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Thermoplastic properties of fish myofibrillar proteins: application to biopackaging fabrication

Bernard Cuq*†, Nathalie Gontard‡ and Stéphane Guilbert†
CIRAD-SAR, BP 5035, 73 rue J.F. Breton, 34090 Montpellier, France
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Thermoplastic properties of fish myofibrillar proteins were studied by dynamic mechanical thermal analysis. Important changes in dynamic mechanical properties, observed when the temperature was increased, were associated with the glass transition of fish myofibrillar proteins. The glass–rubber transition was observed between 215 and 250°C for the dry material. Addition of water or hydrophilic plasticizers (sucrose and sorbitol) induced large decreases in the glass transition temperature. The depressive effect of water content on the glass transition temperature is described with non-linear relationships. The thermodynamic theory of glass transition (i.e. the Couchman–Karasz equation) was adequate to describe partially the plasticizing effect of water on the myofibrillar proteins. Glassy or foamed biopackagings were obtained by a thermomoulding technique when the process temperature was higher than the glass transition temperature at a given moisture content. © 1997 Elsevier Science Ltd.

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INTRODUCTION

Most recent reviews focused on natural polymers have demonstrated the possibility of forming biopackagings from various agricultural raw materials. Biopackagings were developed in an attempt partially to replace some synthetic materials such as food packagings, dinner utensils, containers, trash bags, planting pots, etc.[1]. If biopackagings are collected after use, and pasteurized, ground and pelletized for animal feeds, a significant step toward solid waste reduction could be then expected.

Biopackaging fabrication has been considered using three techniques[2–6]: (i) biopackagings based on mixtures of synthetic polymers and biopolymers, (ii) biopackagings based on microbial polymers produced from agricultural materials used as fermentation substrates, and (iii) biopolymers used as raw materials to form biopackagings. Fabrication of biopackagings based only on biopolymers could be expected from two processes. The 'wet process' requires the biopolymer dispersion in a film-forming solution and has been extensively studied and applied to produce edible or biodegradable films and coatings[7–10]. The 'dry process' is based on thermoplastic properties of some biopolymers (mainly starch and proteins) in low water content conditions and had been applied with success to produce edible and/or biodegradable materials by using common melt processing technologies (e.g. extrusion, moulding or rolling mill procedure)[5,6]. Starch is the most commonly used agricultural raw material since it is inexpensive, widely available and relatively easy to handle, compared with other biopolymers such as microbial polymers[11–13]. Few studies on proteins as thermoplastic raw materials are available. For instance, thermoplastic properties of wheat gluten proteins could be exploited to make biopackagings[14,15].

Thermoplastic properties of biopolymers are defined in relation to the glass transition theory, which is a sufficient tool to explain textural changes that occur during thermoplastic polymer processing. Heating amorphous thermoplastic biopolymers above the glass transition temperature produces soft and rubbery materials, and gives the possibility to form them in specific forms like packaging materials. Cooling at room temperature reconverts the rubbery products into a glassy material, giving a more or less 'rigid' form of the desired structure.

In previous investigations, film packagings based on myofibrillar proteins were developed from a film-forming solution[16] and their main functional properties were studied and characterized as a function of various parameters[17–20]. The present study was undertaken to develop a thermoplastic process for biopackaging fabrication from fish myofibrillar proteins. Thermoplastic properties of myofibrillar proteins were studied by dynamical mechanical thermal analysis (d.m.t.a.) and conditions for biopackagings fabrication by a thermomoulding process were investigated.

EXPERIMENTAL

Preparation of myofibrillar protein-based raw material

Washed fish mince was prepared from Atlantic sardines following a method proposed by Cuq *et al.*[16].

* To whom correspondence should be addressed
† Present address: Ecole Nationale Supérieure Agronomique de Montpellier, 2 Place Viala, 34060 Montpellier, France
‡ Present address: ENSIA-SIARC, 1101 avenue Agropolis, BP 5098, 34033 Montpellier, France

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. Sucrose and sorbitol (each at 17.5 g/100 g dry matter) could be incorporated in fish mince formulation as plasticizer agents before the final chopping. 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. The fish mince was then extruded through a drum, with 2 mm diameter perforations, to form long fibres. Fibres were overlayed on metallic racks in a ventilated oven at 50°C for 5 h. The dry material was then powdered. A complementary drying (for 24 h in a vacuum oven at 50°C) was carried out to reduce the water content at 2.2 (±0.2) g/100 g dry matter.

