Structural Relaxation of Salmon Gelatin Films in the Glassy State

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Abbreviations

Pro	Proline
Hyp	Hydroxyproline
T_g	Glass transition temperature
DSC	Differential scanning calorimeter
E	Elastic modulus
KWW	Kohlrausch–Williams–Watts model
Φ	Decrease in the normalized stress
ϕ	Relaxation function
t	Time
β	Width of the relaxation time distribution spectrum
τ_0	Characteristic relaxation time
RH	Relative humidity
σ	Stress

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γ	Engineering strain
E	Young's modulus
T_a	Storage temperature
R^2	Coefficient of determination
SE	Standard error
ΔH	Enthalpy relaxation
C_p	Heat capacity
ANOVA	Analysis of variance
SD	Standard deviations
CV	Coefficient of variation

1 Introduction

The unique property of gelatin to form networks and induce plasticity and elasticity is considered beneficial in the preparation of biopolymer-based packaging materials. However, its use in food products has been limited by some religious groups (Gudmundsson 2002). Gelatins from marine sources have emerged as an alternative, although they are different from mammalian collagen in terms of their biochemical constituents and therefore in their functional properties. It has been shown that cold-water fish collagen can have a lower content of amino acids (~16–18 %) (Gilsenan and Ross-Murphy 2000). Joly-Duhamel et al. (2002) established a positive correlation of the concentration of amino 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. An aspect that has not been explored in detail for marine gelatin is the structural stability of these systems at temperatures below their glass transition temperature (T_g). 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 a differential scanning calorimeter (DSC). Badii et al. (2005) evaluated the kinetics of enthalpic relaxation of bovine gelatin state as a function of the difference between ageing temperature and T_g. In their later work, the same authors quantified the enthalpic relaxation of the same model system by DSC, correlating enthalpic values with an increase in the elastic modulus (E) obtained by mechanical spectroscopy. Work by Lourdin et al. (2002) described the mechanical relaxation of amorphous potato starch in the glassy state, obtaining characteristic relaxation parameters by the application of the Kohlrausch–Williams–Watts (KWW) model (Eq. 1). 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 toward an absolute relaxed state (Anderssen et al. 2004):

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

where φ is the relaxation function, t is time, β $(0 < \beta \leq 1)$ is the width of the relaxation time distribution spectrum, and τ_0 is the characteristic relaxation time, being dependent on temperature and material structure.

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 looking at the stability of gelatin extracted from salmon skin, an abundant by-product from the fish industry.

2 Materials and Methods

2.1 Preparation of Films

Salmon (*Salmo salar*) gelatin was extracted from skin by an acid–alkaline method giving a ~9 % w/v yield of dry gelatin. The gelatin was suspended at 7 % (w/v) in hot water (70 °C), which was then poured onto rectangular Teflon blocks (30 × 12 × 10 cm) and cold cast at 5 °C in an incubator for 5 days. The obtained films (~0.25 mm in thickness) were then cut (100 mm × ~10 mm) and stored over P_2O_5 (~0 % relative humidity, RH) for 7 days at ambient temperature and later equilibrated under a ~54 % RH (saturated solution of $Mg(NO_3)_2$). The moisture content was determined gravimetrically in triplicates overnight at 105 °C giving ~18.4 % (db.).

2.2 Mechanical Characterization

The stress (σ) versus engineering strain (γ) curves were obtained by a texture analyzer (DO-FBO.5TS, Zwick, Ulm, Germany) using a uniaxial tension method. The application of tensile force was restricted to the linear zone of the stress–strain curve, giving Young's modulus (E). Prior to the measurements, the thermal history of the films was eliminated at 15 °C above T_g in silicone (Down Corning 200R) oil for 10 min, then hermetically packed and quickly cooled to the storage temperature (T_a) (~29 °C), equivalent to 5 °C below T_g, and stored for 0, 4, 8, 16, and 40 h. The stress relaxation of the films was assessed at a constant strain of 1 %. The stress values were normalized and fitted using Eq. (1), obtaining the values β and τ_0 together with the coefficient of determination (R^2), as well as the standard error (SE) of the fits. The moisture content and temperature of the films were kept constant throughout the measurements, using a silicone oil containing inner cylinder in a concentric cylinder chamber previously attached to the texture

analyzer. The outer cylinder was filled with circulating water tempered at the desired temperature ($\pm$0.1 °C).The rate of mechanical relaxation was predicted using the derivative of the function KWW with respect to measuring time (dφ/dt) using the estimated β and τ_0 values. Four replicates were evaluated for each sample.

