mechanical property and specific heat, which are classicaly associated with the glass–rubber transition for amorphous materials. The glass transition was about 20°C broad. Increasing the film water content involved a non-linear decrease in glass transition temperature. The thermodynamic theory of the glass transition (i.e. the Couchman–Karasz equation) was inadequate to describe fully the plasticizing effect of water on the films. © 1997 Elsevier Science Ltd.

**(Keywords: biopackaging; glass transition; myofibrillar proteins)**

## INTRODUCTION

Most recent reviews focused on natural polymers have demonstrated the possibility of forming biopackagings from various hydrocolloid-based materials[1–3]. However, applications for these hydrophilic materials are still delayed because of the sensitivity of their mechanical and barrier properties to external conditions (i.e. ambient temperature and relative humidity). The combined effect of temperature and relative humidity on the functional properties is traditionally interpreted in terms of disruptive water–polymer hydrogen bondings in a hydrophilic network[4–6]. Recognition of the role of water as a plasticizer of amorphous biomaterials could be associated with the significance of the glass transition as a physicochemical event[7,8].

The molecular response of a glassy material as the system transforms from a metastable glassy state to an unsteady rubbery state corresponds to a general increase in disorder, free volume and mobility of macromolecules[9]. The glass transition phenomenon is affected by the macromolecular characteristics, such as flexibility, size, length of chains, size and polarity of lateral groups, molecular weight, presence of intermolecular covalent bonds or crystallites and by the presence and contents of plasticizers.

Modifications in molecular organization induce variations of the material physical properties, and more particularly of their thermal, mechanical and dielectrical properties. The glass transition of amorphous materials displays thus a discontinuous change in specific heat against temperature and can then be characterized by differential scanning calorimetry (d.s.c.)[10]. Most studies of glass transitions in food materials have for the most part used d.s.c. but mechanical spectroscopy should yield additional information on molecular motions, intermolecular interactions or cross-link density. Dynamic mechanical thermal analysis (d.m.t.a.) is particularly used in relating structural and viscoelastic properties to the response to temperature, frequency or deformation[8,11–14]. In addition, when the transition is broad or the change in heat capacity is small, d.m.t.a. may allow measurement of the glass transition temperature when it is not possible by d.s.c..

In previous studies[6,15,16], myofibrillar protein-based films were developed and the effect of hydrophilic plasticizer, water content and temperature on their functional properties were studied. The present study was undertaken to investigate the application of d.m.t.a. and d.s.c. in order to characterize the thermal properties of these films at various water contents, and to obtain a better understanding of the glass transition effect for this kind of material.

## EXPERIMENTAL

### *Preparation of fish mince*

Washed fish mince was prepared from Atlantic sardines following a method proposed by Cuq *et al.*[15]. Gutted and headed fish were passed through a meat bone separator. The fish mince was washed twice with water, strained in a rotary rinser, passed through a refiner and a screw press, and chopped in a cutter. The fish mince was then vacuum packed in polyethylene bags (500 g) and kept at −23°C for a maximum of one month. The samples were thawed for 24 h at 4°C before experiments.

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*Preparation of myofibrillar protein-based films*

Transparent and easily handled films were prepared from a film-forming solution based on fish mince in distilled water and acetic acid. Protein concentration (2.0 g per 100 g solution) and pH (3.0) were adjusted according to previous data[15]. Plasticizers (i.e. equal weight blend of sorbitol and sucrose) were incorporated at 35 g per 100 g of dry matter (Merck, Darmstadt, Germany). All components were mixed at 25°C in a vacuum thermoregulated homogenizer (Stephan UM5, Marne la Vallée, France). The film-forming solutions were stored for 6 h at 25°C before casting on a PVC plate using a thin-layer chromatography spreader to obtain films of 4 mg dry matter $cm^{-2}$. The thin film-forming solution layer was dried in a ventilated oven at 25°C and 50% relative humidity for 15 h.

Film thickness was measured with a hand-held micrometer (Braive Instruments, Checy, France) with 7.5 mm diameter contact faces, to the nearest 1 $\mu$m. Thickness values are means of 10 measurements.