Biopackagings based on myofibrillar proteins

These were prepared from 20 g (dry matter) of myofibrillar protein-based powders, hydrated at various levels by liquid water addition. Hydrated powders were stored for 24 h at 4°C before experiments.

The pilot thermomoulding process apparatus was constituted by a fixed mould overhung by a moving mould (i.e. the hollow punch). A stainless steel saucepan was used to set raw material in the mould and to remove product. Moulds and saucepan faces were coated by Teflon. The moulds were heated (between 100 and 300°C) by electric resistances with a 30°C difference in order to compensate for heat transfer resistance induced by the saucepan. The moulds were preheated for 15 min before experiments. The raw material was laid in the saucepan, which was then placed in the fixed mould. The moving mould was quickly pulled down, defining a chamber of 1.5 mm height. The system was submitted to 8×10^6 Pa pressure for 10 s in order to ensure air-tightness in the chamber, and the hollow punch was then quickly moved up and the biopackagings were obtained.

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 powder samples (disposed in a 18 mm diameter stainless steel cup) by parallel plate probes (15 mm diameter disc). The deformation amplitude was defined with regard to the sample height (0.15% of the sample height). Temperature scans (from −20 to 300°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, 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. Five replicate samples were tested. For each analysis, the d.m.t.a. stored values were storage modulus E', loss modulus E'', $\tan\delta$ and sample height.

Characterization of biopackagings

The specific volume of the biopackagings was determined according to a procedure derived from the Park method[21]. A weighed sample was placed in a vessel of known weight and volume. The free gap was filled up with Fontainebleau sand four times and stricken lightly three times at each stage of filling. The vessel was then weighed and the specific volume (ml g^{-1}) was calculated:

$$\text{Specific volume} = \frac{1}{M_S}\left(V_T - \frac{M_T - (M_V + M_S)}{d}\right) \quad (1)$$

where d is the sand density (g ml^{-1}), M_S is the sample weight (g), M_V is the vessel weight (g), M_T is the weight (g) of the vessel plus sample plus sand, and V_T is the volume (ml) of sand plus sample.

The mechanical properties were determined using a SMS TAXT2 Rheometer (Champlan, France) operated in a perforation mode. Biopackagings were cut in 40 mm diameter discs and stored at 25°C and 57.7% relative humidity for 15 days before testing. The samples were placed over a cylindrical hole (10 mm diameter). A cylindrical probe (3 mm diameter) was displaced perpendicularly to the sample surface at constant speed (0.5 mm s^{-1}) until material break down. The maximum force tolerated by samples (N) and elastic modulus calculated from the origin slope (N m^{-1}) were noted.

RESULTS AND DISCUSSION

Thermomechanical properties

Thermomechanical properties of dry myofibrillar proteins were studied by d.m.t.a. as illustrated by the typical scan in *Figure 1*. The gradual increase in storage and loss modulus, observed when the temperature is up to 100°C, is simultaneous with a slight decrease in sample height. These changes were attributed to a probable drying of samples[1,22]. Drastic changes in storage modulus, loss modulus, $\tan\delta$ and sample height are evident between 215 and 250°C. These variations are similar to those previously observed at the glass transition for myofibrillar protein-based films[20] and for various amorphous biopolymers[15,23–26]. The structural transition observed between 215 and 250°C was thus associated with the glass transition of dry fish myofibrillar proteins. This glass transition temperature (T_g) range is located near T_g values classically observed for collagen, gelatin or starch (*Table 1*). However, lower T_g values characterize corn zein caseins or wheat gluten proteins. Differences in T_g between these biopolymers could be associated with differences in structure, molecular weight or intermolecular interactions' density[39].

Molecular organization and structural characteristics of myofibrillar proteins are probably responsible for the noted differences in thermomechanical properties when compared with common thermoplastic synthetic polymers[40,41]. Specific polydispersity of myofibrillar proteins, heterogeneity of intermolecular interactions within proteins, and the probable presence of some covalent crosslinks or chain entanglements in myofibrillar proteins could be responsible for the high rubbery storage modulus, the lack of 'flow region' and the glass transition breadth ($\Delta T = 35$°C, estimated by the difference between temperature at the top of the $\tan\delta$ peak and the onset of storage modulus drop) observed for myofibrillar proteins.

