

Thermophysical properties of chitosan, chitosan–starch and chitosan–pullulan films near the glass transition

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Abstract

The thermo-mechanical properties of aqueous solution-casted films of chitosan (C), starch–chitosan (SC) and pullulan–chitosan (PC) blends were examined by Dynamic Mechanical Thermal Analysis (DMTA) and large deformation tensile testing. Incorporation of sorbitol (10 and 30% d.b.) and/or adsorption of moisture by the films resulted in substantial depression of the glass transition (T_g) of the polysaccharide matrix due to plasticization. For the composite films there was no clear evidence of separate phase transitions of the individual polymeric constituents or a separate polyol phase; a rather broad but single drop of elastic modulus, E' , and a single peak $\tan\delta$ were observed. The relationship between the T_g and moisture for all films could be modeled with the empirical Gordon–Taylor equation. Apparent activation (E_a) energies for the α -relaxation process, estimated from multifrequency DMTA measurements, were within 225–544 kJ mol⁻¹ depending on film composition; the E_a and ‘fragility’ parameters decreased with increasing moisture content. Analysis of viscoelasticity data using the time–temperature superposition (TTS) principle with the Williams–Landel–Ferry (WLF) equation was successful, provided that the coefficients C_1 and C_2 are optimized and not allowed to assume their ‘universal’ values. Tensile testing of films adjusted at various levels of moisture indicated large drops in Young’s modulus and tensile strength (σ_{\max}) with increasing level of polyol and moisture; the sensitivity of the films to plasticization was in the order of SC > PC > C. Modeling of the modulus data with the Fermi’s equation allowed comparison among samples for the fall in modulus around the glass transition zone as a function of moisture content under isothermal conditions. Relationships between σ_{\max} and water content showed an increase in stiffness of the PCs films from 7–11% moisture, and a strong softening effect at higher water contents. The observed range of σ_{\max} values (20–80 MPa) for most films is comparable to many medium-strength commercial films. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Glass transition; Calorimetry; Pullulan; Starch; Chitosan; Edible films; Mechanical; Tensile; Modulus; Activation energy

1. Introduction

Research interest in the study of thermomechanical behavior and diffusion properties of glassy biopolymers and biopolymers blends, used in foods as edible films and coatings or as bioactive ingredients, has recently increased. The importance of the glass transition temperature, T_g , in understanding the physical state and physicochemical properties of food materials has been extensively discussed by many authors (Fennema, 1996; Karel, Buera & Roos, 1993; Levine & Slade, 1988, 1992; Noel, Ring & Whittam, 1990; Roos, 1995a; Roos, Karel & Kokini, 1996; Schenz, 1995; Slade & Levine, 1991). Glass transition is a second-order phase transition (White & Cakebread, 1966) that occurs over the temperature range at which a glassy material

enters the rubbery domain with a concomitant drop ($\sim 10^3$ times) in the elastic moduli (Young’s or shear moduli). Changes in the mechanical properties of a polymer around T_g are of major practical interest as glasses have high moduli, i.e. they are solid, hard and brittle (Sperling, 1986). At temperature above T_g , various physical properties are significantly affected by a consequent exponential increase in molecular mobility and a decrease in viscosity (Roos & Karel, 1991a,b; Slade & Levine, 1991).

In an amorphous polymer, the α -relaxation, as determined by thermo-mechanical analysis, corresponds to the glass transition and reflects motions of fairly long chain segments in the amorphous domains of the polymer (long-range motions). At the rubber–glass transition the wriggling motion of long chains is frozen out, leaving the chains locked into an entangled mass and leading to limited structural reorganizations. Relaxations at lower temperatures (labeled $\beta, \gamma, \delta, \dots$ with decreasing transition temperature)

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are generally due to local movements of the main chain, or rotations and vibrations of terminal groups or other side chains (short-range motions). They are assumed to occur in regions of the material where the mobility is easier, so called 'islands of mobility' or more recently 'quasi point defects' (Simatos, Blond & Perez, 1995; Montés, Mazeau & Cavallé, 1997). The magnitude of these transitions is much smaller than the main α -relaxation (Sperling, 1986). Overall, the glassy state is frequently described as a state of relative high stability as diffusion-controlled physical and/or chemical processes cease. A small change in temperature in the vicinity of T_g could result in pronounced changes in the mechanical (relevant to the sensory attributes of food systems), thermal and dielectric properties of amorphous materials. In this respect, the glass transition temperature of food ingredients might be of importance to understand and predict many aspects of their stability and processability in model formulations or actual food systems (Simatos et al., 1995).

The mobility of food components is greatly affected by the presence of water; i.e. water is an abundant and a very effective solvent/plasticizer for hydrophilic components (Cocero & Kokini, 1991), bringing about decreasing transition temperatures with increasing amounts of water (Levine & Slade, 1986; Roos, 1987; Roos & Karel, 1991c; Slade & Levine, 1991). On a molecular level, water plasticisation of a polymer leads to increased intermolecular distances (free-volume), decreased local viscosity and increased back-bone chain segmental mobility (Slade & Levine, 1991). Moreover, the addition of low molecular weight plasticizers (e.g. polyols) to an amorphous biopolymer has the same effect as increased temperature on molecular mobility. The relationships between T_g and moisture content may be shown on simplified state diagrams which describe the physical state of food materials as a function of concentration and temperature. Since enhancement of molecular mobility and diffusion above T_g , directly affect kinetics of food deterioration events, information from state diagrams are useful to design improved products with extended shelf life (Karel et al., 1993; Roos & Karel, 1991a; Roos, 1995b,c; Roos et al., 1996; Slade & Levine, 1993).

The application of the glass transition concept can be also a valuable aid in edible films research, as T_g affects their mechanical and barrier (gas, water vapor) properties (Cherian, Gennadios, Weller & Chinachoti, 1995; Biliaderis, Lazaridou & Arvanitoyannis, 1999). The utility of edible films and coatings lies in their capacity to act as an adjunct for improving food quality, extending shelf life, and possibly improving economic efficiency of packaging materials (Kester & Fennema, 1986). Moreover, edible and biodegradable polymer films offer alternative packaging options with no contribution to the environmental pollution (Krochta & De Mulder-Johnston, 1997). The obvious appealing characteristics of edible films include the renewable nature of their ingredients, the film's ability to function as carriers of food additives (e.g. antioxidants, flavors, anti-

microbial agents), and the potential use of such films in the interior of heterogeneous food systems as selective barrier to the transport of vapors, gases, and solutes (Cherian et al., 1995). Polysaccharides (cellulose derivatives, pectin, starch, alginates, chitosan, carrageenans, pullulan etc.) are well known for having good film-forming properties. Coatings made with these biopolymers or their blends are generally considered as effective gas barriers. In contrast, minimal moisture barrier properties can be expected, because of the hydrophilic nature of these materials (Kester & Fennema, 1986). The addition of plasticising agents (e.g. polyols) to edible films (added at concentrations ranging from 10 to 60 g/100 g dry matter depending upon polymer rigidity) is often required to overcome film brittleness caused by extensive intermolecular forces; the plasticizer must be compatible with the polymer matrix. Plasticizers reduce these forces and increase the mobility of polymer chains, thereby improving flexibility and extensibility of the film. This avoids chipping or cracking of the film during subsequent handling and storage (Banker, 1966).

Chitosan is the deacetylated product of chitin, which is the second most abundant naturally occurring biopolymer (after cellulose) and is found in the exoskeleton of crustaceans, in fungal cell walls and in other biological materials. It is a cationic heteropolysaccharide composed mainly of β -(1,4)-2-deoxy-2-amino-D-glucopyranose units, and partially of β -(1,4)-2-deoxy-2-acetamido-D-glucopyranose. Chitosan possess unique functional, nutritional, and biomedical properties and its present and potential uses range from dietary fiber to functional ingredient and processing aid; some of the well known applications of chitosan include its use for prevention of water pollution, medicine against hypertension, antimicrobial and hypocholesterolemic activity, flavor encapsulation, immobilization of whole cells or enzymes, recovery and removal of undesirable compounds from food and food processing wastes, seed coatings, film-forming and controlled release of food ingredients, nutrients and drugs (Dunn, Grandmaison & Goosen, 1997; Knorr, 1984; Muzzarelli, 1985, 1996; Muzzarelli & De Vincenzi, 1997; Onishi, Nagai & Machida, 1997; Sandford & Hutchings, 1987; Shahidi, Arachchi & Jeon, 1999; Struszczyk & Pospieszny, 1997; Winterowd & Sandford, 1995). The low cost and the multiple functional properties of chitosan are the main driving forces pushing studies on new applications of this biopolymer. Numerous publications have reported on studies of films made of chitosan (Bégin & Van Calsteren, 1999; Butler, Vergano, Testin, Bunn & Wiles, 1996; Chen & Hwa, 1996; Singh & Ray, 1998), and chitosan blends with natural (Arvanitoyannis, Nakayama & Aiba, 1998a; Hasegawa, Isogai, Onabe, Usuda & Atalla, 1992; Hosokawa, Nishiyama, Yoshihara & Kubo, 1990; Isogai & Atalla, 1992) or synthetic polymers (Arvanitoyannis, Kolokuris, Nakayama, Yamamoto & Aiba, 1997; Guan, Liu, Fu, Li & Yao, 1998; Ratto, Chen & Blumstein, 1996). Moreover, several researchers coated fruits and vegetables with chitosan films and determined