*Storage conditions*

Film samples (10 mm × 5 mm) were stored at 20°C for five days before testing in desiccators, each maintained at constant relative humidity with appropriate saturated salt solutions[17]. To inhibit microbiological development, the internal atmosphere was saturated with chloroform vapour (no effect on properties). The dried samples were obtained by drying in a vacuum desiccator at 40°C over $P_2O_5$ for one week. The dry matter content was determined by drying to a constant weight in an oven at 104°C.

*D.m.t.a.*

This was carried out with a Perkin Elmer apparatus DMA-7 (Norwalk, CT, USA). A small oscillating uniaxial mechanical strain (frequency = 1 Hz) was impressed on the film samples. The deformation amplitude was defined with regard to the film length (0.40% of the sample length). The extension mode of deformation was chosen for the film sample geometry. The equilibrated film samples were coated with silicone grease to limit water vapour exchange with the external atmosphere during measurements. The silicone coating had no effect on measurements. Temperature scans (from −20 to 200°C) were performed at a heating rate of 5°C $min^{-1}$. The Perkin Elmer DMA-7 was equipped with an Intracooler (FTS Systems, Stone Bridge, NY, USA). The system was calibrated (furnace calibration) using Perkin Elmer calibration software, with indium (mp 156.6°C, Perkin Elmer standard) and distilled water (mp 0°C) samples. The measurement cell was flushed with dry helium, and five replicate film samples were tested. For each analysis, the DMA-7 stored values were storage modulus $E'$, loss modulus $E''$, tan $\delta$ and sample length.

