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ORIGINAL PAPER

Structural Relaxation of Salmon Gelatin Films in the Glassy State

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Abstract Mechanical relaxation of glassy carbohydrates has been reported extensively in the literature; however, little work is available on protein-based systems. This study deals with the structural relaxation of salmon (*Salmo salar*) gelatin in the glassy state. Skin gelatin was obtained by an acid–alkaline extraction method. Molecular weight (M_w) was determined by capillary viscometry. Films prepared by casting (7% *w*/*v*) were equilibrated to a moisture content of ~18.4% (db). The glass transition temperature (T_g) and enthalpic relaxation were determined by differential scanning calorimetry (DSC). Mechanical properties were assessed using a texture analyzer at constant temperature and moisture content. DSC showed a T_g ~34°C, and the selected storage temperature (T_a) was 29°C ($T_g - T_a = 5$°C). The films were aged for 0, 4, 8, 16, and 40 h. Viscometry produced values of M_w ~90.2 kDa. The stress relaxation was modeled by the Kohlrausch–Wlliams–Watts (KWW) equation, reporting an increase in relaxation time (τ_0) as the ageing time increased (τ_0 ~6.41E+03 s for 0 h; τ_0 ~9.01E+05 s for 40 h). β parameter was smaller for the aged films, indicating a spread of relaxation times. The derivative of KWW equation (dφ/dt) indicated a more rapid relaxation in a fresh sample compared with aged films. DSC showed an excess in enthalpy (ΔH) on the aged samples due to the non-equilibrium state of the matrix. ΔH increased with ageing time with values of ΔH ~2.42 J/g for the films aged for 40 h. This work demonstrated molecular relaxation process of gelatin in the glassy state, which must be taken into account if this material is used as a structure forming matrix.

Keywords Salmon gelatin · T_g · Mechanical relaxation · Enthalpic relaxation

Introduction

Gelatin is the most abundant protein in mammals, birds, and fish (Eysturskar et al. 2009). It is extracted by hydrolysis from collagen, which is the structural component of skin, cartilage, and bones in animals (Badii and Howell 2006). Due to its technological versatility, gelatin is used extensively in the pharmaceutical, cosmetic, medical, and photographic industries (Haug et al. 2003). The unique property of proteins to form networks and induce plasticity and elasticity are considered beneficial in the preparation of biopolymer-based packaging materials (Voon et al. 2010). However, its use in food products has been limited by different religious groups due to its origins, since it is produced from porcine and bovine hides (Badii and Howell 2006). In addition, bovine spongiform encephalopathy (BSE) still is a sensitive issue among consumers (Gudmundsson

