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A Comparative Study of the Rheological and Structural Properties of Gelatin Gels of Mammalian and Fish Origins

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Summary: From a long time, the traditional source of collagen has been mammals wastes, mainly bovine and porcine skins and bones. This study undertakes a comparative analysis of the viscoelastic behaviour and the structural properties of gelatin gels from both mammalian and fish origins.
All the rheological measurements were carried out within the linear viscoelastic region for gelatin gels and tentatively related to structural characteristics. At a given concentration the pig gelatine exhibits a high storage modulus, G', which indicates that the gel is stiffer than the fish one. The helical structures content measured by the specific rotation, [α], is also higher for the pig gelatin. Moreover, the gel is denatured in the same range of temperature, namely, around 30°C but the transition is sharper for fish gelatine. These differences between the gelatins lie in the amino-acid composition and molecular weight distribution which may influence the nature of the interchain junctions yielding to various gel mesostruture.

Keywords: AFM; gelatine; gel structure; rheology

Introduction

Gelatin is a major industrial protein, especially in the food and pharmaceutical fields, that is obtained by the thermal breaking of collagen. Its uniqueness lies in the ability to form physical thermo-reversible gels upon cooling. Since the recent ESB crisis, there is a growing interest in finding alternative raw materials of different origin. Among others, gelatin that can be isolated from fish collagen rich skins has a great potential as substitute to mammalian source.[1]

The accurate gelation mechanism of gelatin is not yet fully understood.[2,3] However, it is well accepted that gelation results of a conformational transition of gelatin molecules from random coils to collagen-like triple-helical structures which joins several chains together. As renaturation processed, a three-dimentional network is formed that can exhibit different

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mechanical characteristics according to the origin of the gelatine. In this paper we will focus on the comparative properties of pig and fish gelatine from a rheological point of view.

Experimental

The comparison is made between a gelatin derived from tuna skins and a traditional food grade pork gelatin. The viscoelastic behaviour of the gelatin gels was evaluated using a controlled-strain rheometer (HAAKE Rheostress-1). Thermal sweeps, from 10 to 80 °C, were also performed to evaluate gels thermal stability and melting point. The study were carried out in the elastic domain (4 % strain) at an oscillatory frequency of 1Hz. The viscoelastic parameters G', G''and tanδ, of both gels were monitored for various concentrations. The helicoidal content was determined through their specific optical rotation measured by spectropolarimetry at 589 nm. The helix content is given by the following equation:

$$\chi = \frac{[\alpha] - [\alpha]^0}{[\alpha]^0 - [\alpha]^{100}}$$

where $[\alpha]$, $[\alpha]^0$ and $[\alpha]^{100}$ represents respectively the specific rotation of the sample under study, that of native collagen and that of fully amorphous gelatin. AFM experiments were carried out in tapping mode under ambient conditions (50-60% of Relative Humidity). Sample were prepared by depositing 5mL of a 0,1% gel solution, comming from a 1% diluted mother solution, on freshly cleaved mica. The mica was then allowed to gently evaporate in a Petri dish before AFM observation.

Table 1. Main characteristics of fish and pig gelatines.

Constitution/property	**Fish gelatine**	**Pig gelatine**
Water content (%)	12	13
Total protein content (%)	87	84,4
Proline (%)	11,9	13,7
Hydroxyproline (%)	6,4	7,7
Calcium content	310 mg	11 mg
Molecular weight	96k (large fraction of short chains)	110k (less short chains)
Max helix content in 5% gelatine (%)	28	69

Results

The mechanism of gelatine gelation has been extensively studied,[2,3] however, if the presence of triple helix is not controversial, the formation of the thermoreversible network through the associating helix in junction zones is still unclear. The possible association of three different chains ensuring the direct connection between helical zones or, in contrast, the crystallisation-like phenomena occurring through the formation of fringed micelles are two proposed explanation for the gelation mechanism.