*D.s.c.*

This was performed using a Perkin Elmer DSC-7

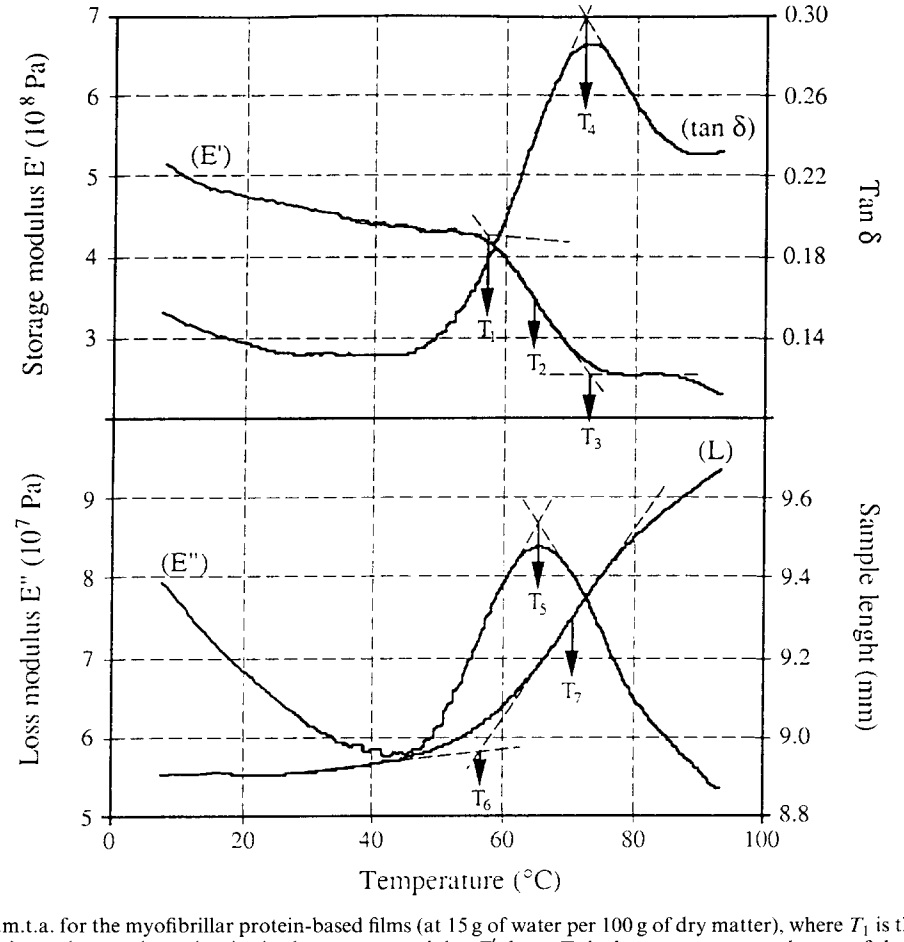


**Figure 1** Typical scan of d.m.t.a. for the myofibrillar protein-based films (at 15 g of water per 100 g of dry matter), where $T_1$ is the temperature at the onset, $T_2$ at the inflection point and $T_3$ at the end-point in the storage modulus $E'$ drop, $T_4$ is the temperature at the top of the tan $\delta$ peak, $T_5$ is the temperature at the top of loss modulus $E''$ peak, $T_6$ is the temperature at the onset and $T_7$ at the inflection point in the sample length drop

instrument (Norwalk, CT, USA). The system was calibrated using Perkin Elmer calibration software, with indium (mp 156.6°C, Perkin Elmer standard) and distilled water (mp 0°C) samples used for calibration of heat flow and two temperature points. The calorimetric cell was cooled with tap water, allowing operation of the instrument at temperatures as low as +20°C. The dry box of the DSC-7 was flushed with dry nitrogen. About 5-mg films samples were sealed in hermetic stainless steel capsules and weighed on a Sartorius microbalance (Type R 2210) with an accuracy of ±0.01 mg. Stainless steel pans with 5 mg of distilled water were used as references. The software supplied by the manufacturer was used to analyse the DSC-7 scans. Three replicate film samples were scanned from 20 to 140°C at a heating rate of 5°C $min^{-1}$.

## RESULTS AND DISCUSSION

### *Thermomechanical properties*

The thermomechanical properties of the myofibrillar protein-based films were determined by d.m.t.a. at several water contents, as illustrated by the typical scan in *Figure 1*. Relatively large variations in mechanical properties can be observed between 50 and 70°C for a film containing 15 g of water per 100 g of dry matter. The