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2002). Gelatins from marine sources have emerged as an alternative for which different methods of extraction, mainly from collagen present in fish skin, have been proposed (Zhang et al. 2007; Gomez-Guillen et al. 2002; Muyonga et al. 2004). Fish collagen is different from mammalian collagen in terms of its biochemical constituents and therefore in its functional properties. Fishes are considered, nonhomoeothermic animals, as their body temperature depends on the temperature of the water in which they live (Joly-Duhamel et al. 2002). Fish collagens therefore exhibit a large biodiversity especially in the amino acid composition in relation to their environment which is not present in mammalian gelatin. It has been shown that cold water fish collagen can have a lower content of imino acids (about 16% to 18%) than the collagen of warm-blooded animals (about 24%) (Gilsenan and Ross-Murphy 2000). In terms of its functionality, gelatin's physical properties depend mainly on two factors: (1) the amino acid composition and (2) the molecular weight distribution (Gomez-Guillen et al. 2002). Indeed, Joly-Duhamel et al. (2002) established a positive correlation of the concentration of imino acids proline (Pro) and hydroxyproline (Hyp) with the melting temperature of native collagen (helix to coil) and gelatin molecular weight with renaturation temperature (coil to helix), suggesting an important effect of biochemical composition on the structure stability of the gel network. Gómez-Guillén et al. (2009) attributed the formation of nucleation zones conducive to the formation of triple helical structures to the imino acid content of Pro and Hyp rich regions in collagen. The same authors reviewed the effect of molecular weight and imino acid content on the physical properties of gelatin films, indicating that in general, fish gelatins had lower tensile strength and higher elongation. An aspect that has not been explored in detail on marine gelatin films is the structural stability of these systems in low moisture content environments or at temperatures below their glass transition temperature (T_g). Some important applications of gelatin in the glassy state include the encapsulation of bioactive compounds such as vitamins, minerals, functional lipids, probiotics, amino acids, peptides, proteins, phytosterols, phytochemicals, and antioxidants (Aguilera and Lillford 2008). Gelatin also encapsulates lipophilic compounds such as oils and other fat-soluble bioactives more effectively than carbohydrate encapsulating materials. During isothermal storage, spontaneous and kinetically controlled relaxation phenomena occur, leading to the reduction of molecular mobility, which affects the physical properties of the material such as oxygen and water permeability, texture, fragility, brightness, and transparency which have great technological importance (Badii and Howell 2006). Relaxation has been successfully studied using calorimetric and mechanical techniques (Badii et al. 2005, 2006). Some of the early studies in glassy carbohydrates by Noel et al. (1999) described the ageing kinetics of maltose in the glassy state in terms of an overshoot in heat capacity on consecutive heating runs using differential scanning calorimetry (DSC). Badii et al. (2005) evaluated the kinetics of enthalpic relaxation of bovine gelatin in both the amorphous and semi-crystalline state as a function of the difference between ageing temperature and T_g. In later work, the same authors quantified the enthalpic relaxation of the same model system by differential calorimetry, correlating enthalpic values with an increase in the elastic modulus (E) obtained by mechanical spectroscopy. Work by Lourdin et al. (2002) described the structural relaxation of amorphous potato starch in the glassy state by a three point bending test, obtaining characteristic relaxation parameters by the application of the Kohlrausch–Williams–Watts (KWW) model. Anderssen et al. (2004) discussed the KWW equation in terms of a spectrum of relaxation times describing the ageing of a polymer. The KWW is an equation that can quantitatively describe the kinetics of the relaxation process towards an absolute relaxed state (Anderssen et al. 2004).

$$\varphi(t) = \exp\left[-\left(\frac{t}{\tau_0}\right)^{\beta}\right] \tag{1}$$

Where φ is the relaxation function, t is time, β ($0<\beta\leq1$) is a measure of non-exponentiality or width of the relaxation time distribution spectrum (Liu et al. 2006), and τ_0 is the characteristic relaxation time, being dependent on temperature and material structure. Kim et al. (2003) used the KWW approach to successfully describe the enthalpic relaxation in amorphous potato starch stored at temperatures below its T_g, detecting an increase in τ_0 when the difference between the ageing temperature and T_g increased.

The lack of information on the structural relaxation kinetics of marine gelatin with known differences in mechanical properties from those from mammalian sources encouraged this work to focus on the stability of gelatin extracted from salmon skin, an abundant by-product from the fish industry.

Materials and Methods

Preparation of Films

Salmon (*Salmo salar*) gelatin was extracted from skin by an acid–alkaline method following the protocol proposed by Adriazola (2007), with a ~9% yield (weight to volume *w*/*v*) of dry gelatin. The film preparation consisted of the dissolution of gelatin in hot water (70°C) at a concentration of 7% (*w*/*v*), which was then poured onto rectangular teflon blocks (30×12×10 cm) and cold cast at 5°C in a incubator for 5 days. Special care was taken to keep the containers level in order to get a uniform thickness for all films. The films were then cut; ~100 mm in length, ~10 mm in width, and ~0.25 mm in thickness. Acknowledging the well-known T_g dependence of biopolymers on moisture content (Enrione et al. 2010), the films formed were stored over P_2O_5 (0% relative humidity, RH) for 7 days at ambient temperature. For practical purposes, in terms of T_g and ageing temperature, the films were later equilibrated in desiccators over a saturated solution of Mg $(NO_3)_2$ giving an environmental RH of 54%. Equilibrium was considered to have been reached when the change in weight was less than 0.1%. The moisture content of the equilibrated samples was determined gravimetrically in triplicates by heating in an oven at 105°C for 12 h (Enrione et al. 2010). The moisture content of films was ~18.4% with a standard deviation of ~0.5% (dry weight basis, db).