Table 1
Polysaccharide (% dry matter) film composition

Sample	Composition			
	Chitosan	Gelatinized corn starch	Pullulan	Sorbitol
C	100	–	–	–
Cs10	90	–	–	10
Cs30	70	–	–	30
SC	50	50	–	–
SCs10	45	45	–	10
SCs30	35	35	–	30
PC	50	–	50	–
PCs10	45	–	45	10
PCs30	35	–	35	30

their effects on gas permeability, internal gas composition, and shelf-life of these fresh commodities. These studies indicated that chitosan coatings have the potential to prolong storage life of fresh strawberries (Ghaouth, Arul, Ponnampalam & Boulet, 1991), peach, Japanese pear, kiwi-fruit (Du, Gemma & Iwahori, 1997), and tomatoes (Ghaouth, Ponnampalam, Castaigne & Arul, 1992), as they inhibited fungi growth, reduced ethylene production, increased internal CO₂ and decreased O₂ levels. The findings of Makino and Hirata (1997) supported the applicability of a biodegradable laminate composed of a chitosan–cellulose–polycaprolactone as a packing material of head lettuce, cut broccoli, whole broccoli, tomatoes, and sweet corn.

Besides the above discussed applications of chitosan in the food industry, the use of starch (either as native or chemically modified) and pullulan (a microbial polysaccharide) as coating and film forming materials has been long recognized (Whistler, BeMiller & Paschall, 1984; Wurzburg, 1986; Yuen, 1974; Biliaderis et al., 1999). The objective of this work was to study the thermal and mechanical properties of chitosan, starch–chitosan, and pullulan–chitosan based films, plasticized with water and sorbitol and prepared by casting and evaporation of aqueous solutions of these biopolymers.

2. Experimental

2.1. Materials and sample preparation

Native corn starch granules (amylose content 26%) were rendered amorphous by gelatinizing ~30% w/w starch slurries in a roller drier (one process) at 140°C (roller–drier speed 10 rpm; pressure 32 psi–221 kPa; gap 1 mm; diameter 165 mm; width 150 mm). Pullulan was a food grade preparation from Hayashibara Biochemical Laboratory, (Okayama, Japan) and chitosan was obtained from Seikagaku Corporation (Tokyo, Japan). Sorbitol (analytically pure) was obtained from Sigma Chemical (St. Louis, MO, USA). Inorganic salts (reagent grade) used for adjusting the relative humidity were from Merck KGaA (Darmstadt, Germany).

The chitosan solutions were prepared by dissolving chitosan (1% w/v) in water and adjusting pH to 4.0 with a mixture of water: concentrated acetic acid of 4:1 (v/v). Mixed biopolymer solutions were made by mixing 1% (w/v) chitosan with 2% (w/v) starch or 4% (w/v) pullulan solutions at 40°C. The polyol-plasticized samples were prepared by adding sorbitol to the polysaccharide solutions and mixing until its dispersion. The solutions were then filtered to remove any unsolubilized material and casted on plastic frames (10 × 10 × 1.5 cm³). They were subsequently stored at room temperature for several days allowing them to dry. The preparation of films (with thickness of 0.1 ± 0.05 mm) for large deformation mechanical tests (tensile tests) was carried out by single casting and evaporation of solutions, while the preparation of thicker samples (2.3 ± 0.5 mm), suitable for small deformation mechanical tests, by multiple casting and repeated evaporation of solutions in suitable plastic frames. The compositions of different samples (% of dry matter) along with their symbols are given in Table 1.

Hydration of the biopolymer films to various moisture contents was brought about by either conditioning in different relative humidity chambers (saturated salt solutions) or exposing the samples to 100% relative humidity for different periods of time. The moisture content of samples used for this study was determined by drying at 130°C for 1 h.

2.2. Small deformation mechanical tests

Dynamic mechanical thermal analysis (DMTA) was performed on thick specimens (~3.8 × 5.0 × 3.3 mm), conditioned to various levels of moisture content. A Mark III analyzer (Polymer Laboratory, Loughborough, UK) was used in the single cantilever bending mode, with a standard heating rate of 2°C min⁻¹, at frequencies of 1, 3 and 10 Hz and a strain × 2, corresponding to a maximum displacement (amplitude) of 32 μm.

For constructing the state diagrams, the T_g (or T_α) was determined as the peak in $\tan\delta$ ($\tan\delta = E''/E'$, where E'' and E' are the loss and storage modulus, respectively), derived from DMTA measurements at 1 Hz frequency. Data analysis to fit the experimental values of T_g to the empirical Gordon–Taylor (G–T) equation (Gordon & Taylor, 1952) was performed using the Table Curve™ software (Jandel Scientific) and a non-linear least-square curve fitting routine

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2}$$

where w_1 and w_2 are the respective weight fractions of the polymer blend and water, T_{g1} is the T_g of the composite polymer matrix, T_{g2} is the T_g of amorphous water and k is a constant related to the strength of polymer–diluent interaction. The constructed G–T plots were based on the best data fitting to the equation (i.e. optimization for both parameters, k and T_{g1}) of data derived from the DMTA traces (i.e. the peak $\tan\delta$ values were used as T_g); a T_g of –138°C was used for water (Sugisaki, Suga & Seki, 1968).

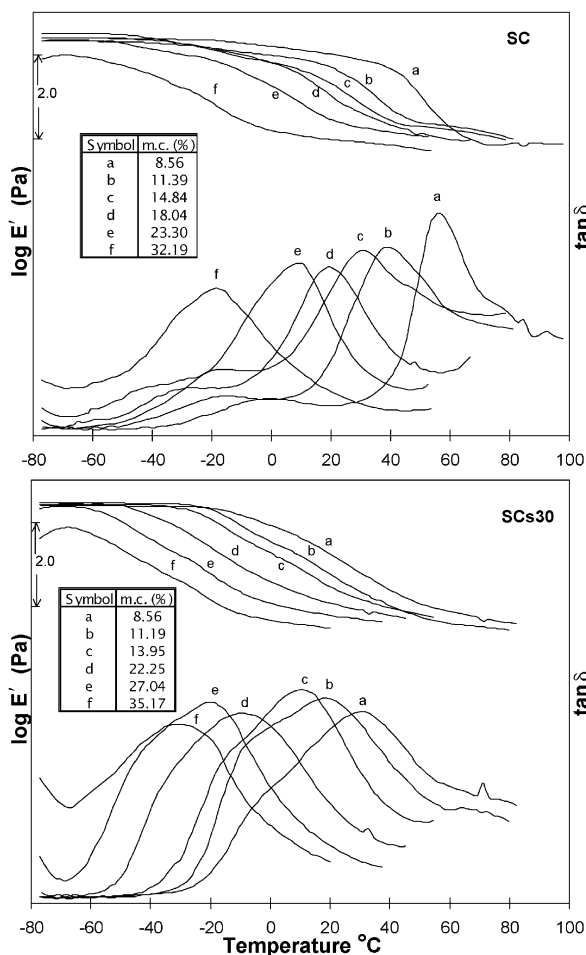


Fig. 1. DMTA $\log E'$ and $\tan \delta$ for starch/chitosan (SC) and starch/chitosan/sorbitol (SCs30) blends at different moisture content; single cantilever bending mode, heating rate 2°C min^{-1} , frequency 1 Hz.

For some specimens, DMTA measurements at a variety of frequencies (0.3, 1, 2, 3, 5, 10, 20, 30 and 50 Hz) were performed at several temperature steps (isothermally using increments of 5°C between 30 and 80°C). The data collected from these tests were used to generate master curves by employing the time/superposition principle (Ferry, 1980).