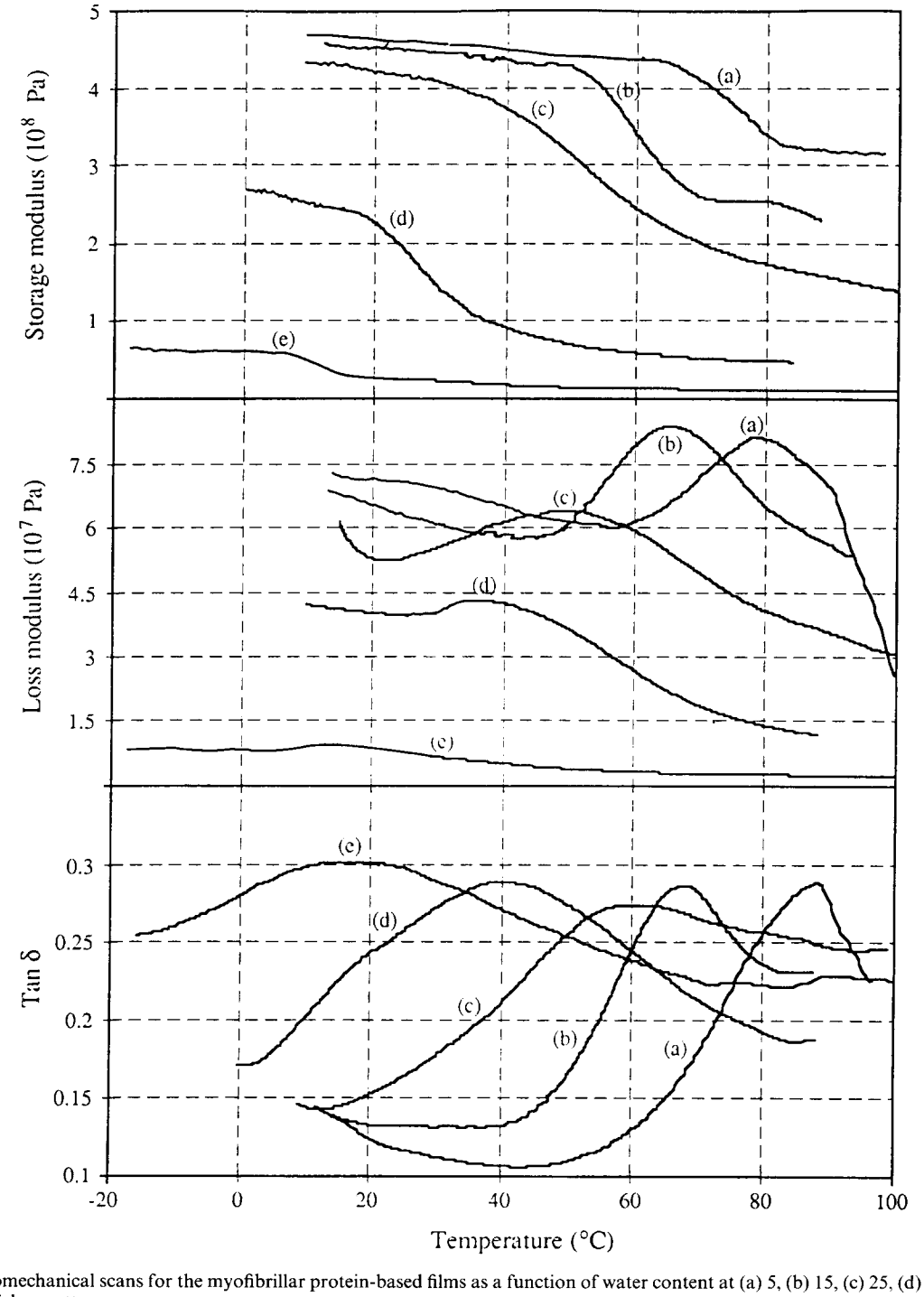


**Figure 2** Thermomechanical scans for the myofibrillar protein-based films as a function of water content at (a) 5, (b) 15, (c) 25, (d) 45, and (e) 68 g of water per 100 g of dry matter

**Table 1** Characteristic temperatures (±5°C) of the glass transition range for myofibrillar protein-based films, determined by d.m.t.a. and d.s.c.

| | Dynamical mechanical thermal analysis | | | | | | | | Differential scanning calorimetry | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Water content [a] | $T_1$ | $T_6$ | $T_2$ | $T_5$ | $T_3$ | $T_4$ | $T_7$ | $\Delta T$ | $T_i$ | $T_f$ | $\Delta T'$ |
| 0 | 123 | 119 | 135 | 128 | 139 | 142 | 139 | 23 | 116 | 130 | 14 |
| 5 | 71 | 65 | 79 | 76 | 88 | 89 | 82 | 24 | 63 | 78 | 15 |
| 8 | 60 | 60 | 70 | 69 | 78 | 80 | 76 | 20 | 55 | 69 | 14 |
| 15 | 55 | 53 | 63 | 64 | 72 | 69 | 69 | 19 | 50 | 65 | 15 |
| 25 | 40 | 35 | 50 | 52 | 59 | 58 | 54 | 24 | 42 | 57 | 15 |
| 45 | 25 | 31 | 36 | 28 | 44 | 42 | 43 | 19 | 25 | 41 | 16 |
| 68 | 1 | 3 | 10 | 8 | 20 | 18 | 21 | 20 | — | — | — |

$T_1$ is the onset, $T_2$ is the inflection point and $T_3$ at the end-point in storage modulus $E'$ drop, $T_4$ is the top of the tan $\delta$ peak, $T_5$ is the top of loss modulus $E''$ peak, $T_6$ is the onset and $T_7$ the inflection point in sample length drop, $T_i$ is the onset and $T_f$ the end-point in endothermic shift in apparent specific heat; $\Delta T = (T_3, T_4 \text{ or } T_7) - (T_1 \text{ or } T_6)$ and $\Delta T' = T_f - T_i$[a]. Expressed in g of water per 100 g of dry matter

sudden decrease in storage modulus, peaks in loss modulus and tan $\delta$, and increase in sample length, correspond to a typical transition from a 'rigid' to a 'rubbery' structure. Similar variations were classically observed for amorphous macrobiomolecules and attributed to the glass transition of the material[7,8,18–20].