Determination of Molecular Weight (M_w) of Gelatin

The molecular weight of gelatin was determined by capillary viscometry. Viscometric measurements have been used to probe fundamental properties of food macromolecules such as conformation in dilute solutions, molecular weight, and interaction properties (Harding 1998). The dependence of the reduced viscosity (η_{red}) and inherent viscosity (η_{inh}) of a dilute suspension on the concentration (*c*), due to the effect of non-ideality, is well established. At infinite dilution ($c \rightarrow 0$), η_{red} and η_{inh} are defined as the intrinsic viscosity [η]. The relation between [η] and M_w can be described in terms of the Mark–Houwink–Kuhn–Sakurada (MHKS) equation (Harding 1998):

$$[\eta] = K \times M_w{}^a \tag{2}$$

where *K* and *a* are dependent on the nature of the solvent and the polymer conformation (Harding 1997). The determination of M_w of the extracted salmon gelatin was carried out using the values of *a* and *K* used by Aguirre (2009) for fish and poultry gelatins.

Salmon gelatin was diluted in a 0.1 M NaCl solution, at eight concentrations ranging from 0.6 to 1.8 g/l (Wulansari 1999) and left overnight at 4°C for complete hydration. The flow time of the gelatin suspension was measured by an Ostwald U-tube (Cannon-Fenske Calibrated CFRC (9721-B50)) immersed in a thermoregulated water bath at 50°C. Aliquots of the suspension (2 ml) were placed in the U-tube with the help of a manual pump. The flow time of four replicates of each gelatin suspension was determined by measuring the time required for the suspension to flow from the top mark to the bottom mark of the U-tube. Subsequently, the reduced (η_{red}) and inherent (η_{inh}) viscosities against concentration were plotted, and the limit near zero concentration was taken as the intrinsic viscosity [η].

Mechanical Characterization

Prior to the mechanical studies, the thermal history of the films was eliminated by immersing them in silicone oil at a temperature 15°C above T_g (first DSC trace) for 10 min (Lourdin et al. 2002). The films were then sealed hermetically in an impermeable foil-laminated bag and quickly cooled to the ageing temperature T_a (~29°C), equivalent to 5°C below T_g. Finally, the films were stored at constant temperature for 0, 4, 8, 16, and 40 h. The stress (σ) versus engineering strain (γ) curves for each of the aged films were obtained by a texture analyzer (Zwick DO-FBO.5TS, August-Nagel-Str. D-89079 Ulm, Germany) using a uniaxial tension method. The application of the tensile force was restricted to the elastic or linear zone of the stress–strain curve. The slope was defined as the Young's modulus (*E*). The mechanical relaxation of the films aged in the glassy state, was assessed by tensioning the film to a deformation (strain) of 1% and obtaining a force (*N*) versus time (*s*) curve. The force was recorded every 5 s. In order to maintain the control over the moisture content (~ 18.4% db) and temperature (~29°C) of the sample's constant throughout the mechanical relaxation measurements, a specially designed device was attached to the texture analyzer. Each film was submerged in a silicone oil (Down Corning 200R) containing inner cylinder of a concentric cylinder chamber. The outer cylinder was filled with circulating water tempered at the desired temperature (±0.1°C). The force values were then converted to stress, which was later normalized (divided by stress at analysis time equal 0 s) and fitted using Eq. 1 producing the values of the parameters β and τ_0. The KWW model fitting was performed using the software Curve Expert 1.40 (Freeware), obtaining the correlation coefficient (R^2) as well as the standard error (SE) of the fits. For each ageing time, four replicates were measured (20 experiments). Furthermore, the rate of mechanical relaxation was estimated using the derivative of the function KWW with respect to measuring time ($d\varphi/dt$) using the estimated β and τ_0 values.