2.3. Large deformation mechanical tests

Films were cut with a scalpel or scissors into strips ($\sim 3.3 \times 0.7 \times 0.1$ mm) and after conditioning to various moisture contents they were analyzed with a TA-XT2i instrument (Stable Micro systems, Godalming, Surrey, UK) in the tensile mode operated according to ASTM D828-88 (ASTM, 1989) at 25°C . Cross head speed was set at 50 mm min^{-1} . The thickness of each sample was measured at three different points with a micrometer and an average value was obtained. Measurements calculations of tensile modulus (E), tensile strength (σ_{max}) and percentage elongation were made as described elsewhere (Arvanitoyannis & Psomiadou, 1994). The Young's modulus (E)

was calculated from the initial slope of the stress–strain curves, while σ_{max} and (%) elongation correspond to the tensile strength and percentage elongation at break, respectively. Each of the reported moduli and σ_{max} values represent an average of at least eight samples similarly conditioned to a certain moisture level.

For the tensile modulus values, the following Fermi's empirical model (Peleg, 1996a; Harris & Peleg, 1996) was applied to fit the sigmoidal curves of E vs. moisture content (m)

$$E(m) = (E_0 - E_r) / \{1 + \exp[(m - m_c)/b]\} + E_r$$

where $E(m)$ is the magnitude of modulus, m the moisture content of the sample, E_0 the initial modulus (dry or glassy state), m_c the characteristic water content where $E(m) = E_0/2$; b is a constant describing the steepness of the modulus drop around the m_c ; and E_r is the residual magnitude of modulus after the transition.

3. Results and discussion

3.1. Thermomechanical properties

The thermomechanical behavior of chitosan and its blends with starch or pullulan, either alone or with sorbitol (plasticized at a 30% d.b. level) in the glass transition zone was examined by DMTA. The effect of water on DMTA traces of two representative samples (SC and SCs30) is demonstrated in Fig. 1. A large drop in storage modulus E' and a peak in $\tan \delta$ are used as indicators of the glass transition (also denoted as α -relaxation); the $\tan \delta$ peak is normally found at a temperature higher than the onset or the midpoint temperature of the modulus (E') drop. The location of glass transition shifted to lower temperatures with increase of water content or addition of sorbitol due to plasticisation of the polymer matrix.

The measurement of the glass transition temperature of a polymer mixture is often claimed as a criterion to establish its miscibility. For all the water- and sorbitol-plasticized SC and PC blends, one-step drop in elastic modulus (E') at T_g ($\sim 10^{2.0} - 10^{2.5}$ Pa) as well as a single peak in $\tan \delta$ was observed in the DMTA traces (Fig. 1); i.e. there was no evidence for separate phase transitions of the two polymeric components in the blends. However, this behavior does not necessarily imply miscibility between starch and chitosan or between pullulan and chitosan at a molecular or segmental level. In fact, some preliminary FTIR microspectroscopic imaging of thin chitosan–pullulan films (1:1) (carried out by Farhat and his colleagues at the University of Nottingham) have clearly indicated that there is a microphase separation between the two polymers in the blend; i.e. distinct domains ($\sim 30 \times 70 \mu\text{m}$) enriched in one or the other polysaccharide appeared throughout the composite film matrix. Nevertheless, the manifestation of a single glass transition in the DMTA curves of the blends may be attributed to a close

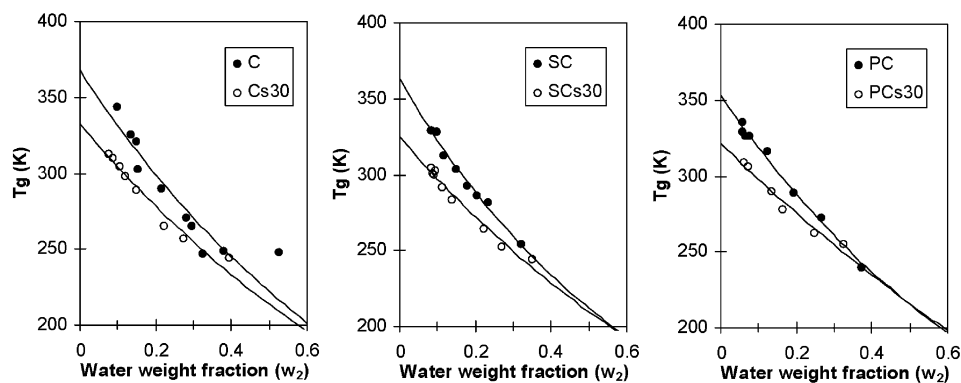


Fig. 2. State diagrams of chitosan, starch/chitosan and pullulan/chitosan blends; the solid lines give the G–T plots of experimental data (best fit of DMTA peak $\tan\delta$ values at 1 Hz).

proximity of the T_g s of the individual polymeric components and a similar plasticization behavior as previously reported for other biopolymer blends, such as sodium caseinate-soluble starch (Arvanitoyannis & Biliaderis, 1998), methyl cellulose-soluble starch (Arvanitoyannis & Biliaderis, 1999) and pullulan–starch (Biliaderis et al., 1999). For a series of polysaccharides, widely differing in their molecular structure, branching, and conformation of glycosidic linkages, Bizot, Le Bail, Leroux, Davy, Roger, and Buleon (1997) have shown that their glass transition temperature varies only by 5–20°C over a moisture content range of 5–25% w/w. Moreover, these polymers exhibited parallel trends in the T_g — moisture content plots, indicative of similar plasticisation responses. In a previous study on gelatin–chitosan blends (Arvanitoyannis et al., 1998a) a single glass transition has been revealed by DSC (differential scanning calorimetry) and DMTA tests. A single α -relaxation was also found for white bread (a composite matrix of starch and gluten) by mechanical spectroscopy (Roudaut, 1998), clearly indicating the difficulty of separating individual glass transition events of individual polymeric constituents in composite food products by thermomechanical measurements. Similarly, Kalichevsky and Blanshard (1992) have observed only one glass transition for amylopectin–casein blends, whereas for gluten–amylopectin and gluten–casein mixtures two separate glass transitions of the two polymers were seen in the DSC traces; for the latter, substantial differences in chemical structure and hydrophobicity between gluten and amylopectin (or casein) could account for the observed difference in the T_g s (~20–50°C) of these materials, making detectable the glass transition of each of the two polymers separately.

With increasing water content an increased breadth of the transition (drop in elastic modulus and peak of $\tan\delta$) was observed (Fig. 1), implying a broader distribution of relaxation times. For the polyol-plasticized polysaccharides (Cs, SCs and PCs), even at the high level of sorbitol (30% d.b.) used in the composite films, there was no evidence for two individual α -relaxations that could be assigned to the polyol

and polymer components. Also, previous studies on sugar–polymer blends did not reveal a separate transition for the polyol component in the blend up to a level of 20% d.b. These studies were on blends of sugars with amylopectin (Kalichevsky & Blanshard, 1993), hydroxypropyl starch–gelatin (Arvanitoyannis, Nakayama & Aiba, 1998b), starch–pullulan (Biliaderis et al., 1999), chitosan–poly(vinyl alcohol) (Arvanitoyannis et al., 1997), chitosan–gelatin (Arvanitoyannis et al., 1998a). It was only at high sugar contents, i.e. a sugar/polymer (amylopectin, gluten, caseinate) ratio of 1:2, where two transitions (from the polymer-rich and polyol-rich phases) was observed, implying a phase separation between the polymer and the plasticizer (Kalichevsky & Blanshard, 1993; Kalichevsky, Jaroszkiwicz & Blanshard, 1992a; Kalichevsky, Blanshard & Tokarczuk, 1993a). However, the addition of sorbitol to chitosan and chitosan blends with pullulan or starch, as shown in Fig. 1, made broader the α -transition, which is in agreement with previous findings. Kalichevsky and co workers (Kalichevsky et al., 1992a; Kalichevsky, Jaroszkiwicz & Blanshard, 1993b) also reported that sugar broadens the transition of gluten and amylopectin, probably due to sample microheterogeneity. Georget and Smith (1995) ascribed the increased breadth of the $\tan\delta$ peak for wheat-flakes (a composite material of flaked wheat, malt, sodium chloride, sucrose and water) to the heterogeneity of the mixture and the immiscibility of the components.