2.3   Determination of Enthalpy Relaxation

Tg and enthalpy relaxation (ΔH) were determined by DSC (Diamond DSC, Perkin Elmer), calibrated with indium $T_m = 156.6$ °C and $\Delta H = 28.4$ J/g. Ten milligram of each sample was placed in 30 μL aluminum capsules and hermetically sealed. T_g was defined as the midpoint of the change in heat capacity (C_p) on the second DSC scan giving a value of ~34 °C. The capsules were stored for the same time and temperature as the mechanical relaxation studies. The thermal history was erased by holding the sample at 10 °C above T_g for 15 min (Lourdin et al. 2002) and then cooled to the storage temperature (29 °C). The methodology used was the following: holding at −40 °C for 2 min, heating from −40 to 90 °C at 10 °C/min, holding for 2 min at 90 °C, cooling from 90 to −40 °C at 40 °C/min, and finally heating from −40 to 90 °C at 10 °C/min. The first DSC thermogram indicated denaturation and melting like endotherms at ~55 and ~70 °C, respectively (data not shown). The ΔH was calculated from the area generated from subtracting the first and second DSC scans. Each sample was evaluated in triplicate.

The statistical significance of the experimental data was evaluated using the analysis of variance (ANOVA) with $\alpha = 0.05$ using Data Analysis Suite in Excel (Office 2003. Microsoft Corp.). Standard deviations (SD) were calculated for replicates and plotted as error bars. Coefficients of variation (CV) were calculated and presented when necessary.

## 3   Results and Discussion

3.1   Mechanical Characterization

A significant increase in E ($p < 0.05$) was detected after 40 h of storage, from ~1.2E + 03 to ~1.6E + 03 MPa (~33 %) (Fig. 1). The increase in stiffness was attributed to a decrease in specific volume of the amorphous polymeric structure by movement of the chains toward 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 was possible to fit the data with the equation: $E = 9.8*t + 1315.2 (R^2 = 0.94)$.

Figure 2 shows 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. A statistical analysis of the variances indicated significant

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

$$y = 9{,}77x + 1315{,}2 \quad R^2 = 0{,}94$$
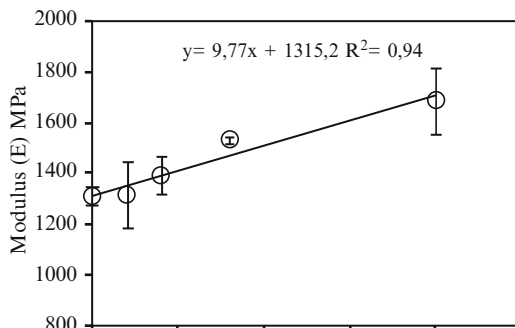
Fig. 2 Stress relaxation, φ (normalized) for salmon gelatin films in glassy state aged at 29 °C ($T_g - T_a = 5$ °C) for 0, 4, 8, 16, and 40 h. Significant differences ($p < 0.05$) in φ at measurement time > 50 s
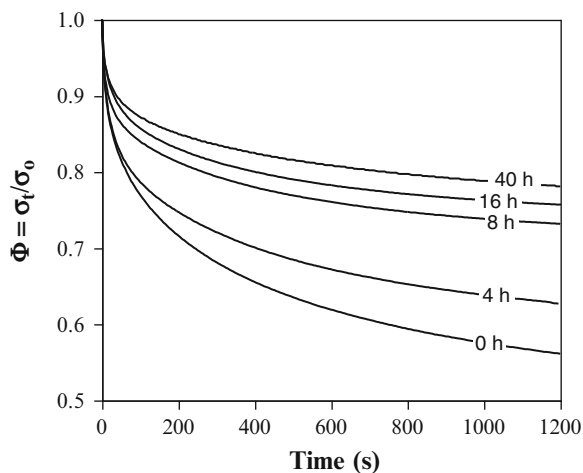
Table 1 Parameters β and τ_0, percentage of mechanical relaxation (A), and relaxation enthalpy ΔH, for storage times 0–40 h

| Ageing time (h)	τ_0 (s)	$SD_{\tau 0}$	β	SD_β	SE	A (%)	ΔH (J/g)	$SD_{\Delta H}$
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

differences ($p < 0.05$) in stress between fresh aged films after 50 s of tensioning. Indeed, the amplitude of the decrease (A) between φ at time 0 and 1200 s (Table 1) was ~46.0 % for the fresh sample and ~21.9 % for the film stored for 40 h. Such behavior was represented by the KWW model, whose parameters β and τ_0 were obtained individually (Table 1). β 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), this 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 and to an increase in spread of the distribution of relaxation times (Lourdin et al. 2002). The decrease in β was 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. τ_0 increased significantly ($p < 0.05$) from 6.1E +03 to 9.0E+05 s with ageing time. β versus ageing time (t_{ageing}) was well fitted (SE < 0.01) by the exponential, $\beta = 0.32 - 0.037* \ t_{ageing}^{0.32} (R^2 = 0.97)$, whereas τ_0 followed the equation, $\tau_0 = -3.2E+04 + 2.4E+04 \cdot t_{ageing}$ ($R^2 = 0.99$) (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}.

Figure 3 shows the variation in the normalized relaxation rate, $(d\varphi/dt)_t/(d\varphi/dt)_{t=0}$ generated from the derived 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 stored for 40 h was the first to reach the minimum value of ~0.01, followed by the samples with 16, 8, 4, and 0 h storage.