Determination of Enthalpy Relaxation

The glass transition temperature T_g and enthalpy relaxation were determined by differential scanning calorimetry (Perkin Elmer Diamond DSC, 710 Bridgeport Avenue, Shelton, CT 06484-4794, U.S.A.). The equipment was calibrated with indium as standard (T_m=156.6°C and ΔH=

28.4 J/g). Ten milligram of sample was placed in aluminum capsules of 30 μL and hermetically sealed. T_g was defined as the midpoint of the change in heat capacity (C_p) from the second run giving a value of ~34°C. The capsules were stored under the same conditions of temperature and time as the mechanical relaxation studies. However, in this case, the removal of thermal history was achieved on the same equipment by holding the sample at 15°C above T_g for 10 min (Lourdin et al. 2002; Badii et al. 2006), and then cooled to the storage temperature (T_g-T_a=5°C). Heating over T_g would allow the film structure to recover the lost enthalpy during ageing due to the increase in molecular mobility (Liu et al. 2006). On fast cooling, the local viscosity of the polymer changed progressively (Lourdin et al. 2002) leading to re-vitrification at T_g.

The method was: holding at −40°C for 2 min, heating from −40°C to 90°C at a heating rate of 10°C/min, holding for 2 min at 90°C, cooling from 90°C to −40°C at 40°C/min, and finally heating from −40°C to 90°C at a heating rate of 10°C/min. The first DSC scan thermogram indicated denaturation and melting like endotherms at ~55°C and ~70°C, respectively (data not shown). The ageing was performed after the second DSC scan.

The excess in enthalpy (ΔH) generated on ageing was calculated from the area under the endotherm produced after subtracting the first and second DSC scans. Each sample stored at the different ageing times was evaluated in triplicate giving a total of 15 experiments.

The statistical significance of the experimental data was evaluated using analysis of variance (ANOVA) with α= 0.05 using the Data Analysis Suite in Excel (Office 2003. Microsost Corp). Standard deviations (SD) were calculated for replicates and plotted as error bars. Variation of coefficient (VC) were calculated and presented when necessary.

Results and Discussion

Determination of Gelatin Molecular Weight

In Fig. 1, the reduced (η_{red}) and inherent (η_{inh}) viscosities obtained by capillary viscometry are presented as function of the salmon gelatin concentration. Both values increased ($p<0.05$) when the concentration of the suspensions varied from 0.6 to 1.4 g/dl.

Eq. 2 was used to determine the molecular weight of the gelatin. The value of the constants K and a used were 8.57×10^{-5} and 0.74, respectively (Aguirre 2009); therefore, Eq. 2 becomes: $[\eta]=8.57\times10^{-5}\times M_w^{0.74}$, where $[\eta]$=0.40 (determined by the intercept of the two lines on Fig. 1), giving M_w=90.2 kDa with a standard deviation (SD)= 6.8 kDa.

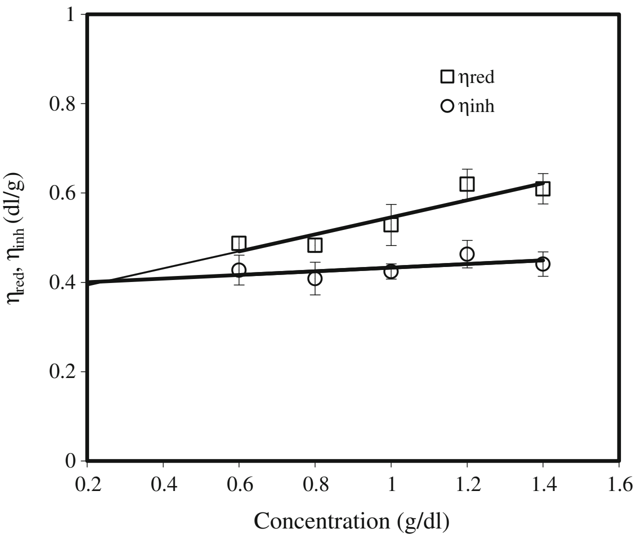

Fig. 1 Double extrapolation of (*empty square*) η_{red} and (*empty circle*) ln η_{inh}→zero concentration as the intrinsic viscosity [η] of salmon gelatin. *Vertical error bars* represent ±1 standard deviation (SD)

This value is in good agreement with the previous studies published by Arnesen and Gildberg (2007) who claim that for salmon gelatin (*S. salar*) extracted at 65°C, the M_w distribution had a mean value of ~100 kDa using HPLC as a fractionation method. Recently, Aguirre (2009) reported a M_w of about ~97 kDa using a similar viscometry protocol for a similar gelatin type.