A β -relaxation was also observed in some of the DMTA traces (e.g. $\tan\delta$ profiles of SC in Fig. 1) of water-plasticized samples. This transition appeared at sub- T_g temperatures and sifted to lower temperatures with increase in moisture content. In the past, mechanical spectroscopy and DMTA data revealed secondary relaxations for amorphous cellulose, chitosan/gelatin blends, extruded and baked low-moisture breads, that were dependent more or less on the water (and/or another plasticizer) content (Montés et al., 1997; Arvanitoyannis et al., 1998a; Le Meste, Roudaut & Davidou, 1996; Kelley, Rials & Glasser, 1987). The β -relaxation, often attributed to rotation of hydrated methylol groups or local motions of chain segments in the

Table 2

Estimated glass transition temperatures of dry chitosan, starch/chitosan and pullulan/chitosan blends using Gordon–Taylor equation (best fit)

Sample	T_{g1} (K)	k	r^2
C	367.9	1.68	0.93
Cs30	332.6	1.52	0.99
SC	363.7	1.97	0.99
SCs30	325.1	1.55	0.99
PC	353.5	1.72	0.99
PCs30	321.7	1.31	0.99

disordered regions (Bradley & Carr, 1976), seems to be an important phenomenon in food technology, because it is connected with molecular mobility at temperatures below T_g (Simatos et al., 1995). Using DMTA Ratto et al. (1996) have also reported a water-induced β -relaxation for chitosan.

Fig. 2 shows the composition dependence of T_g (T_α) of chitosan and its blends with starch and pullulan. The state diagrams presenting experimental data from DMTA (peak $\tan\delta$, frequency 1 Hz) demonstrate the plasticising effect of water and sorbitol on the samples. It was observed that the plasticising action of sorbitol is greater at lower levels of water content; instead, at high water contents (above $\sim 30\%$ w/w) the sorbitol-plasticized samples seemed to have T_g values close to those of samples without polyol and of equivalent water content. This is in agreement with the findings of Kalichevsky and Blanshard (1993) on plasticization effects of fructose and water on amylopectin. A stronger effect of fructose on the T_g of amylopectin at lower water contents was attributed by these workers to the greater volume fraction of fructose in samples of reduced water content. On the other hand, because of its low molecular

weight the plasticising effect of water dominates that of fructose as the water content is increased. Modeling the T_g depression by water for all specimens, using the empirical G–T equation, has proven convenient in fitting the experimental T_g data with the moisture content. It should be noted, however, that application of this equation as well as that of Couchman and Karasz (1978) assumes component miscibility at a segmental level, a condition that is unlikely to be met with the water- and sorbitol-plasticized specimens. Moreover, with the existing limitations in sample preparation it is not feasible to obtain data for water weight fractions in the specimens between 0.4 and 1.0, thus making uncertain the interpolation of the results in this moisture content range. Despite the theoretical limitations, the G–T equation can be applied in a purely empirical sense to describe the water content dependence of T_g for composite matrices, allowing comparisons among samples. The fitting of G–T equation to the experimental T_g data is illustrated in Fig. 2, while the estimated parameters of all G–T plots, T_{g1} of dry matrices (T_{g1}) and k constants, are summarized in Table 2. Very good fits ($r^2 > 0.93$) were obtained in all cases with estimated T_{g1} and k values in the range of 368–322 K and 1.97–1.31, respectively. As it was expected, the T_{g1} of dry chitosan, chitosan/starch and chitosan/pullulan blends was higher (~ 30 – 40 K) than the T_{g1} of the corresponding specimens with sorbitol. Moreover, dry chitosan/pullulan blends exhibited lower T_g s, compared to the respective dry chitosan/starch blends, whereas dry chitosan samples exhibited the highest T_g s. Among the various polysaccharide film compositions tested by DMTA, the chitosan preparations seemed to be the least sensitive to water plasticization. Bizot et al. (1997) have found the T_g of pullulan lower than starch polymers and interpreted this phenomenon in terms of internal plasticisation by α -(1–6) branching; this glycosidic linkage

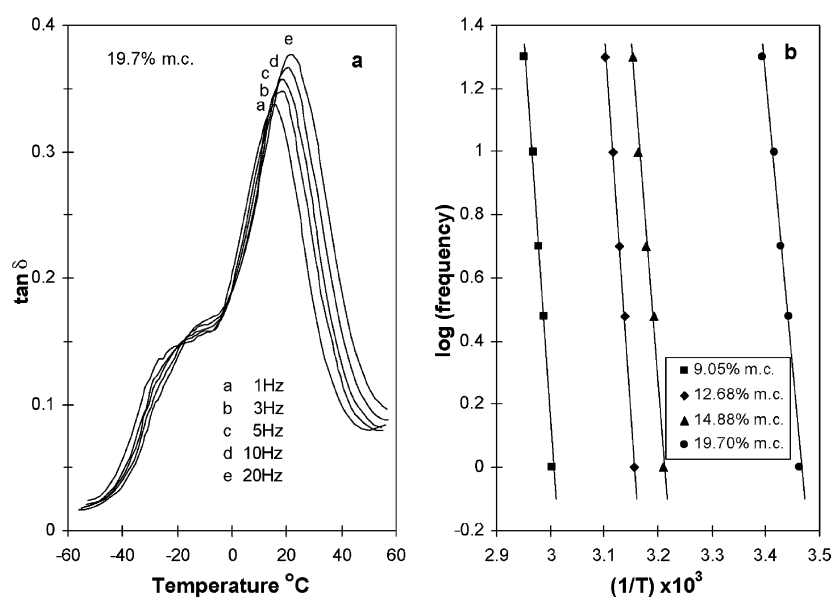


Fig. 3. Multifrequency DMTA $\tan\delta$ of thermal scans for chitosan (a) and plots of $\log f$ against reciprocal temperature for chitosan at different moisture contents to determine the activation energy of the α -relaxation process (b).

Table 3

Apparent activation energy (ΔH_a) of the α -relaxation process and “fragility” parameter (m) estimated for chitosan, starch/chitosan, and pullulan/chitosan films at different moisture levels from Arrhenius plots applied to multifrequency DMTA data

Sample	Moisture content (% w/w)	ΔH_a (kJ mol ⁻¹)	r^2	m
C	9.05	482.6	0.98	75.3
C	12.68	450.6	0.99	73.0
C	14.88	410.3	0.99	68.0
C	19.70	356.0	0.99	62.0
SC	6.97	400.3	0.95	62.5
SC	7.99	329.3	0.99	52.1
SC	14.84	259.9	0.97	44.5
PC	5.16	544.5	0.99	84.9
PC	10.05	344.1	0.99	56.5
PC	17.89	225.1	0.99	40.0

offers three rotational degrees of freedom instead of two for α -(1–4) linkages, introducing more flexibility in the polymer chain. Similar studies on pullulan and pullulan/starch blends showed lower T_g values for pullulan than pullulan/starch at equivalent water contents (Biliaderis et al., 1999).

The dynamic mechanical $\tan\delta$ peaks shift to higher temperatures with increasing frequency (Fig. 3(a)) as expected for any thermally activated relaxation process. This sensitivity of the α -relaxation process to frequency permits its discrimination from other structural changes such as crystallization or melting. The apparent activation energy (ΔH_a) for α -relaxation can be thus determined from the Arrhenius rate relationship

$$\ln f = -(\Delta H_a/RT)$$

Fig. 3(b) shows the variation of T_g (peak $\tan\delta$) as a function of the frequency of mechanical oscillation (1–20 Hz) for chitosan films at different moisture levels. The regression coefficients (r^2) of Arrhenius plots and the calculated activation energies (ΔH_a) for chitosan, starch/chitosan and pullulan/chitosan blends at different moisture levels are given in Table 3. The activation energies of the examined samples varied within the range 225 and 545 kJ mol⁻¹. These values are close to those reported for synthetic polymers in the absence of plasticizer (351 kJ mol⁻¹) (Sperling, 1986) and amorphous wood components (\sim 340 kJ mol⁻¹ at 15% moisture) (Kelley et al., 1987). Over similar frequency ranges, for white bread samples with 0 and 5% moisture, ΔH_a values of 424 and 370 kJ mol⁻¹, respectively, were reported (Le Meste et al., 1996). Moreover, for amylopectin with 10% moisture (Kalichevsky, Jaroszkiwicz, Ablett, Blanshard & Lillford, 1992b) and gluten with 12.9% moisture (Kalichevsky et al., 1992a) apparent activation energy estimates of 300 kJ mol⁻¹ and 242 kJ mol⁻¹, respectively, were shown. Recently, values of 296 and 226 kJ mol⁻¹ for pullulan/corn starch blends plasticized (at a 20% d.b. level) with sorbitol (8.8% moisture) and xylose (13.6% moisture), respectively, have been determined from DMTA multifrequency data (Biliaderis et al., 1999). MacInnes (1993) has

found activation energies of 200–300 kJ mol⁻¹ for glass transitions of frozen 80% (w/w) sucrose solutions and Simatos, Blond, Roudaut, Champion, Perez and Faivre (1996) have estimated a mean apparent activation energy of 253 kJ mol⁻¹ for sorbitol using mechanical spectroscopy data. The relatively high activation energy values obtained for the α -relaxation process of many biopolymer and food systems, compared to β - and γ -transitions, are indicative of large degrees changes in segmental mobility and a high degree of co-operativity. As it was expected, the ΔH_a values in Table 3 show a decrease with increasing moisture content of all samples examined.