Thermal degradation of myofibrillar proteins was observed to occur up to 250°C. It was interesting to note that the glass transition for the dry proteins (between 215 and 250°C) occurred just before their thermal degradation, so that the workable temperature

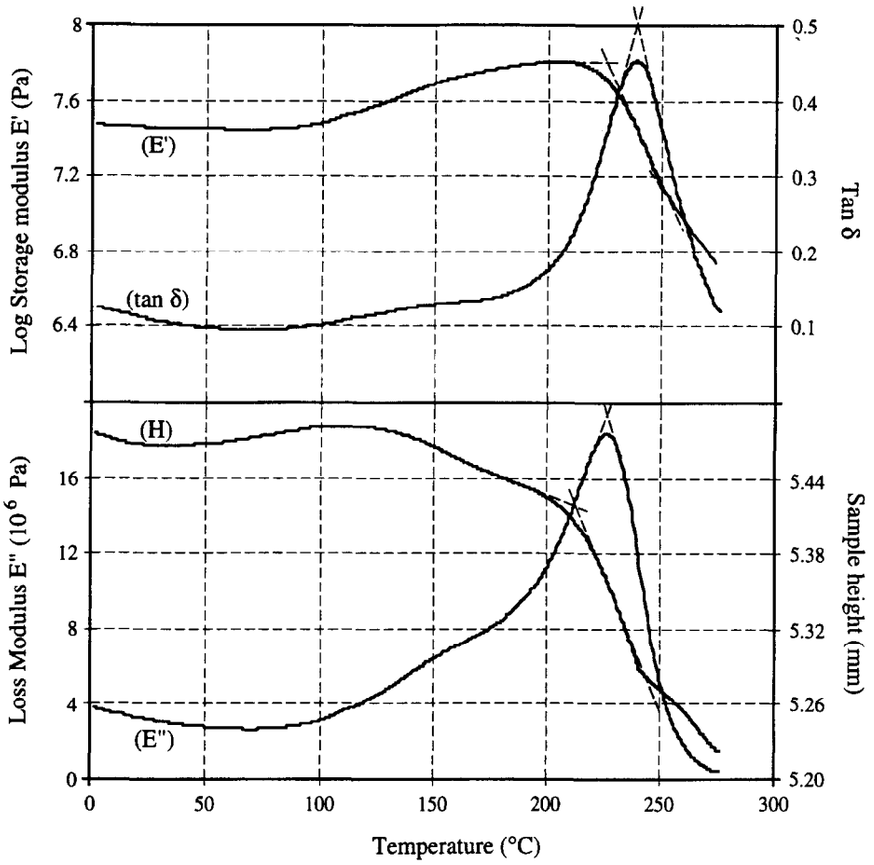

Figure 1 Typical scan of d.m.t.a. for the myofibrillar proteins (at 2.2 g water/100 g dry matter)

Table 1 Glass transition temperature of various natural polymers at 0 g water/100 g dry matter and of various synthetic polymers

Polymer	Method	T_g (°C)	Reference
Polycarbonate	—	150	27
Polystyrene	—	94	27
Poly(vinyl chloride)	—	90	27
Polypropylene	—	−15	27
Polyethylene	—	−110	27
Starch	d.s.c.	250	28
Starch	d.s.c.	243	29
Myofibrillar proteins	d.m.t.a.	215–250	Current study
Amylopectin	d.s.c.	227	30
Dextran	d.s.c.	223	31
Gelatin	d.s.c.	210	32
Collagen	d.s.c.	200	33
Elastin	d.s.c.	197	33
Gelatin	d.t.a.	190	34
Gluten	d.m.t.a.	162	22
Gluten	d.m.t.a.	160	35
Glutenin	d.m.t.a.	160	36
Casein	d.m.t.a.	144	37
Zein	d.m.t.a.	139	38
Gliadin	d.m.t.a.	121	38

d.s.c. = differential scanning calorimetry; d.t.a. = dynamic thermal analysis; d.m.t.a. = dynamic mechanical thermal analysis

range for biopackaging thermomoulding was very restricted.