Mechanical Characterization

The modulus versus ageing time is presented on Fig. 2. A significant increase in E ($p<0.05$) was detected for the aged gelatin films. After 40 h of storage, the modulus increased from ~1.2E+03 to ~1.6E+03 MPa (~33%). As moisture content was kept constant during the experiment, the increase in stiffness was attributed to a decrease in specific volume of the amorphous polymeric structure by movement of the chains towards a lower energy state. The increase in modulus with time was linear-like, with a slight decrease in the slope at long ageing times. It is possible to fit the data

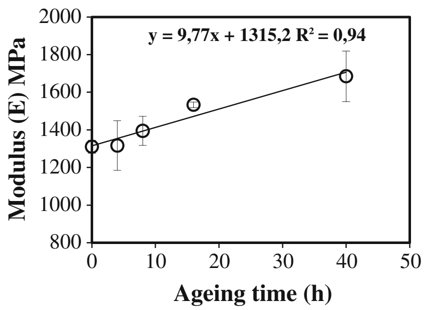

Fig. 2 Modulus versus ageing time at 29°C (T_g-T_a=5°C). *Vertical error bars* represent ±1 standard deviation (SD)

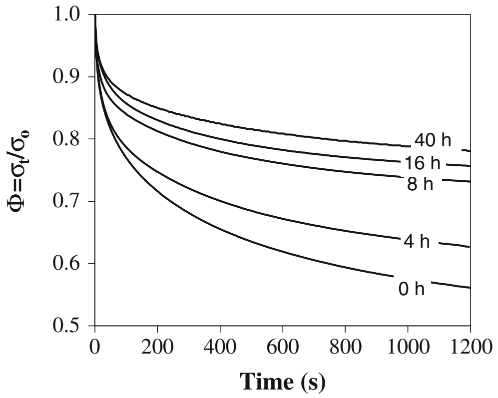

Fig. 3 Stress relaxation, φ (normalized) for salmon gelatin films in glassy state aged at 29°C (T_g-T_a=5°C) for 0, 4, 8, 16, and 40 h. Significant differences ($p<0.05$) in φ at measurement time >50 s

with a curve of the form: $E=9.8*t+1315.2$ ($R^2=0.94$). This increase in stiffness on storage has also been reported for carbohydrate-based systems. Lourdin et al. (2002) described a significant increase in strength at break of potato starch extrudate from ~112 to ~150 N (~34% increase) after 96 h of storage at temperatures below T_g.

Mechanical Relaxation

Figure 3 shows the mechanical relaxation of the aged gelatin films represented by a decrease in the normalized stress (φ) at constant deformation. The reduction in φ follows an exponential behavior with a marked decrease during the first 200 s after tensioning at constant strain. A statistical analysis of the variances between ageing times indicated significant differences ($p<0.05$) in stress between fresh and aged films after ~50 s of tensioning. These results show greater mechanical relaxation in the fresh gelatin films compared to aged samples. Indeed, if the amplitude of the decrease (A) is calculated from the difference between φ at time 0 and at 1,200 s, it was found that A decreased with aging time (Table 1). An A value of ~46.0% was obtained for the fresh sample (0 h storage), with the lowest value of A ~21.9% for the 40 h storage film. Such behavior was represented by the KWW model, whose parameters β and τ_0 were obtained individually. The mean and standard deviations are shown in Table 1.