In comparison with thermoplastic synthetic polymers, the thermomechanical properties of the myofibrillar protein-based films distinguished by two points:

(i) The rubbery storage modulus (at high temperature) was higher than those usually observed for synthetic thermoplastic polymers[21]. The resulting moderated variations in storage modulus drop, classically observed for various thermoset polymers[22] and for various protein materials[8,18] were associated with the probable presence of some covalent cross-links or chain entanglements in the myofibrillar protein-based films. This probable molecular organization was also responsible for the lack of 'flow region' on thermomechanical scans.

(ii) The decrease in storage modulus for the myofibrillar protein-based films occurred over a relatively large temperature range (*Figure 1*). This glass transition breadth ($\Delta T$ about 20°C) could be associated with the specific polydispersity of myofibrillar proteins and with the heterogeneity of structure within proteins, which tends to yield significant broadening of the glass transition. These reflected the different types of intermolecular interactions between proteinic chains.

The glass transition breadth could be described from the shape of the storage modulus drop as proposed by Peleg[23–25] or by taking into consideration several characteristic temperatures[8,26,27]. The description of glass transition breadth for the myofibrillar protein-based films was achieved from several temperature points (*Figure 1*): the onset ($T_1$), inflection point ($T_2$) and end-point ($T_3$) in storage modulus drop, the top of the tan $\delta$ peak ($T_4$) and loss modulus ($T_5$) peaks, and the onset ($T_6$) and inflection point ($T_7$) in sample length drop. The difference between the lowest ($T_1$ or $T_6$) and highest temperatures ($T_3$, $T_4$ or $T_7$) was then a reliable indicator of glass transition breadth for the myofibrillar protein films (*Table 1*).

The effect of water content on the thermomechanical properties for the myofibrillar protein-based films is presented in *Figure 2*. The decrease in intermolecular interaction density due to replacement of polymer–polymer bondings by polymer–water bondings involves a decrease in intensity of storage modulus before and

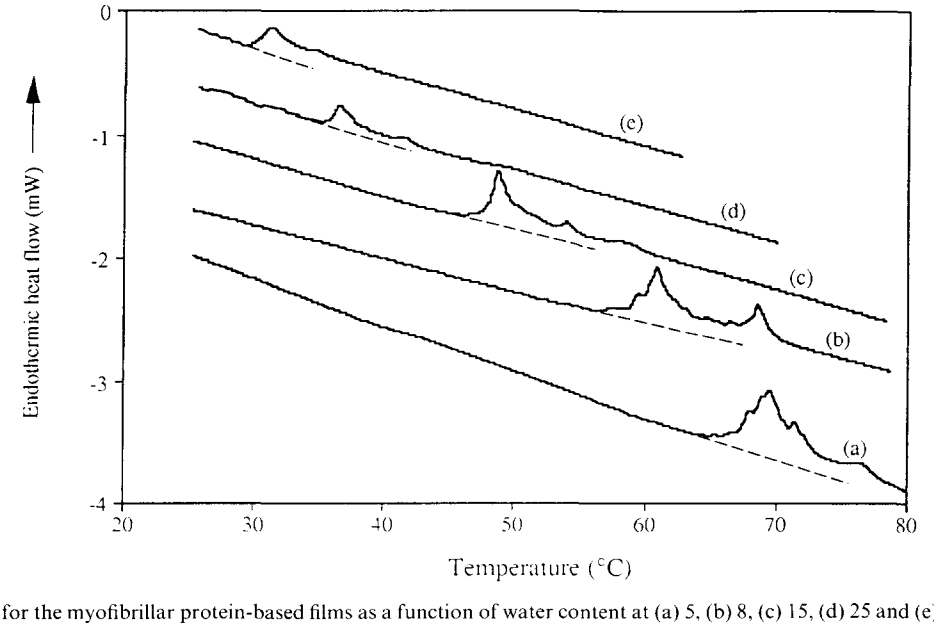


**Figure 3** D.s.c. scans for the myofibrillar protein-based films as a function of water content at (a) 5, (b) 8, (c) 15, (d) 25 and (e) 45 g of water per 100 g of dry matter