Hydrophilic plasticizer incorporation

The thermomechanical properties of plasticized (with sucrose and sorbitol) myofibrillar proteins (*Figures 2* and *3*) are close to those previously observed for unplasticized raw materials. Drastic changes in dynamic mechanical properties observed between 130 and 185°C thus characterize the glass transition for dry plasticized proteins.

Relative complexity in the protein–plasticizer system could be responsible for the larger glass transition breadth ($\Delta T = 55$°C). As expected, incorporation of hydrophilic plasticizer into myofibrillar proteins involves a large decrease (>75°C) in the T_g value. The depressive effect of hydrophilic plasticizer incorporation on T_g is typical for thermoplastic hydrophilic biopolymers. For instance, Kalichevski and co-workers[23,37] have observed a 40°C decrease in T_g for wheat gluten proteins, caseins or amylopectin, when 33 g sucrose/100 g dry matter were added.

Effects of water content

The effects of water content on the thermomechanical properties plasticized (with sucrose or sorbitol) myofibrillar proteins are illustrated in *Figures 4* and *5*. Nearly similar scans were previously observed with unplasticized myofibrillar proteins (*Figure 1*) and with sucrose and sorbitol plasticized materials: irrespective of water content, drastic changes in dynamic mechanical properties characterize the glass transition of hydrated myofibrillar proteins. Increasing water content involves decreases in glass and rubbery storage modulus and in height of loss modulus and tan δ peaks. These variations express a decrease in molecular interaction density for the myofibrillar protein network due to the replacement of protein–protein bonds by protein–water bonds.

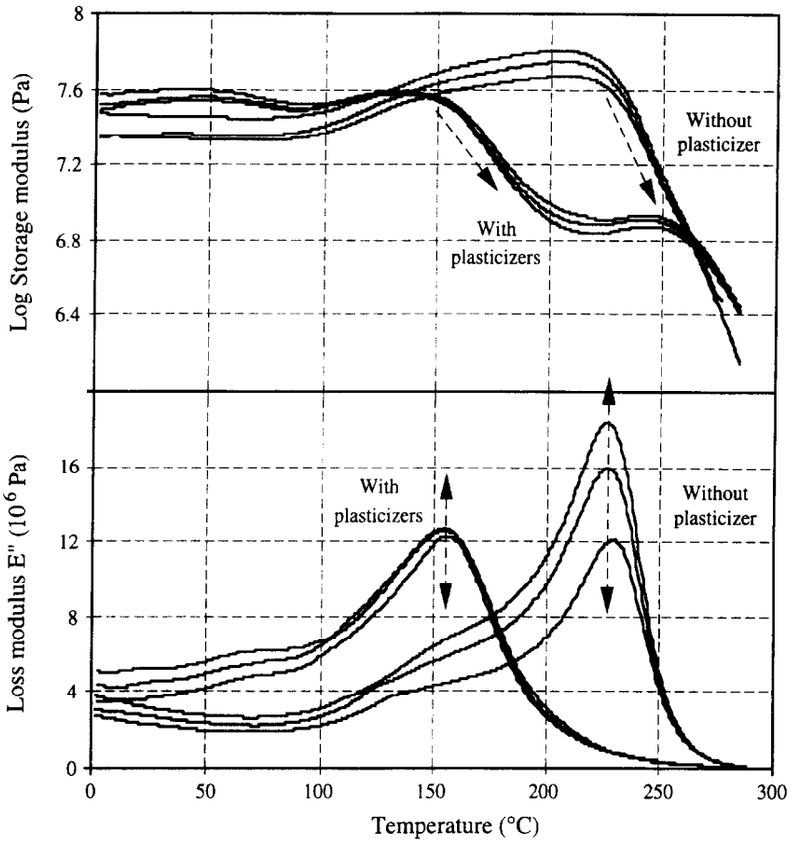

Figure 2 Thermomechanical scans (storage modulus and loss modulus) for unplasticized or plasticized (equal mixture of sucrose and sorbitol at 35 g/100 g dry matter) myofibrillar proteins (at 2.2 g water/100 g dry matter)

It is interesting to note (*Figures 4* and *5*), that these moist samples were sensitive to drying above 100°C after the glass transition of hydrated materials. Whatever the initial water content, dehydration of samples above 100°C allowed us to observe the glass transition of dry myofibrillar proteins between 130 and 180°C. Moreover, incorporation of water involved broadening of the glass transition range, and more particularly for unplasticized proteins. The glass transition breadth for the most hydrated myofibrillar proteins (22.2 g water/100 g dry matter) was found to be close to 60°C.