The value of the β parameter decreased from 0.30 to 0.21 ($p<0.05$) when the ageing time increased to 40 h. According to the attachment theory proposed by Ngai (1998), a decrease in the parameter β can be interpreted as strengthening of the coupling between the relaxing species and its close surroundings, which is related to an overall decrease in molecular mobility (Mano and Viana 2001) and to an increase in spread of the distribution of relaxation times (Lourdin et al. 2002). It is proposed that the decrease in β is attributable to the densification of the polymeric structure with increased storage time. As the intermolecular space is reduced, the mobility of the polymer chains becomes more restricted, decreasing the relaxation during tensioning. Kim et al. (2003), using the KWW approach to describe the enthalpic relaxation in amorphous potato starch below T_g, obtained values of β ~0.30, which was related to a reduction in molecular motion of the polymeric structure. The relaxation time τ_0 increased significantly ($p<0.05$) from 6.1E+03 to 9.0E+05 s with ageing time. This parameter represents the average value for all relaxation times occurring in the polymer, defined by the heterogeneity of its structure. Lourdin et al. (2002) detected an increase in one order of magnitude in τ_0 for potato starch extrudates upon storage, where τ_0 went from ~1.0E+03 to ~1.0E+04 s for a storage time of ~20 h at T_g-T_a=18°C. These authors reported that the progressive changes in mechanical properties occur in a time scale similar to that of densification of an amorphous material, which is characteristic of structural relaxation in the glassy state.

β versus ageing time (t_{ageing}) was well fitted (SE<0.01) by the equation: $\beta=0.32–0.037*t_{\mathrm{ageing}}^{0.32}$ ($R^2=0.97$), whereas the time τ_0 followed the equation: $\tau_0=-3.2\mathrm{E}+04+2.4\mathrm{E}+04\cdot t_{\mathrm{ageing}}$ ($R^2=0.99$) (SE<0.01) (figures not shown). Thus, the mechanical relaxation kinetics of the salmon gelatin could also be modeled by the KWW model with only one parameter, the ageing time, t_{ageing}.

Mechanical Relaxation Rate

Figure 4 shows the variation in the normalized relaxation rate, $(\mathrm{d}\varphi/\mathrm{d}t)_t/(\mathrm{d}\varphi/\mathrm{d}t)_{t=0}$ generated from the derivative of Eq. 1 using the calculated β and τ_0 values.

In all cases, a decrease in relaxation rate during the tension test was observed. The gelatin film annealed for

Table 1 Parameters β and τ_0, percentage of mechanical relaxation (A) and relaxation enthalpy ΔH, for storage times 0 to 40 h

Ageing time (h)	τ_0 (s)	$\mathrm{SD}_{\tau0}$	B	SD_β	SE	A (%)	ΔH (J/g)	$\mathrm{SD}_{\Delta H}$
0	6.1E+03	3.1E+02	0.32	0.03	6.4E−02	45.9	0	–
4	1.7E+04	1.9E+03	0.28	0.01	4.1E−02	40.4	1.1	0.40
8	1.3E+05	5.3E+03	0.24	0.03	6.4E−02	25.6	2.3	0.20
16	3.8E+05	1.9E+04	0.22	0.03	5.6E−03	24.5	2.3	0.25
40	9.0E+05	2.1E+04	0.21	0.01	3.0E−03	21.9	2.4	0.22

SE standard error from KWW equation fitting, *SD* standard deviation

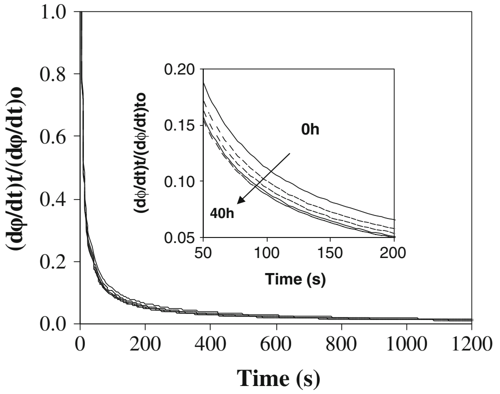

Fig. 4 Normalized mechanical relaxation rate versus time for salmon gelatin films aged at 29°C (T_g-T_a=5°C) for 0, 4, 8, 16, and 40 h

40 h was the first to reach the minimum value of ~0.01, followed by the samples with 16, 8, 4, and 0 h of holding time. It is interesting to note that the relaxation rate was lower for the aged samples compared to fresh gelatin at all times. It was also noted that approximately 90% of the variation in relaxation rate occurred before 100 s for all the samples, indicating that most of the changes in the matrix occurred at a small fraction (~10%) of the overall relaxation time. The films stored for 40 h showed the lower relaxation rate throughout the test time (see insert in Fig. 4). As mentioned earlier, this could be explained by the densification, in relation to the thermodynamic properties of the system. Thus, in a fresh material, the intermolecular volume is maximum as the loss of enthalpy is zero; therefore, the mobility of chains is significant at the time scale studied. In the case of the aged material, the intermolecular volume loss will have already occurred, the loss depending on the magnitude of the storage.