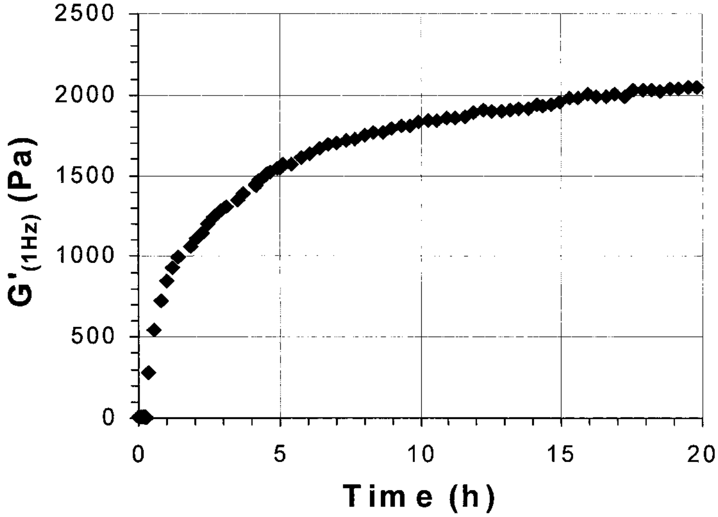

Fig. 1. Time evolution of the storage modulus for 5% fish gelatine at 10°C.

The sol-gel transition can be followed by rheology as observed in Figure 1 by the increase of G'. The kinetic is often described as the sum of two distinct diffusion process, the faster related to cooperative diffusion of the entangled chains in solution and the slower one to the perturbed diffusion of a few aggregates present in the solution. The kinetic of organisation of chains in triple helix as well as the association of these locally organised sequences depends on several factors such as gelatine chemical structure, concentration and temperature. Much more information on the structure of the final gel network can be deduced from the melting behaviour of the gelatine.

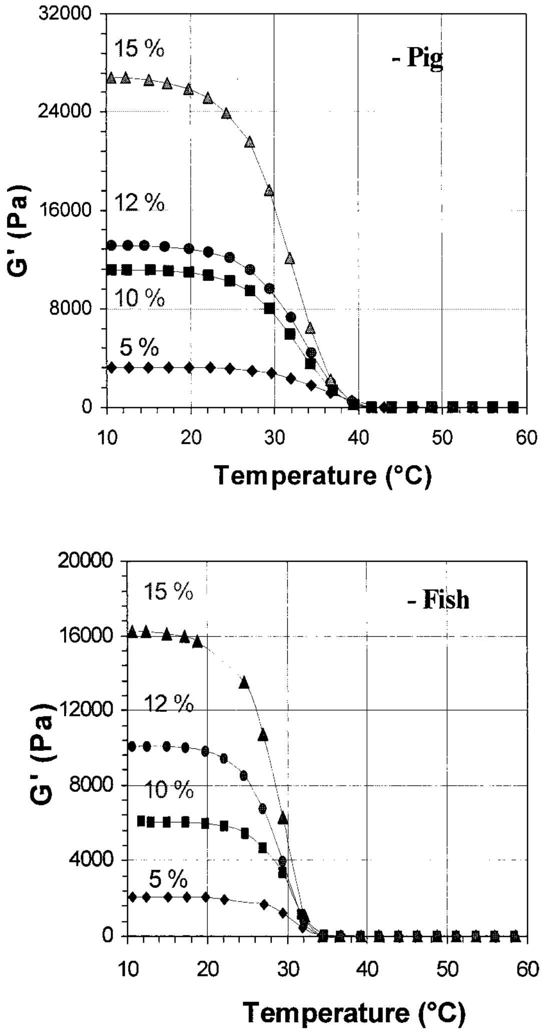

Fig. 2. Storage modulus, G', as function of temperature for various gelatine concentration in water (in %) after 24 h maturation at 10°C. The top curve corresponds to pig gelatine and the bottom one to fish gelatine.