As previously observed for myofibrillar protein-based films[20], the depressive effect of water on the T_g for myofibrillar proteins (*Figure 6*) is very important at low water contents (below 10–15% water). For higher water contents, the T_g seems to be stabilized between 25 and 60°C. The T_g decrease as a function of water content has been described with non-linear relationships. The thermodynamic theory of glass transition was tested to describe the plasticizing effect of water on myofibrillar proteins. The Couchman–Karasz (CK) equation[42], which relates the T_g of homogeneous blends of several elements to their fractional concentrations and the thermodynamic characteristics of the 'pure' compounds (i.e. T_g and specific heat of 'pure' compounds), was then tested.

The unplasticized myofibrillar proteins were considered as a binary system constituted by proteins and water. The CK equation applied to binary systems is as follows:

$$T_g = \frac{w_1 \Delta C_{p1} T_{g1} + w_2 \Delta C_{p2} T_{g2}}{w_1 \Delta C_{p1} + w_2 \Delta C_{p2}} \quad (2)$$

where T_g is the glass transition temperature of the system, T_{gi} is the glass transition temperature of the 'i' pure compounds [proteins ($i = 1$); water ($i = 2$)], ΔC_{pi} is the specific heat of the 'i' pure compounds, w_i is the fractional weight concentration of the 'i' compounds in the system ($\sum w_i = 1$). From characteristics of 'pure' water[43] ($\Delta C_{p2} = 1.94\,J\,g^{-1}\,K^{-1}$ and $T_{g2} = 134\,K$) and from the experimental T_g value of 'pure' myofibrillar proteins ($T_{g1} = 508\,K$), it was possible to describe the plasticizing effect of water on myofibrillar proteins (*Figure 6*). The more suitable description was obtained with an optimized ΔC_{p1} value ($\Delta C_{p1} = 0.32\,J\,g^{-1}\,K^{-1}$).

For the plasticized proteins, the CK equation is given by the following equation:

$$T_g = \frac{w_1 \Delta C_{p1} T_{g1} + w_2 \Delta C_{p2} T_{g2} + w_3 \Delta C_{p3} T_{g3} + w_4 \Delta C_{p4} T_{g4}}{w_1 \Delta C_{p1} + w_2 \Delta C_{p2} + w_3 \Delta C_{p3} + w_4 \Delta C_{p4}} \quad (3)$$

[myofibrillar proteins ($i = 1$), sucrose ($i = 2$), sorbitol ($i = 3$) and water ($i = 4$)]. ΔC_{pi} and T_{gi} for sorbitol and sucrose were found in ref. 44 (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 $T_{g3} = 270\,K$). Resolving equation (3) when $w_4 = 0$ and $T_g = 432\,K$ (average experimental temperature data) allowed us to calculate the ΔC_{p1} ($\Delta C_{p1} = 0.72\,J\,g^{-1}\,K^{-1}$). The CK equation was then used to model the effect of water content on T_g for plasticized myofibrillar proteins (*Figure 6*).

It was evident that, irrespective of the ΔC_{p1} value, the thermodynamic theory allows us to describe partially