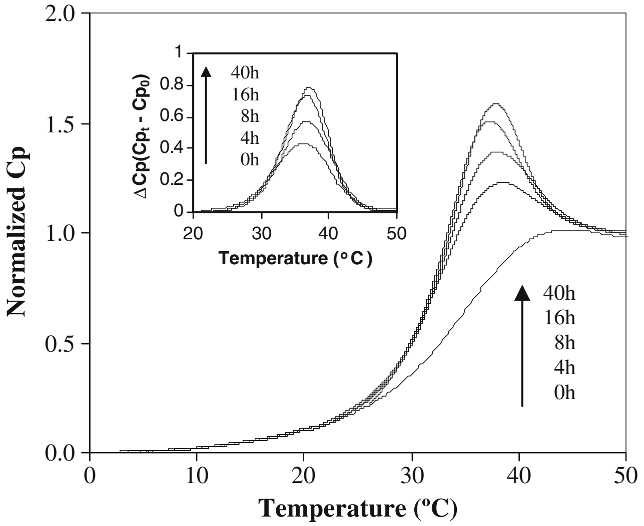

Fig. 5 Normalized C_p versus temperature in salmon gelatin-based films aged at 29°C (T_g-T_a=5°C) for 0, 4, 8, 16, and 40 h. VC of C_p replicates<10% of each ageing time

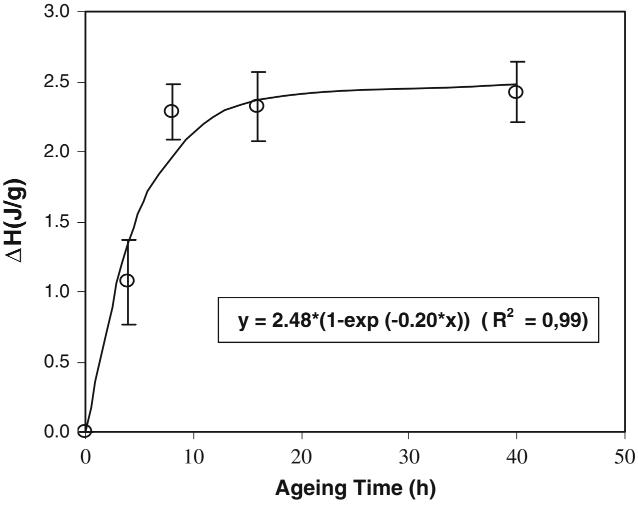

Fig. 6 Enthalpy relaxation (Δ*H*) vs. aging time for salmon gelatin films aged at T_g-T_a=5°C for holding times of 0, 4, 8, 16, 40 h. *Error bars* correspond to ±1SD

Therefore, from these results, it can be seen that the normalized relaxation rate has an inverse relationship with the storage time (see Fig. 4). This was related to the β parameter, were the 0 h storage film had a large relaxation amplitude with β ~0.32, compared with a stored film at 40 h which had a lower relaxation amplitude giving a β ~0.21.

Enthalpic Relaxation

Figure 5 shows the normalized heat capacity C_p as a function of temperature for all the gelatin films. The resulting variation coefficient (VC) at each ageing time was <10% indicating low data dispersion among replicates. An overshoot in heat flow at T_g was observed for the aged samples, which was related to the increase in storage time. This behavior was similar to that observed in synthetic and natural polymers where overshoots in heat flow at

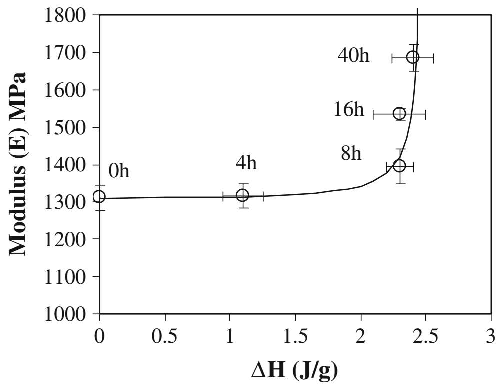

Fig. 7 Modulus (*E*) vs. enthalpy relaxation (Δ*H*). Each point corresponds to a specific ageing time from 0 to 40 h at 29°C (T_g-T_a= 5°C). *Error bars* correspond to ±1SD

temperatures close to T_g are reported (Badii et al. 2005, 2006; Bailey et al. 2001; Lourdin et al. 2002; Surana et al. 2005).