**Table 2** Glass transition temperatures of various natural polymers at 0 g of water per 100 g of dry matter

| Polymer | Method | $T_g$ (°C) | Reference |
|---|---|---|---|
| Starch | d.s.c. | 243 | 32 |
| Amylopectin | d.s.c. | 227 | 33 |
| Dextran | d.s.c. | 223 | 34 |
| Gelatin | d.s.c. | 210 | 10 |
| Collagen | d.s.c. | 200 | 35 |
| Elastin | d.s.c. | 197 | 35 |
| Gluten | d.m.t.a. | 162 | 18 |
| Casein | d.m.t.a. | 144 | 30 |
| Zein | d.m.t.a. | 139 | 36 |
| Gliadin | d.m.t.a. | 121 | 36 |

after the glass transition range. Increasing the water content induces also an important decrease in height for loss modulus and tan $\delta$ peaks. The exact type of molecular movements associated with variations in thermomechanical properties for amorphous polymers are still not well known[28]. For protein-based materials, these movements could be associated with specific regions in molecules that are particularly rich in polar amino acids.

It is important to note that application of d.m.t.a. for hydrated materials was difficult to interpret due to difficulties in controlling the atmosphere around samples. The drying phenomenon was generally significant beyond ambient temperature and more particularly above the peak in tan $\delta$[29,30]. Because of their specific thin layer structure, the myofibrillar protein-based films are potentially sensitive to drying in spite of their silicone grease coatings. Glass transition temperatures could thus be slightly overestimated, especially at low water contents.

*Calorimetric properties*

The calorimetric properties for the myofibrillar protein-based films at different water contents are presented in *Figure 3*. In addition to slight thermal events, a clear endothermic transition in the heat flow is evident and does not disappear upon rescan. This expresses a discontinuous behaviour in specific heat and characterizes a second-order endothermic transition (i.e. the glass transition).

The glass transition breadth for the myofibrillar protein-based films was characterized by temperatures noted at the onset ($T_i$) and end-point ($T_f$) of specific heat change (*Table 1*). The glass transition breadth ($\Delta T'$ about 15°C) is of the same magnitude of order as those determined from d.m.t.a. for the myofibrillar protein-based films and as published data for various proteinic materials[30,31]. When the water content is increased, the temperature noted at endothermic heat drop flow is lowered (*Figure 3*).

Due to the glass transition breadth, comparison between d.m.t.a. and d.s.c. results is difficult. Moreover, glass transition depends on the thermal history of the product and sollicitation frequency. In agreement with published data, changes in endothermic heat flow for the myofibrillar protein-based films is observed between onset of decrease in storage modulus $E'$ and top of the tan $\delta$ peak [30,31].

*Water content effect*

The glass transition is observed for the dry myofibrillar protein-based films between near 120 and 140°C (*Table 1*). This temperature range is lower as compared with already published glass transition temperatures for various proteinic materials (*Table 2*) and corresponds to the presence of large amounts of plasticizer in the films (35 g of sorbitol and sucrose per 100 g of dry matter). Differences in glass transition temperatures observed for these natural polymers are frequently attributed to differences in structure, molecular weight and interactions density[37].

Non-linear relationships are able to describe the decrease in glass transition temperatures ($T_1-T_7$, $T_i-T_f$) for myofibrillar protein-based films as a function of water content (*Figure 4*). The plasticizer effect of water on the glass transition is very important at low water content (below 10 g per 100 g of dry matter), with a decrease close to 8°C/1% water. For higher water