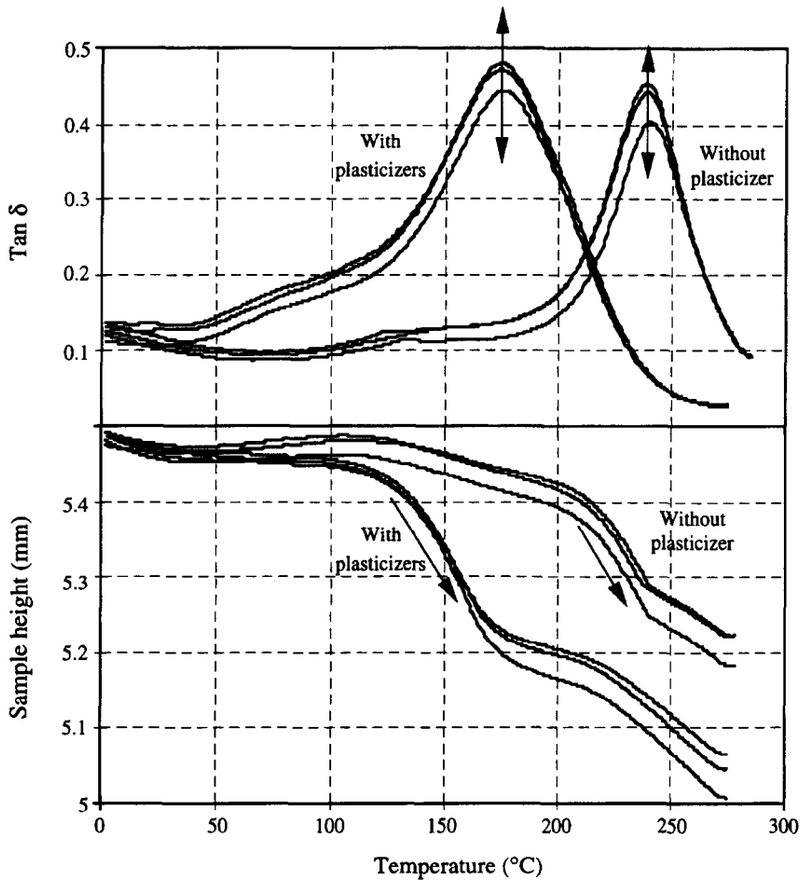

Figure 3 Thermomechanical scans (tan δ and sample height) for unplasticized or plasticized (equal mixture of sucrose and sorbitol at 35 g/100 g dry matter) myofibrillar proteins (at 2.2 g water/100 g dry matter)

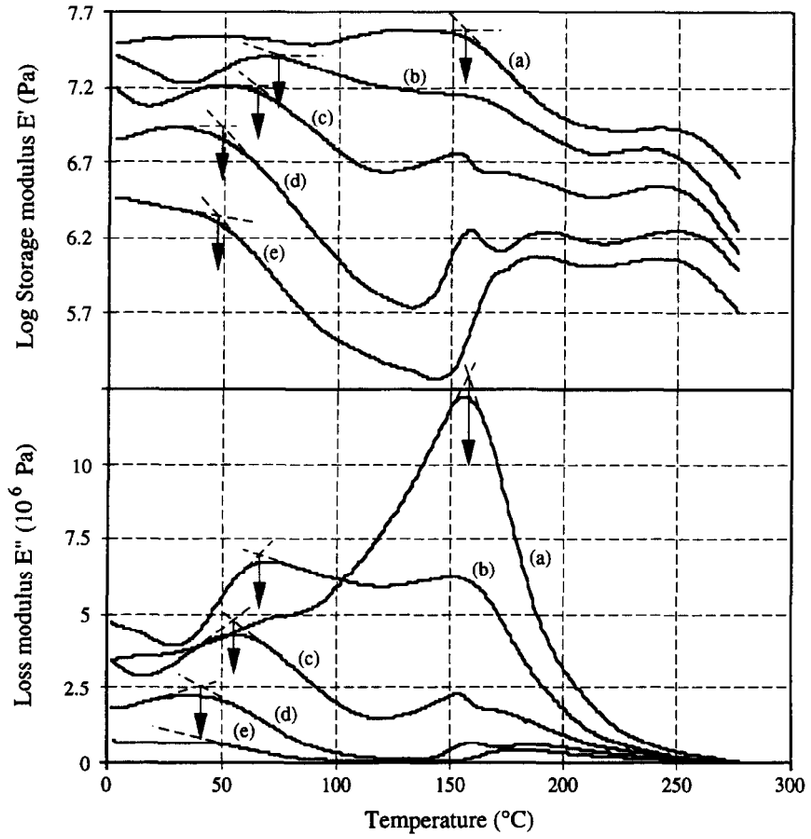

Figure 4 Thermomechanical scans (storage modulus and loss modulus) for plasticized (equal mixture of sucrose and sorbitol at 35 g/100 g dry matter) myofibrillar proteins as a function of water content: at (a) 2.2, (b) 7.2, (c) 12.2, (d) 17.2 and (e) 22.2 g water/100 g dry matter