From the apparent activation energies, the fragility parameter, m , can be calculated using the following relationship

$$m = (-\Delta H_a/2.303RT_g)$$

This parameter, introduced by Angell to simplify the applicability of the concept of ‘strong/fragile’ glass-forming liquids, is the slope of the T_g -scaled Arrhenius plot of any relaxation time (or any property, such as viscosity, which is proportional to a relaxation time) measured at T_g (Angell, Bressel, Green, Kanno, Oguni & Sare, 1994). ‘Strong’ materials are those for which the temperature coefficient for a mechanical property does not vary much with increasing temperature above T_g . In contrast, fragile materials show a strong decline in their mechanical properties above T_g and then a rapid degradation of their microstructure. Using the given equation, the derived ΔH_a values from the DMTA data and the glass transition temperatures estimated from the G–T plots, the parameter m was calculated for chitosan and starch/chitosan, pullulan/chitosan blends at different moisture levels (Table 3). The estimated values for SC at moisture levels above 8.0% and for PC at moisture levels above 10.0% are in close agreement with findings for other biopolymers: $m = 40.5 \pm 2.0$ for poly-L-asparagine (15–24% m.c.) (Angell et al., 1994); $m = 43$ for elastin (\sim 22% m.c.) (Lillie & Gosline, 1993); $m = 40$ for gluten (\sim 13% m.c.) (Kalichevsky et al., 1992a); $m = 51$ and $m = 42$ for pullulan/corn starch blends plasticized (to a 20% d.b. level) with sorbitol (\sim 9% moisture) and xylose (\sim 13.5% moisture), respectively (Biliaderis et al., 1999). Such values of m are characteristic of relative strong materials according to Angell’s classification; i.e. the materials are structurally resistant when heated above their T_g s. Fragility decreased with increasing moisture content of the biopolymers, as shown by the decreased values of m in Table 3. Angell et al. (1994) have made similar observations on solutions of hydrazine and sucrose. Moreover, it seems that the SC and PC blends for a small change in moisture content exhibit a considerable change in the fragility parameter, which might be of importance for the mechanical stability of these materials. From the definition of fragility and its characterizing parameter m (slope at T_g of the scaled Arrhenius plots), we may expect that small variation in the value of m among materials would result in large differences of their stability on storage for an ‘equivalent’ increase in temperature (T/T_g)

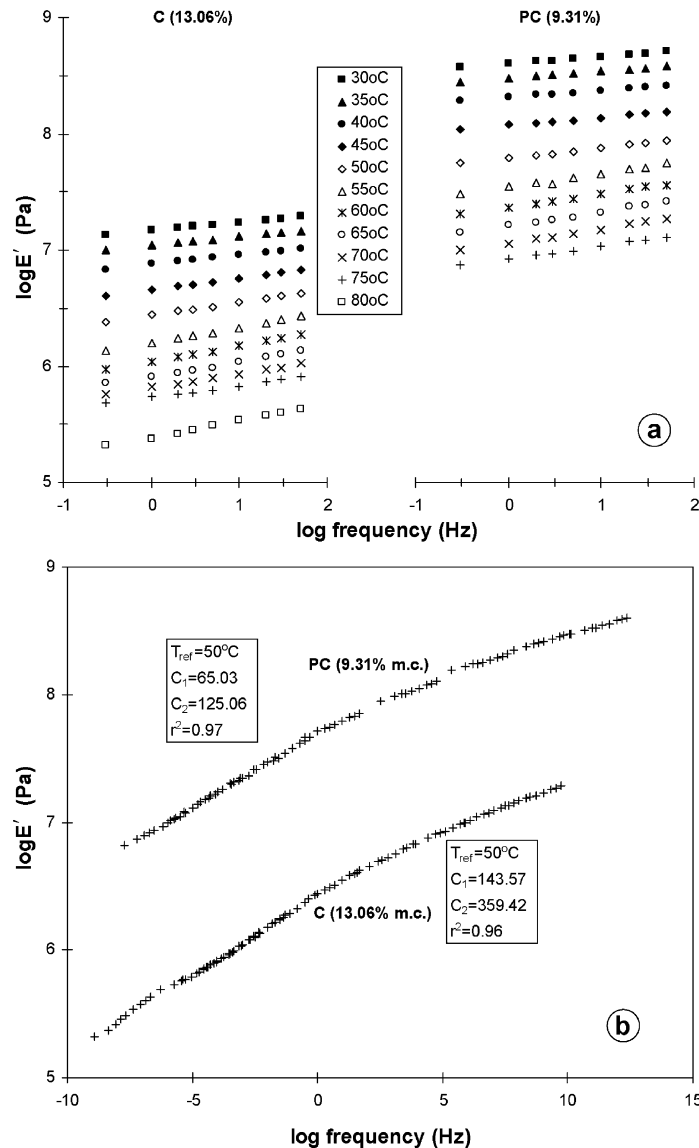


Fig. 4. Modulus-frequency curves (a) and master curves of $\log E'$ against frequency for chitosan (C) and Pullulan-chitosan (PC) blend at specified moisture contents.

(Simatos et al., 1995). However, as pointed out by LeMeste (1995) measurements of the dynamic behavior of glass-forming polymers in the glass transition zone should be complemented by diffusion data to derive at reliable relationships and predictions of molecular diffusivities from knowledge of T_g and m values.

The viscoelastic properties of polymers are dependent on temperature as well as time (Ferry, 1980). The effects of time can also be observed by varying the frequency of deformation applied to a polymer. It has been often demonstrated that viscoelastic data collected at one temperature (and multiple frequencies) can be superimposed upon data collected at a different temperature simply by shifting one of the curves along the time (or frequency) axis (Williams, Landel & Ferry, 1955; Ferry, 1980); i.e. there is equivalency between time (frequency) and temperature as they affect material properties of polymers. The TTS principle is

based on the premise that processes involved in molecular relaxations or rearrangements occur most rapidly at high temperatures. Thus the time over which these processes occur can be significantly reduced by conducting the measurements at elevated temperatures, then transposing the data to lower temperatures. In this respect, viscoelastic changes which occur relative quickly at higher temperatures can be made to appear if they have occurred at lower temperatures simply by shifting the data with respect to time. Fig 4(a) depicts the traces of storage modulus E' plotted logarithmically against the frequency at several temperature steps. In general, there seemed to be very little dependence of E' on frequency; however, the modulus values did show an increasing sensitivity to the applied frequency with increasing temperature. By selecting a reference temperature, shifting the other data with respect to time and superimposing them upon the selected reference curve,

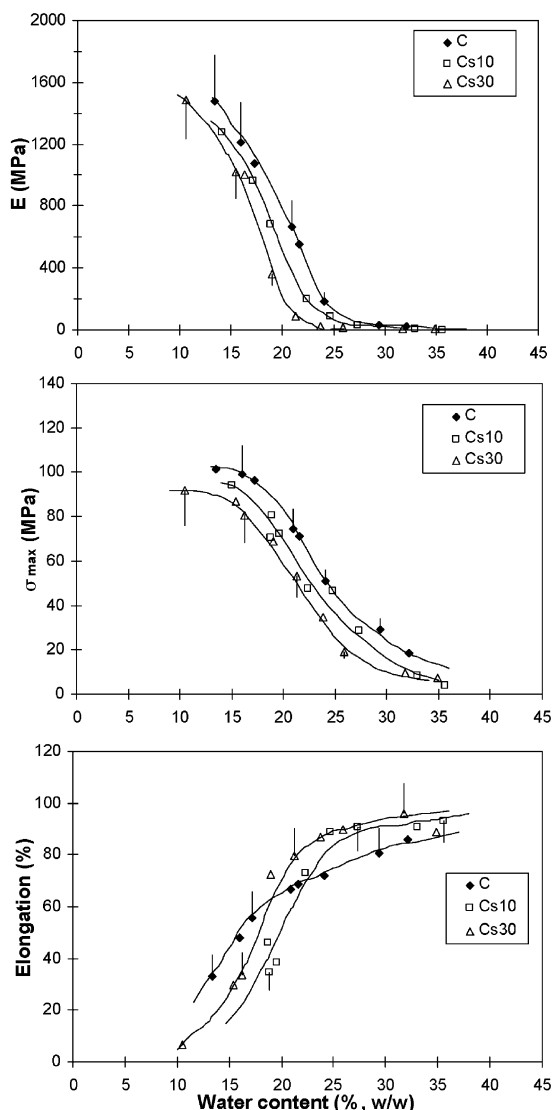


Fig. 5. Effect of water content on tensile modulus (top), tensile strength (middle) and percentage elongation (bottom) as determined from tensile tests of C, Cs10 and Cs30; points are means of at least eight measurements and half-bars show the standard deviation values.