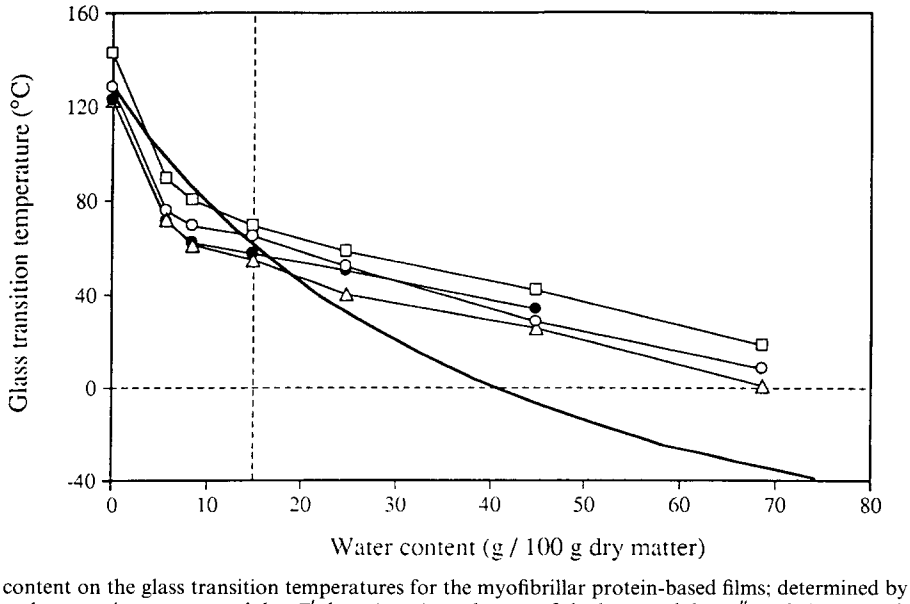


**Figure 4** Effect of water content on the glass transition temperatures for the myofibrillar protein-based films; determined by the temperature at the top of tan $\partial$ peak (–□–), at the onset in storage modulus $E'$ drop (–△–), at the top of the loss modulus $E''$ peak (–○–) and at the end-point of the endothermic shift in the apparent specific heat (–●–); where (—) represents the calculated curve from the Couchman–Karasz equation

contents, the decrease in glass transition temperatures is less important, about 1°C/% water. The depressing effect of water content on the glass transition has been previously reported for a variety of natural water-compatible polymers[20,38–42]. For instance, Cocero and Kokini[31] reported a decrease close to 9°C/1% water for glutenin-based materials when the water content was below 14 g per 100 g of dry matter.

The thermodynamic theory of the glass transition was applied to describe the plasticizing effect of water on the myofibrillar protein-based films. The description of glass transition temperature variations as a function of water content is achieved with the Couchman–Karasz (CK) equation[43], which relates the glass transition temperature of an homogeneous blend of several elements to their fractional concentrations and thermodynamic characteristics of the 'pure' compounds (i.e. 'pure' compound glass transition temperature and specific heat)[44]. This equation was applied to our system constituted by four compounds—myofibrillar proteins (1), sucrose (2), sorbitol (3) and water (4):

$$T_g = \frac{w_1\Delta C_{p1}T_{g1} + w_2\Delta C_{p2}T_{g2} + w_3\Delta C_{p3}T_{g3} + w_4\Delta C_{p4}T_{g4}}{W_1\Delta C_{p1} + w_2\Delta C_{p2} + w_3\Delta C_{p3} + w_4\Delta C_{p4}} \quad (1)$$

where $T_g$ is the glass transition temperature of the system, $T_{g_i}$ are the glass transition temperatures of the '$i$' pure compounds, $\Delta C_{pi}$ are the specific heats of the '$i$' pure compounds, $w_i$ are the fractional weight concentrations of the '$i$' compounds in the system ($\sum w_i = 1$); $\Delta C_{pi}$ and $T_{gi}$ for sorbitol, sucrose and water were found in published data[45,46] (sucrose: $\Delta C_{p2} = 0.60\,J\,g^{-1}\,K^{-1}$ and $T_{g2} = 343$ K; sorbitol: $\Delta C_{p3} = 0.96\,J\,g^{-1}\,K^{-1}$ and $T_{g3} = 270$ K; water: $\Delta C_{p4} = 1.94\,J\,g^{-1}\,K^{-1}$ and $T_{g4} = 134$ K). Because the dry matter composition of the film is a constant irrespective of water content, the ratios $w_2/w_1$ and $w_3/w_1$ are constant (= 0.269) and equation (1) could be written as follows:

$$T_g = \frac{w_1\left[T_{g1} + \frac{w_2}{w_1}\left(\frac{\Delta C_{p2}}{\Delta C_{p1}}T_{g2} + \frac{\Delta_{p3}}{\Delta C_{p1}}T_{g3}\right)\right] + w_4\frac{\Delta C_{p4}}{\Delta C_{p1}}T_{g4}}{w_1\left[1 + \frac{w_2}{w_1}\left(\frac{\Delta C_{p2}}{\Delta C_{p1}} + \frac{\Delta C_{p3}}{\Delta C_{p1}}\right)\right] + w_4\frac{\Delta C_{p4}}{\Delta C_{P1}}} \quad (2)$$

With the glass transition temperature for the 'pure' myofibrillar proteins ($T_{g1} = 508$ K)[47] and the glass transition temperature for the films when $w_4 = 0$ (i.e. the dry films) ($T_g = 403$ K), equation (2) allowed us thus to calculate $\Delta C_{p1}$ for the myofibrillar proteins in the films: $\Delta C_{p1} = 0.42\,J\,g^{-1}\,K^{-1}$. Replacing $\Delta C_{pi}$ and $T_{gi}$ by the published or calculated values allowed us to calculate the glass transition temperature from $w_1$ and $w_4$.

*Figure 4* shows that the CK equation is not adapted to describe fully the plasticizing effect of water on the myofibrillar protein-based films. The calculated glass transition temperatures are slightly higher than experimental values when the water contents are below 15 g per 100 g of dry matter. For higher water contents, the decrease in calculated values is larger that those obtained for experimental data.

Some factors could limit the validity of the CK equation applied to myofibrillar protein-based films. On the one hand, it is possible that the film composition does not stay constant between formulation and fabrication, in consequence of partial hydrolysis of compounds (e.g. myofibrillar proteins of sucrose) in the film-forming solution[15], inducing a more complex system. On the other hand, the specific heat values for plasticizers and water used in the calculations were determined in binary systems and their extrapolation in other systems still remains subject to discussion[18]. From published papers, satisfactory correlations of the CK equation have been reported for homogeneous binary food systems based on water and water-soluble molecules or macromolecules[20,32,33].

Many workers have studied the decrease in glass transition temperatures for natural fully or partially water-insoluble polymers at high water contents. For instance, Biliaderis *et al.*[48] and Kalichevski *et al.*[26] have observed stabilization in glass transition temperatures for native rice starch and amylopectin (near 60°C) when water contents were above 30 g per 100 g of dry matter. However, discussions about deviation from thermodynamic laws (such as the CK equation) were often avoided and the main part of the studies were limited to low water contents[10,36,38,49]. There is then no clear explanation to describe the behaviour of hydrophilic polymers at high water content.

Two hypotheses were thus proposed to explain the variations in glass transition temperatures for the myofibrillar protein-based films as a function of water content:

(i) The films could be considered as heterogeneous systems at higher water contents. Indeed, when hydration is higher that the 'monolayer moisture content' (about 10 g water per 100 g of dry matter) and the added moisture layer of slightly bound water[6], films could be considered as constituted by a hydrated insoluble protein network and by an aqueous phase. The CK equation can only be applied to homogeneous systems; it is then normal to expect that it has not yet been adapted.

(ii) A hypothesis based on modifications of water repartition between polymer and plasticizers as a function of water content could also be proposed. At low water activity, sugars and polyols are low hygroscopic materials contrary to hydrophilic macromolecules. The shapes of water sorption isotherms are very different and intersect at water contents close to 20 g per 100 g of dry matter[6]. Below this limit, water preferentially plasticizes myofibrillar proteins and the thermodynamic theory is thus quite acceptable. At water contents above 20 g per 100 g of dry matter, water fixes preferentially on sucrose and sorbitol to the detriment of proteins; the thermodynamic description of the plasticizing effect of water on the proteinic network is thus not valid. A similar hypothesis was proposed by Kalichevski *et al.*[18] and by Gontard and Ring[8] to explain the effect of plasticizer on various sugars and of water on the glass transition of wheat gluten.

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