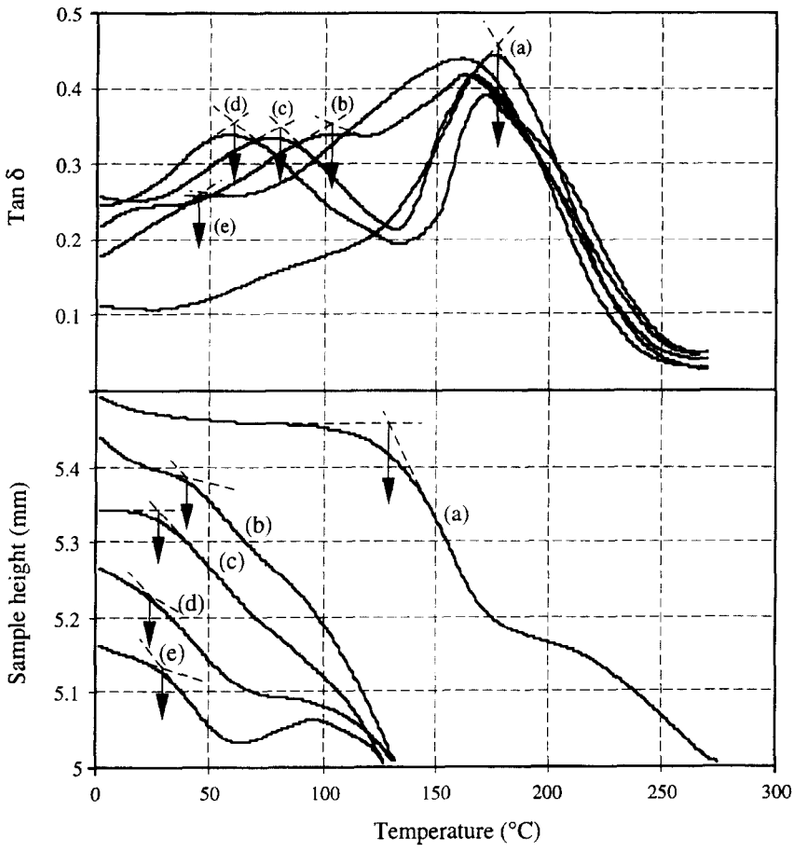

Figure 5 Thermomechanical scans (tan δ and sample height) for plasticized (equal mixture of sucrose and sorbitol at 35 g/100 g dry matter) myofibrillar proteins as a function of water content: at (a) 2.2, (b) 7.2, (c) 12.2, (d) 17.2 and (e) 22.2 g water/100 g dry matter