master curves were obtained, as shown in Fig. 4(b). An important benefit of generating a master curve is the ability to extend the measurements in mechanical properties over a much broader frequency range or time scale, often inaccessible by practical experiments; i.e. data collected over ~ 2 decades of frequency can be transformed to cover 17 orders of magnitude in our case. The degree of horizontal (time) shifting required to superimpose a given set of data with respect to a reference curve can be described by the WLF equation

$$\log \alpha_T = \frac{-C_1(T - T_0)}{C_2 + (T - T_0)},$$

where α_T is the shift factor, C_1 and C_2 are constants (for many amorphous polymers they generally assume the universal values of 17.4 and 51.6, respectively), and T_0 is

a reference temperature (e.g. T_g). This equation is typically used to describe the time/temperature behavior of polymers in the glass transition region, whereas the Arrhenius model is applied to describe the viscoelastic events associated with β - or γ -relaxations. Using the superposition (WLF) software of the DMTA analyzer, master curves of $\log E'$ (dynamic elastic modulus) were generated for chitosan ($r^2 = 0.96$) and pullulan/chitosan blend ($r^2 = 0.97$) at specified moisture contents (Fig. 4(b)). In these plots the T_g was used as a reference temperature for two samples, which was $\sim 50^\circ\text{C}$ in both cases. The derived best fit values for C_1 and C_2 of the C and PC samples largely differ from the universal values of the WLF constants. Similarly, master curves have been generated for water- and polyol-plasticized pullulan/starch blends (Biliaderis et al., 1999). The TTS analysis was also applied to dimethyl sulphoxide-plasticized amylose (Nakamura & Tobolsky, 1967) and ethyl formamide-plasticized wood (Kelley et al., 1987). In all these cases the applicability of the WLF equation was feasible only over a limited range of temperatures and optimization of the C_1 and C_2 coefficients.

3.2. Tensile properties and physical state

Biodegradability and good gas barrier properties are the main features of polysaccharide-based films. However, the mechanical properties of such materials are equally important to maintain the structural integrity of a product as well as provide physical protection and controlled rates of release of additives in the food. Plasticizers, such as polyols, are often added to modify the mechanical properties of films and these may also cause significant changes in the barrier properties of the material (e.g. gas and water vapor permeabilities). Figs. 5–7 show the effect of water content on tensile modulus (top), tensile strength or stress at break (middle) and percentage elongation (bottom), determined by the tensile tests at 25°C , of the C, Cs10, Cs30 (Fig. 5), SC, SCs10, SCs30 (Fig. 6), and PC, PCs10, PCs30 (Fig. 7) films. At moisture contents less than 10–15%, films exhibited very high tensile modulus, high tensile strength, and low elongation values, at room temperature, typical of glassy materials. With increasing plasticizer content (water and/or sorbitol) there was a gradual transition from brittle to ductile failure for all films. The latter is characteristic of polymers going through their glass transition. This state transition is accompanied by sharp decreases in modulus and tensile strength, and an increase in elongation as shown in Figs. 5–7. At this point it is of interest to note that the strength of biopolymer films depends on a number of different factors; i.e. besides the nature of polymer and the plasticizer type and concentration used, crystallite formation, chemical cross-linking, orientation and ageing effects, and macrostructure are important determinants of the film strength. A comparison of the tensile data among the various film compositions examined indicated that chitosan alone was the least sensitive to plasticization effects

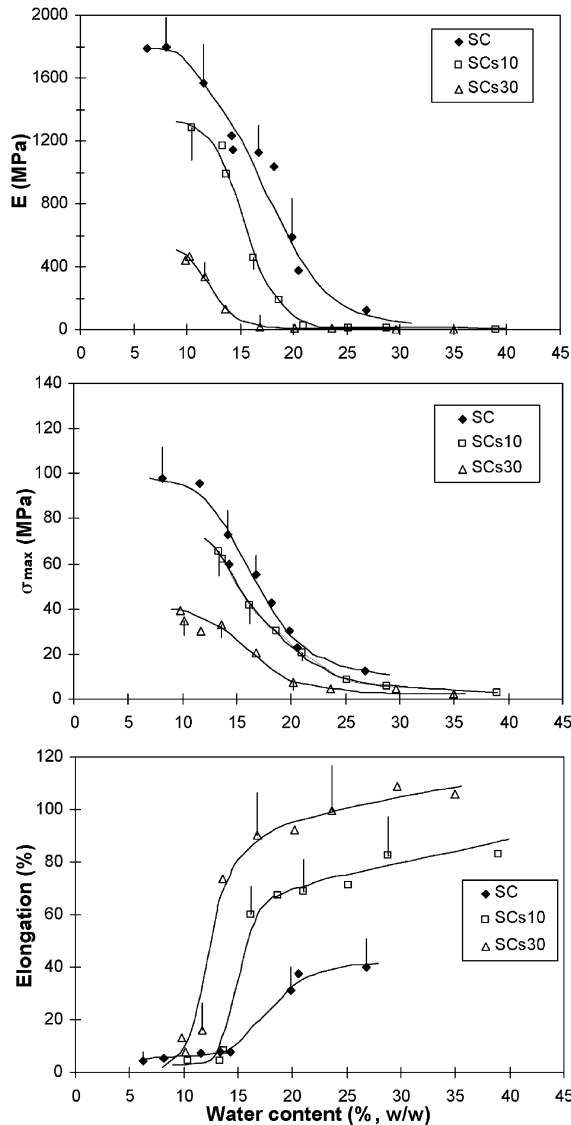


Fig. 6. Effect of water content on tensile modulus (top), tensile strength (middle) and percentage elongation (bottom) as determined from tensile tests of SC, SCs10 and SCs30; points are means of at least eight measurements and half-bars show the standard deviation values.

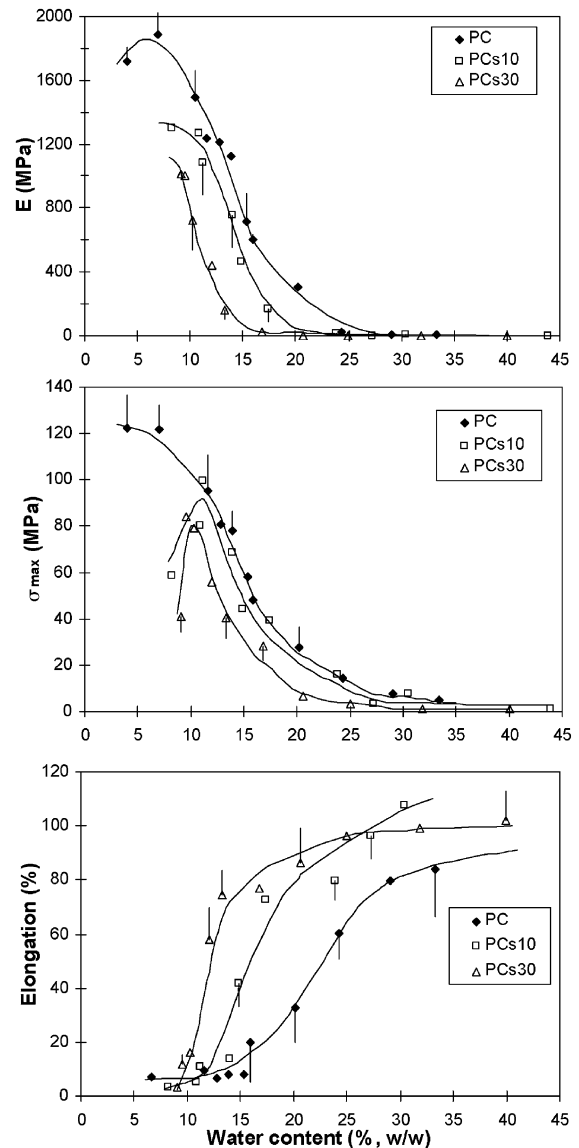


Fig. 7. Effect of water content on tensile modulus (top), tensile strength (middle) and percentage elongation (bottom) as determined from tensile tests of PC, PCs10 and PCs30; points are means of at least eight measurements and half-bars show the standard deviation values.

brought about by water and/or sorbitol; e.g. the tensile strength of chitosan films was the least affected by the addition of 10 and 30% sorbitol (d.b.) compared with the SC or PC blends. The observed range of σ_{\max} values (20–80 MPa) are similar to the tensile strength values (8–45 MPa) reported for glycerin-plastized chitosan films by Butler et al. (1996) and comparable to many medium-strength commercial films, e.g. HPDE and LDPE films (MPE, 1988).