It can be observed in Figure 2 in the lower temperature range that the storage modulus of the gel is strongly related to the gelatine concentration in water. The minimum gelatine content for the network formation has not been measured since it strongly depends on the sensitivity of the technique, but might be above the critical concentration, c*, of the solution which is 0,5%. Whatever the concentration, the pig gelatine exhibits a higher G' than the fish gelatine and the evolution of the storage modulus with concentration follows in both cases a power law with an exponent equal to 1,8 as shown. The stiffness of the gel is therefore higher for the pig gelatine than for fish one but evolve identically with concentration.

The gelatine denaturation, corresponding to the decrease of G' with temperature, occurs around 30°C whatever the gel origin. However, the range of temperature for the gel-sol transition is 15° for the pig gelatine and only 10° for fish gelatine. The exact meaning of this sharper transition for fish gelatine already reported in literature[4] is not yet understood but may be related to the degree of cooperativity required for the process.[5] Actually, it is difficult to draw direct structural information from these rheological observations. The combination of these thermorheological results with the temperature evolution of the helix content might provide further insights.

It can be observed from Figure 3 that the gel-sol transition probed by G' is approximately concomitant with a modification of the specific rotation [α] due to the loss of gelatin chains having helical conformation and, therefore, active optical power. Actually, the temperature induced loss of stiffness is preceded by only a few degrees by quasi-total denaturation of the gelatin. The delay between the rupture of the collagen-like structure and the decrease of the gel modulus is perhaps due to the ability of the gel to partially preserve its network stiffness even with a low remaining degree of junction sections. Other workers have suggested that gelatine sol could retain a certain extent of interchain interaction after melting[6] and that this phenomena is especially observed for pig gelatin. This difference between the sol viscosity of the two gelatins is not observed in our work probably due to a low detection threshold of our rheometer.

The fish gelatin show in Figure 3 a lower maximum helix content (28%) than pig gelatin (69%). This is mainly ascribed to the lower hydroxyproline (Hyp) and proline (Pro) concentration in fish gelatin as shown in Table 1 as compared to pig gelatin. Indeed, the stability of the helical structures has been reported to be proportional to the content of Pro + Hyp rich zones[7] that are important for the helix stabilization by H-bonds an also for the nucleation of intra-chain larger scale organized sequences. Even though, for the two gelatin under investigation the Pro + Hyp content is not strongly different, namely, 18,3 % and 21,4% for fish and pig gelatin, respectively, the effect on the content of collagen-like structures in the gels is significantly enhanced. The high helix content of pig gelatin is consistent with the high G' recorded for the gel at room temperature as previously reported in the literature.

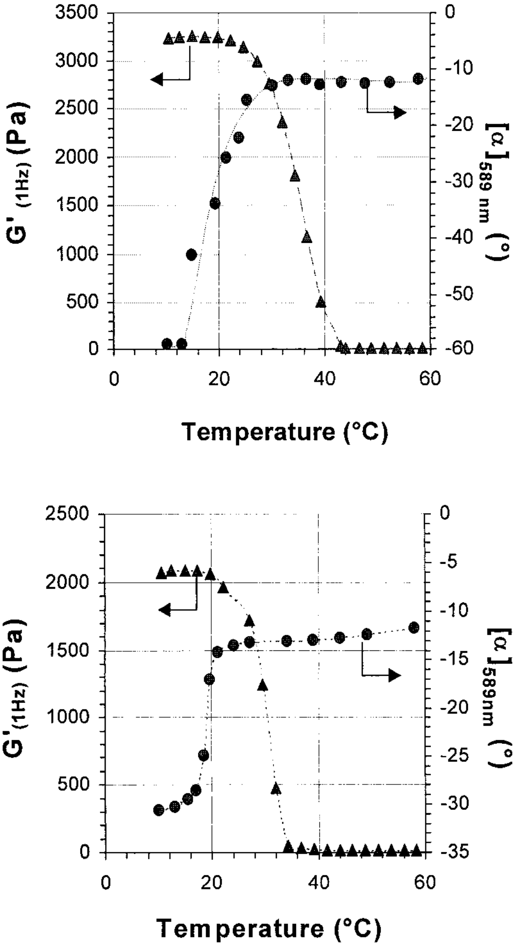

Fig. 3. Evolution of the storage modulus G' (triangles) and the specific rotation [α] (circles) as a function of temperature of various gelatine at 5% in water. The top curve corresponds to pig gelatine and the bottom one to fish gelatine.