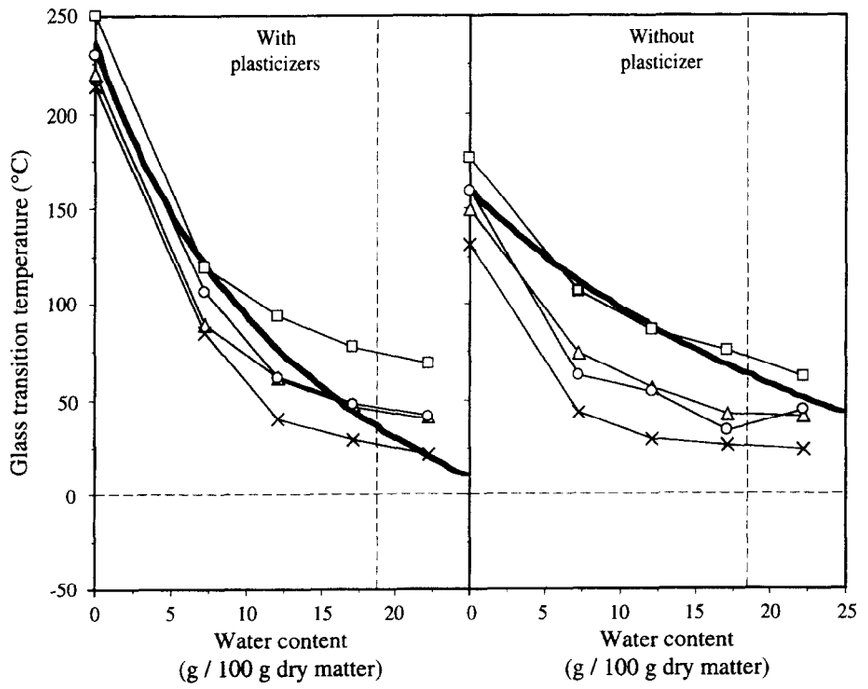

Figure 6 Effect of water content on the glass transition temperatures for unplasticized or plasticized (equal weight mixture of sucrose and sorbitol at 35 g/100 g dry matter) myofibrillar proteins, determined from the top of the tan δ (—□—) and loss modulus (—○—) peaks, and the onset in storage modulus (—△—) and sample height (—×—) drops. The theoretical curves (——) were calculated from the Couchman–Karasz equation

Table 2 Main characteristic of biopackagings based on plasticized myofibrillar proteins and of various biopackagings (at 25°C and 57.7% relative humidity)

	Specific volume ($ml\,g^{-1}$)	Maximum force (N)	Elastic modulus ($10^{-3}\,N\,m^{-1}$)
Myofibrillar protein			
—glassy structure[a]	1.0 (±0.1)	170 (±10)	70 (±6)
—foamed structure[b]	2.6 (±0.4)	60 (±7)	20 (±3)
Foamed starch[c]	9.0 (±0.1)	47 (±5)	4.6 (±0.4)
Foamed polystyrene	13.5 (±0.1)	23 (±1)	3.6 (±0.3)

[a] Formed at 150°C from myofibrillar proteins at 2.2 g water/100 g dry matter
[b] Formed at 200°C from myofibrillar proteins at 22.2 g water/100 g dry matter
[c] Formed at 175°C from pregelatinized cassava starch at 13 g water/100 g dry matter

the plasticizing effect of water on the myofibrillar proteins (*Figure 6*). The calculated T_g values are slightly overestimated when water contents are below 15–20%. For higher water contents, the decrease in calculated T_g seems to be more important than the observed experimental variations, especially with plasticized proteins. The goodness of fit of the CK thermodynamic theory applied to describe the plasticizing effect of water on myofibrillar proteins is nearly the same as those previously presented and discussed for the myofibrillar proteins-based films[20]. Differences between experimental data and thermodynamic theory were then associated with the fact that hydrated myofibrillar proteins could be considered as heterogeneous systems as high water contents.

Application to biopackaging fabrication

Unplasticized and plasticized myofibrillar proteins were used as raw materials for biopackaging fabrication by a thermomoulding process. Experiments were carried out at 100, 150, 200 or 250°C with proteins at 2.2, 12.2 or 22.2% water.

Above 250°C, biopackaging fabrication was impossible due to thermal degradation of myofibrillar proteins. Forming biopackagings was also impossible at 100 and 150°C from unplasticized proteins (at 2.2% water) and at 100°C from plasticized proteins (at 2.2% water); the thermomoulded product was still a powder under these conditions.

Under any other experimental conditions, the thermomoulding process allowed fabrication of homogeneous biopackagings from myofibrillar proteins. In relation to the previously determined T_g values (*Figure 6*), it appears that thermomoulding of myofibrillar proteins was only possible when the process temperature was higher than the maximum T_g value at a given moisture content. The d.m.t.a. results were thus useful to predict temperature and water content conditions required for biopackaging fabrication from myofibrillar proteins by a thermomoulding process.

As expected and according to thermoplastic extrusion of starch-based materials[45–47], the structure of myofibrillar protein-based biopackagings depended on processing conditions. For instance, biopackagings formed at 150°C from proteins at 2.2% water were characterized by a specific structure like translucent rigid glass; when biopackagings formed at 200°C from proteins at 22.2% water, they were characterized by a foamed rigid structure like foamed polystyrene. The main functional properties of these biopackagings were compared with those of starch or polystyrene foamed materials (*Table 2*). The myofibrillar protein biopackagings were characterized by lower specific volume due to a lower expansion rate during the thermomoulding process. These low specific volumes were then responsible for the higher mechanical resistance and rigidity in comparison with materials based on starch or polystyrene.

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