For some composite films (PCs10 and PCs30) a bell-shape curve describing the relationship between maximum stress (σ_{\max}) and moisture was found (Fig. 7, middle). Such behavior has been previously reported for mechanical parameters of foods and their components (Attenburrow, Davies, Goodband & Ingman, 1992; Biliaderis et al., 1999; Cuq,

Gontard, Aymard & Guilbert, 1997; Fontanet, Davidou, Dacremont & Le Meste, 1997; Gontard, Guilbert & Cuq, 1993; Harris & Peleg, 1996; Nicholls, Appelqvist, Davies, Ingman & Lillford, 1995). The most salient feature of these plots is the apparent increase in stiffness as the moisture rises from 8 to 12%, whereas the softening/plasticizing effect of water becomes dominant above this level. Several suggestions have been made to explain such material toughening on partial plasticisation with water. According to Harris and Peleg (1996), glassy biopolymers at low moisture are extremely brittle and very fragile, offering no resistance to applied load. With low levels of hydration, the plasticized matrix becomes more cohesive, more structural elements remain intact (offering more resistance to fracture), and

Table 4

Parameters of the Fermi's distribution function applied to tensile modulus (E) data derived from tensile tests of water- and sorbitol-plasticized chitosan and starch/chitosan, pullulan/chitosan films

Sample	m_c	b	r^2
C	19.3	2.80	0.99
Cs10	18.6	2.10	0.99
Cs30	17.1	1.66	0.99
SC	17.6	3.29	0.94
SCs10	15.4	1.46	0.99
SCs30	12.5	1.03	0.99
PC	14.3	2.78	0.98
PCs10	14.1	1.57	0.99
PCs30	11.4	1.10	0.99

the material would deform rather than disintegrate on compression. On the basis of compression data of extruded flat bread, Fontanet et al., (1997) have ascribed the hardening phenomenon to short range reorganization of the material as a result of increased molecular mobility by adding small amounts of water; i.e. partial plasticization with water would foster aging phenomena in the glass, leading to a denser structure and thereby higher strength. These authors have also offered an alternative hypothesis, attributing material toughening to formation of micro-shear bands caused by slight increase in free-volume during compression testing. Moreover, the improvement of puncture resistance for gluten films (Gontard et al., 1993) and the increment of force at break for myofibrillar protein-based films (Cuq et al., 1997) at low moisture levels have been attributed to the formation of supplementary hydrogen bonds between the protein chains. In contrast, at higher levels of hydration water molecules interact with protein chains and probably these interactions dominate over the bonds between the polymer chains. A decreasing density of intermolecular interactions loosens the protein network and decreases its cohesiveness and elasticity.

Modeling of the modulus data with the Fermi's equation enables the comparison among samples for the fall in modulus (around the glass transition zone) as a function of moisture content under isothermal conditions (Peleg, 1996a,b). The regression parameters (m_c and b) of the Fermi's distribution function for all samples are summarized in Table 4. Very good fits ($r^2 > 0.94$) were obtained when applying this equation on the modulus data of the polysaccharide films. For each of the C, SC, and PC systems, the m_c value decreased (i.e. the fall in E at ambient temperature occurred at a lower moisture content) and the transitions became sharper (lower value of b) with increasing level of sorbitol. The former is expected due to plasticising effect of sorbitol. Similar observations on Fermi's equation parameters have been made for flexural modulus data of polyol-plasticized pullulan/starch blends (Biliaderis et al., 1999). Moreover, the fall in modulus centered at a lower m_c in the following sequence: PC, SC, C, at equivalent sorbitol level (0 or 10 or 30% d.b.). This is consistent with the small

deformation DMTA measurements. The observed changes in modulus as a function of water content in Figs. 5–7 clearly show a succession of processes and material properties around the glass transition, adding further support to the notion that this transition is not an abrupt event, particularly in the case of biopolymer mixtures and complex food systems (Harris & Peleg, 1996).

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References

- Angell, C. A., Bressel, R. D., Green, J. L., Kanno, H., Oguni, M., & Sare, E. J. (1994). *Journal of Food Engineering*, 22, 115–142.
- Arvanitoyannis, I., & Biliaderis, C. G. (1998). *Food Chemistry*, 62, 333–342.
- Arvanitoyannis, I., & Biliaderis, C. G. (1999). *Carbohydrate Polymers*, 38, 47–58.
- Arvanitoyannis, I., & Psomiadou, E. (1994). *Journal of Applied Polymer Science*, 51, 1863–1899.
- Arvanitoyannis, I., Kolokuris, I., Nakayama, A., Yamamoto, N., & Aiba, S. (1997). *Carbohydrate Polymers*, 34, 9–19.
- Arvanitoyannis, I. S., Nakayama, A., & Aiba, S. (1998a). *Carbohydrate Polymers*, 37, 371–382.
- Arvanitoyannis, I., Nakayama, A., & Aiba, S. (1998b). *Carbohydrate Polymers*, 36, 105–119.
- ASTM (1989). *Annual Book of ASTM standards*, D 828-88, American Society for Testing and Materials, Philadelphia, USA.
- Attenburrow, G. E., Davies, A. P., Goodband, R. M., & Ingman, S. I. (1992). *Journal of Cereal Science*, 16, 1–12.
- Banker, G. S. (1966). *Journal of Pharmaceutical Science*, 55, 81–89.
- Bégin, A., & Van Calsteren, M. R. (1999). *International Journal Biological Macromolecules*, 26, 63–67.
- Biliaderis, C. G., Lazaridou, A., & Arvanitoyannis, I. (1999). *Carbohydrate Polymers*, 40, 29–47.
- Bizot, H., Le Bail, P., Leroux, B., Davy, J., Roger, P., & Buleon, A. (1997). *Carbohydrate Polymers*, 32, 33–50.
- Bradley, S. A., & Carr, S. H. (1976). *Journal of Polymer Science*, 14, 111–124.
- Butler, B. L., Vergano, P. J., Testin, R. F., Bunn, J. M., & Wiles, J. L. (1996). *Journal of Food Science*, 61, 953–955 See also page 961.
- Chen, R. H., & Hwa, H. D. (1996). *Carbohydrate Polymers*, 29, 353–358.
- Cherian, G., Gennadios, A., Weller, C., & Chinachoti, P. (1995). *Cereal Chemistry*, 72, 1–6.
- Cocero, A. M., & Kokini, J. L. (1991). *Journal of Rheology*, 35, 257–270.
- Couchman, P. R., & Karasz, F. E. (1978). *Macromolecules*, 11, 117–119.
- Cuq, B., Gontard, N., Aymard, C., & Guilbert, S. (1997). *Polymer Gels and Networks*, 5, 1–15.
- Du, J., Gemma, H., & Iwahori, S. (1997). *Journal of the Japanese Society of Horticultural Science*, 66, 15–22.
- Dunn, E. T., Grandmaison, E. W., & Goosen, M. F. A. (1997). In M. F. A. Goosen, *Applications of Chitin and Chitosan* (pp. 3–29). Lancaster, PA: Technomic Publishing.
- Fennema, O. R. (1996). In O. R. Fennema, *Food Chemistry*, (3rd ed.). (pp. 17–94). New York: Marcel Dekker.
- Ferry, J. D. (1980). *Viscoelastic Properties of Polymers*, (3rd ed.) New York: Wiley.