It is interesting to note that the relaxation rate was slower for the aged samples at all times. It was also noted that approximately 90 % of the variation in relaxation rate occurred before 100 s, indicating that most of the structural changes in the matrix occurred at a small fraction of the overall relaxation time.
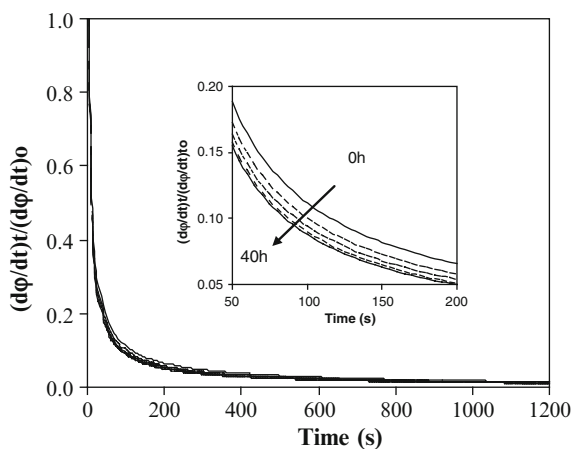

Fig. 3 Normalized mechanical relaxation rate versus time for salmon gelatin films aged at 29 °C ($T_g - T_a = 5$ °C) for 0, 4, 8, 16, and 40 h

3.2 Enthalpic Relaxation

Figure 4 shows the normalized values of heat capacity (C_p) as a function of temperature for all gelatin films. An overshoot at T_g was observed for the aged samples, which was correlated to the increase in storage time. This behavior was similar to that observed in synthetic and natural polymers, where overshoots in heat flow at temperature close to T_g have been reported (Badii et al. 2006).

ΔH versus ageing time is shown in Fig. 5, where a two-stage behavior is observed, with a rapid increase in ΔH from 0 J/g to ~2.3 J/g (0–8 h) ($p < 0.05$),
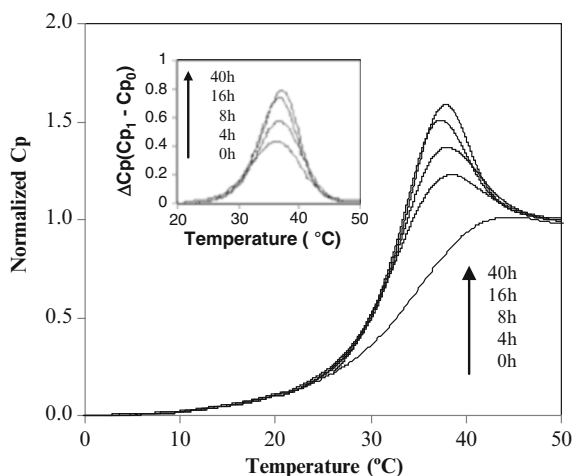
Fig. 4 Normalized C_p versus temperature in salmon gelatin-based films aged at 29 °C ($T_g - T = 5$ °C) for 0, 4, 8, 16, and 40 h. CV of Cp replicates <10 % of each ageing time

Fig. 5 Enthalpy relaxation (ΔH) v/s ageing time for salmon gelatin films aged at $T_g - T_a = 5$ °C for holding times of 0, 4, 8, 16, and 40 h. Error bars correspond to ±1 SD

$$y = 2.48*(1-\exp(-0.20*x)) \quad (R^2 = 0{,}99)$$
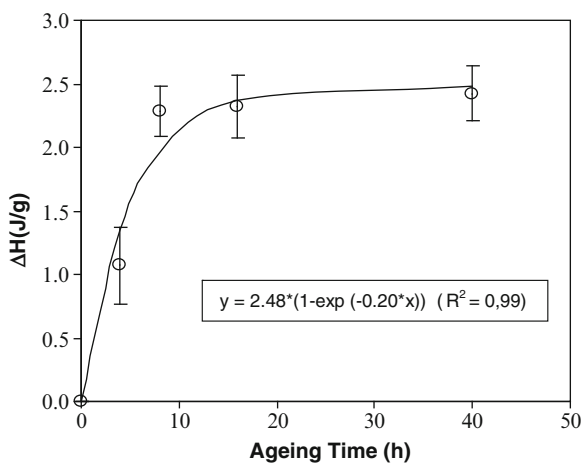
after which ΔH leveled off ($p > 0.05$). These results support the mechanical data in terms of a loss in the internal energy from the system by the spontaneous densification of a polymeric matrix approaching equilibrium. In terms of the structure stability, these data suggest that fish gelatin can undergo similar relaxation processes to those reported for biopolymers. In the future, comparative studies of structure relaxation in gelatin from different biological origins would help to understand the effect of biochemical composition on the kinetic of this phenomenon.

4   Conclusions

A spontaneous structural relaxation of salmon gelatin in the glassy state was observed by thermal and mechanical techniques. This behavior was related to a reduction in molecular mobility, as indicated by an increase in relaxation times (τ_0) and a decrease in relaxation 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 in aged films was associated with lower-energy states as thermodynamic equilibrium was approached. This work shows that significant changes in structure can occur within a short time frame. This work will be continued using predictive models for the enthalpy 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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