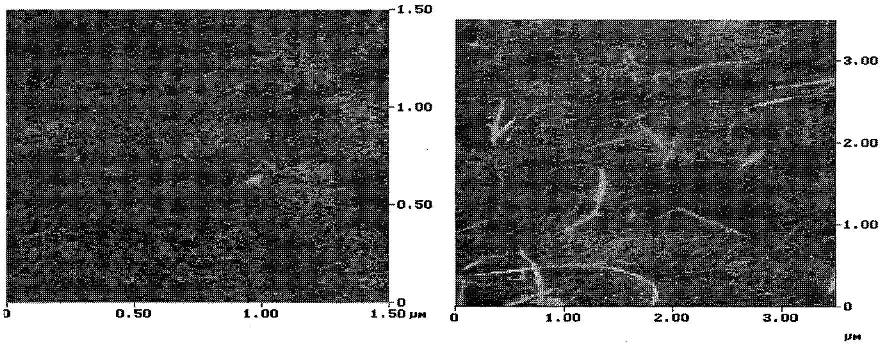

Fig. 4. AFM pictures of thin-films of pig (left) and fish (right) gelatin deposited on mica from a 0.1% gelatin solution.

However, if the storage modulus of a 5% gelatin solution is normalized by the helix content, values of 73,9 and 47 are obtained for the G'/[α] ratio for fish and pig gelatin, respectively. From this point of view, fish gelatin is, therefore, stiffer than pig gelatin per helix unit. This result evidences the importance of others factors than the only helix content on the rheological behaviour of gels. Parameters such as the nature of the helix, namely, type I or type II, presence of short chains, as well as the nature of interchain stabilization that are not taken into account here, must be considered. Actually, the mesostructure of the physical network is the main parameter that might be further investigated. Some structural information might be obtained by AFM investigation of gelatine ultra-thin films. As shown in Figure 4, at low solution concentration of 0.1%, obtained from the dilution of a 5% gel, the surface is covered by the gelatine nano-clusters. In the surface plane, a continuous and homogeneous gel with small aggregates are observed for pig gelatin whereas for fish gelatin, heterogeneous fibrillar structures, with typical lengths between 50 and 500 nm and heights of 5nm remain after denaturation of the gel by dilution.: The presence for fish gelatine of large clusters on the mica surface could result from the a strong structural heterogeneity of the polymer chains in the 0.1% solution). Strong interactions and therefore local order between helical segments seems to prevent total denaturation of the fish gelatin in diluted solution and can be partially correlated with the normalized relative stiffness (G'/[α] ratio) of this gel. Even though the exact nature of the interaction cannot be experimentally determined yet, the large amount of calcium and sodium ions (as shown in table 1) in the fish gelatin can support the assumption of strong ionic interactions favoured between helical sequences in the fish gelatin aggregates. This has to be evidenced in further experiments.

Conclusion

Mammalian and fish gelatins exhibit significant differences in their mechanical (G'), thermal (sharpness of the transition) behaviour and gel structure (helix content). Although, the higher the helix content, the higher the stiffness of the gel, the helix factor cannot alone explain the differences in the thermorheological behaviour of the gels. Differences in the mesostructure arise from the aggregation mechanism between helix containing chains. From first AFM investigations the aggregates present a very different size and shape according to the gelatin origin. Coupling rheology and AFM seems to be a promising approach for a better understanding of gel structure.

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