- Fontanet, I., Davidou, S., Dacremont, C., & Le Meste, M. (1997). *Journal of Cereal Science*, 25, 303–311.
- Georget, D. M. R., & Smith, A. C. (1995). *Cereal Chemistry*, 28, 305–311.
- Ghaouth, A. E., Arul, J., Ponnampalam, R., & Boulet, M. (1991). *Journal of Food Science*, 56, 1618–1620 (see also page 1631).
- Ghaouth, A. E., Ponnampalam, R., Castaigne, F., & Arul, J. (1992). *HortScience*, 27 (9), 1016–1018.
- Gontard, N., Guilbert, S., & Cuq, J. -L. (1993). *Journal of Food Science*, 58, 206–211.
- Gordon, M., & Taylor, J. S. (1952). *Journal of Applied Chemistry*, 2, 493–500.
- Guan, Y., Liu, X., Fu, Q., Li, Z., & Yao, K. (1998). *Carbohydrate Polymers*, 36, 61–66.
- Harris, M., & Peleg, M. (1996). *Cereal Chemistry*, 73, 225–231.
- Hasegawa, M., Isogai, A., Onabe, F., Usuda, M., & Atalla, R. H. (1992). *Journal of Applied Polymer Science*, 45, 1873–1879.
- Hosokawa, J., Nishiyama, M., Yoshihara, K., & Kubo, T. (1990). *Industrial Engineering and Chemical Research*, 29, 800–805.
- Isogai, A., & Atalla, R. H. (1992). *Carbohydrate Polymers*, 19, 25–28.
- Kalichevsky, M. T., & Blanshard, J. M. V. (1992). *Carbohydrate Polymers*, 19, 271–278.
- Kalichevsky, M. T., & Blanshard, J. M. V. (1993). *Carbohydrate Polymers*, 20, 107–113.
- Kalichevsky, M. T., Jaroszkiewicz, E. M., & Blanshard, J. M. V. (1992a). *International Journal Biological Macromolecules*, 14, 257–266.
- Kalichevsky, M. T., Jaroszkiewicz, E. M., Ablett, S., Blanshard, J. M. V., & Lillford, P. J. (1992b). *Carbohydrate Polymers*, 18, 77–88.
- Kalichevsky, M. T., Blanshard, J. M. V., & Tokarczuk, P. F. (1993a). *International Journal of Food Science and Technology*, 28, 139–151.
- Kalichevsky, M. T., Jaroszkiewicz, E. M., & Blanshard, J. M. V. (1993b). *Polymer*, 34, 346–347.
- Karel, M., Buera, M. P., & Roos, Y. (1993). In J. M. V. Blanshard & P. J. Lillford, *The glassy state in foods* (pp. 13–34). Loughborough, UK: Nottingham University Press.
- Kelley, S. S., Rials, T. G., & Glasser, W. G. (1987). *Journal of Material Science*, 22, 617–624.
- Kester, J. J., & Fennema, O. R. (1986). *Food Technology*, 40 (12), 47–59.
- Knorr, D. (1984). *Food Technology*, 38 (1), 85–96 See also page 140.
- Krochta, J. M., & De Mulder-Johnston, C. (1997). *Food Technology*, 51 (2), 61–74.
- LeMeste, M. (1995). In V. Barbosa-Canovas & L. Welti-Chanes, *Food preservation by moisture control* (p. 209). Lancaster, PA: Technomic Publishing.
- Le Meste, M., Roudaut, G., & Davidou, S. (1996). *Journal of Thermal Analysis*, 47, 1361–1375.
- Levine, H., & Slade, L. (1986). *Carbohydrate Polymers*, 6, 213–244.
- Levine, H., & Slade, L. (1988). In J. M. V. Blanshard & J. R. Mitchell, *Food structure: its creation and evaluation* (pp. 149–180). London: Butterworths.
- Levine, H., & Slade, L. (1992). In H. G. Schwartzberg & R. W. Hartel, *Physical chemistry of foods* (pp. 83–221). New York: Marcel Dekker.
- Lillie, M. A., & Gosline, J. M. (1993). In J. M. V. Blanshard & P. J. Lillford, *The glassy state in foods* (pp. 281–301). Loughborough, UK: Nottingham University Press.
- MacInnes, W. M. (1993). In J. M. V. Blanshard & P. J. Lillford, *The glassy state in foods* (pp. 223–248). Loughborough, UK: Nottingham University Press.
- Makino, Y., & Hirata, T. (1997). *Postharvest Biology and Technology*, 10, 247–254.
- Modern Plastics Encyclopedia (1988). In R. Juran, *Modern plastics encyclopedia* (pp. 550–559). New York: McGraw-Hill.
- Montés, H., Mazeau, K., & Cavaillet, J. Y. (1997). *Macromolecules*, 30, 6977–6984.
- Muzzarelli, R. A. A. (1985). In G. O. Aspinall, *The polysaccharides* (pp. 417–450). London, UK: Academic Press.
- Muzzarelli, R. A. A. (1996). *Carbohydrate Polymers*, 29, 309–316.
- Muzzarelli, R. A. A., & De Vincenzi, M. (1997). In M. F. A. Goosen, *Applications of chitin and chitosan* (pp. 115–127). Lancaster, PA: Technomic Publishing.
- Nakamura, S., & Tobolsky, A. V. (1967). *Journal of Applied Polymer Science*, 11, 1371–1386.
- Nicholls, R. J., Appelqvist, I. A. M., Davies, A. P., Ingman, S. J., & Lillford, P. J. (1995). *Journal of Cereal Science*, 21, 25–36.
- Noel, T. R., Ring, S. G., & Whittam, M. A. (1990). *Trends in Food Science and Technology*, 1 (9), 62–67.
- Onishi, H., Nagai, T., & Machida, Y. (1997). In M. F. A. Goosen, *Applications of chitin and chitosan* (pp. 205–231). Lancaster, PA: Technomic Publishing.
- Peleg, M. (1996a). *Critical Reviews in Food Science and Nutrition*, 36, 49–67.
- Peleg, M. (1996b). *Cereal Chemistry*, 73, 712–715.
- Ratto, J. A., Chen, C. C., & Blumstein, R. B. (1996). *Journal of Applied Polymer Science*, 59, 1451–1461.
- Roos, Y. H. (1987). *Journal of Food Science*, 52, 146–149.
- Roos, Y. H. (1995a). *Phase transitions in foods*, New York: Academic Press.
- Roos, Y. H. (1995b). *Journal of Food Engineering*, 24, 339–360.
- Roos, Y. H. (1995c). *Food Technology*, 30 (10), 97–102.
- Roos, Y. H., & Karel, M. (1991a). *Food Technology*, 26 (12), 68–71 See also pages 66 and 107.
- Roos, Y. H., & Karel, M. (1991b). *Journal of Food Science*, 56, 1676–1681.
- Roos, Y. H., & Karel, M. (1991c). *Journal of Food Science*, 56, 38–43.
- Roos, Y. H., Karel, M., & Kokini, J. L. (1996). *Food Technology*, 31 (11), 95–108.
- Roudaut, G. (1998). *Mobilité moléculaire dans des verres de produits céréaliers de cuisson: incidence sur la texture*. PhD Thesis, Univ. de Bourgogne, ENSBANA, Dijon, France.
- Sandford, P. A., & Hutchings, G. P. (1987). In M. Yalpani, *Industrial polysaccharides: genetic engineering, structure/property relations and applications* (pp. 363–376). Amsterdam: Elsevier.
- Schenz, W. (1995). *Food Hydrocolloids*, 9 (4), 307–315.
- Shahidi, F., Arachchi, J. K. V., & Jeon, Y. J. (1999). *Trends in Food Science and Technology*, 10, 37–51.
- Simatos, D., Blond, G., & Perez, J. (1995). In V. Barbosa-Canovas & L. Welti-Chanes, *Food preservation by moisture control* (pp. 3–31). Lancaster, PA: Technomic Publishing.
- Simatos, D., Blond, G., Roudaut, G., Champion, D., Perez, J., & Faivre, A. L. (1996). *Journal of Thermal Analysis*, 47, 1419–1436.
- Singh, D. K., & Ray, A. R. (1998). *Carbohydrate Polymers*, 36, 251–255.
- Slade, L., & Levine, H. (1991). *Critical Reviews in Food Science and Nutrition*, 30, 115–360.
- Slade, L., & Levine, H. (1993). In J. M. V. Blanshard & P. J. Lillford, *The glassy state in foods* (pp. 35–101). Loughborough, UK: Nottingham University Press.
- Sperling, L. H. (1986). *Introduction to physical polymer science*, New York: Wiley.
- Struszczyk, H., & Pospieszny, H. (1997). In M. F. A. Goosen, *Applications of chitin and chitosan* (pp. 171–184). Lancaster, PA: Technomic Publishing.
- Sugisaki, M., Suga, H., & Seki, S. (1968). *Bulletin of the Chemical Society of Japan*, 41, 2591–2599.
- Whistler, R. L., BeMiller, J. N., & Paschall, E. F. (1984). *Starch: chemistry and technology*, New York: Academic Press.
- White, G. W., & Cakebread, S. H. (1966). *Journal of Food Technology*, 1, 73–82.
- Williams, M. L., Landel, R. F., & Ferry, J. D. (1955). *Journal of the American Chemical Society*, 77, 3701–3707.
- Winterowd, J. G., & Sandford, P. A. (1995). In A. M. Stephen, *Food polysaccharides and their applications* (pp. 441–462). New York: Markel Dekker.
- Wurzburg, O. B. (1986). *Modified starches: properties and uses*, Boca Raton, FL: CRC Press.
- Yuen, S. (1974). *Process Biochemistry*, 9 (11), 7–9 (see also page 22).