The excess in enthalpy or ΔH was calculated from the area under each curve after subtraction of the thermogram corresponding to 0 h storage. ΔH versus ageing time is shown in Fig. 6, where a two-stage behavior is observed, with a rapid increase in ΔH from 0 to ~2.3 J/g (0 to 8 h) ($p < 0.05$) after which ΔH leveled off ($p > 0.05$). These results support the mechanical data in terms of a loss in the internal energy from the system by the spontaneous densification of a polymeric matrix approaching equilibrium. Chung and Lim (2003) reported that the rate of relaxation process for waxy and normal rice starches gradually decreased with aging time. They found values of excess enthalpy ΔH for 40 h annealing of 1.2 and 1.1 J/g for waxy and normal starch (moisture content ~15% d. b.), respectively. In this work, the highest value of enthalpy for 40 h annealing of gelatin films was ~2.4 J/g (Table 1). In terms of the structure stability, these data suggest that biopolymers of different compositions such as carbohydrate and proteins can undergo similar energy relaxation processes to those reported for synthetic polymers.

The molecular weight of salmon gelatin reported in this work (M_w=90.2 kDa) had lower values compared to those reported in the literature for mammalian gelatins (Joly-Duhamel et al. 2002). Smaller gelatin molecules would generate lower thermal transitions temperatures associated with helix to coil configurational changes and T_g (Gomez-Guillen et al. 2002). These differences could have a significant effect on the relaxation kinetics of the material by its dependence on the difference between ageing temperature and T_g (Badii et al. 2006).

Modulus Versus Enthalpy Relaxation

Looking for possible correlations between the mechanical and thermal data, the values of modulus were plotted against ΔH for each ageing time on Fig. 7. DSC seemed to detect significant changes in ΔH ($p<0.05$) at earlier ageing times compared with mechanical measurements (E). Indeed, significant changes in modulus ($p<0.05$) were detected only after 8 h of annealing with a ΔH ~2.3 J/g. A possible explanation of this difference could be related to various mechanisms; (1) known differences in T_g from calorimetric and mechanical measurements (Enrione 2005), resulting in different ageing due to a difference in $T_g - T_a$; (2) the extent of densification of the gelatin matrix required for significant variation in E; (3) differences in molecular structuring of the polymer chains. In this study, calorimetric data showed the presence of ordered fractions in the films conditioned for mechanical analysis compared to the samples aged in the DSC which were amorphous in structure. These differences in structure could have an effect on the relaxation kinetics but literature suggests otherwise. Badii et al. (2005) found no significant differences in the enthalpic relaxation between semi-crystalline and amorphous bovine gelatin-based films aged at $T_a - T_g$ from 70°C to 15°C for 12 and 168 h.

Conclusions

A spontaneous structural relaxation of salmon gelatin in the glassy state was observed by thermal and mechanical techniques. During the ageing of the films, the material became stiffer as indicated by a significant increase in modulus over time. This behavior was related to a reduction in molecular mobility as indicated by increasing relaxation times (τ_0) and rates estimated from the stress relaxation data. The reduction in β on ageing suggests a spreading of relaxation times occurring in the matrix.

The excess in enthalpy (ΔH) in aged films were associated with lower energy states as thermodynamic equilibrium was approached. The correlation analysis between mechanical and calorimetric data showed variations in thermal properties at shorter ageing times implying possible differences in relaxation mechanisms.

This work shows significant changes in structure can occur in a short time frame. This work will be continued using predictive models for the enthalpic data and will attempt to confirm or refute these findings. A direct comparison with gelatin from other sources, assessing the significance of biochemical structure on the kinetics associated with ageing, will also be carried out.

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