Korea-Australia Rheology Journal
Vol. 22, No. 2, June 2010 pp. 119-127

Rheological properties of fish skin collagen solution : Effects of temperature and concentration

Min Zhang[1], Yihui Chen[1], Guoying Li[1,*] and Zongliang Du[2]
[1]*National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University, Chengdu 610065, PR China*
[2]*Textile Institute, Sichuan University, Chengdu 610065, PR China*
(Received February 18, 2010; final revision received April 9, 2010; accepted April 19, 2010)

Abstract

To use collagen from freshwater fish skin as an alternative source of mammalian collagens for biomedical applications, we tested the rheological and structural properties of collagen from the skin of largefin longbarbel catfish (*Mystus macropterus*) by dynamic viscoelastic measurements. Fish skin collagen solution (FSCS) exhibited a shear-thinning flow behavior. The complex viscosity (η^*), loss tangent (tan δ) and relaxation time of 1.5% FSCS decreased with the increase of temperature. Also, tan δ decreased while the relaxation time increased with the increase of FSCS concentration. FSCS had considerably lower storage modulus (G'), loss modulus (G''), η^* values and relaxation time and a higher tan δ value than those of bovine skin collagen solution (BSCS). However, FSCS behaved without regularity above 27.5°C, which was in agreement with the result that the dynamic denaturation temperature of this collagen was approximately 29.5°C. These results indicated that temperature and concentration could be tools suitable for adjusting FSCS viscosity. The Arrhenius-type time-temperature superposition (TTS) was applied. In addition, the activation energy of 149.6 KJ mol^{-1} for 1.5% FSCS was calculated according to the Arrhenius equation, indicating a weaker entanglement effect amongst fish skin collagen molecules than that amongst bovine skin collagen molecules, which was in agreement with the results from AFM measurement.

Keywords **:** fish skin, collagen, rheological properties, temperature, concentration

1. Introduction

Collagen is a fibrous protein and the most abundant protein in animals, constituting approximately 30% of total protein (Haug *et al*., 2004). Although the relative amounts of these components vary substantially across tissue types, type I collagen is the chief structural component of most mammalian connective tissue such as tendons, bones and skins (Yang and Kaufman, 2009). It is widely used in fields including foods, medicines, cosmetics, and cell cultures (Yoshimura *et al*., 2000). In most situations, collagen processing involves liquid aqueous preparations. For instance collagen solution was used for medial injection for the repair of dermatological defects (Ford *et al*., 1992) or used to be drug carriers for local application (Toung *et al*., 1999). On the other hand, solutions may also be transferred into solid implants such as sheets, tubes, fibers, powders, eeces, sponges and membranes (Friess, 1998).

Considering that the physical properties of these medical products are closely related to the rheological properties of collagen, it is necessary to carry out rheological measurement to provide some information for processing technique. Amis *et al*. (1985) investigated the effect of pH on collagen flexibility by dilute solution viscoelastic measurements. Friess and Schlapp (2001) showed the influence of pH, temperature, collagen concentration and chemical cross-linking on the rheological behavior of insoluble collagen fiber dispersions with different isoelectrical point. Forgacs *et al*. (2003) studied the assembly of collagen matrices as a sol-gel phase transition by means of rheometry. Yang and Kaufman (2009) studied the self-assembly of bovine collagen initiated by raising the temperature by rheological measurements. Yoshimura *et al*. investigated the viscoelastic properties of alkali-solubilized collagen (2000) and pepsin-solubilized collagen (1999) exacted from shark skin. Lai *et al*. (2008) investigated the effect of concentration and temperature on the rheological behavior of bovine skin collagen solutions, besides, the rheological behavior of collagen dispersion/poly (vinyl alcohol) blends was studied (Lai *et al*., 2007).

At present, the main sources of type I collagen in many fields are limited to those of bovine or porcine dermis. Nevertheless, bovine spongiform encephalopathy (BSE) or

*Corresponding author: liguoyings@163.com

transmissible spongiform encephalopathy (TSE) is a horrifying threat to collagen medical products based on bovine material. Compared to mammalian collagens, fish collagens have relatively low risk of possessing pathogens.

In our previous work, collagen from largefin longbarbel catfish (a temperate freshwater fish) was exacted and partially characterized. Some physicochemistry characteristics such as the composition of amino acid were found to be different between this fish collagen and mammalian collagen (Zhang *et al*., 2009). It is well known that amino acid composition could influence the structure of collagen molecules, while the physical properties such as rheological properties of collagen are significantly influenced by their structures and conformations in solution (Ramachandran, 1988). Therefore, the rheological behaviors between this freshwater fish collagen and mammalian collagen might be quite different. However, there is no information about the different rheological behaviors between a temperate freshwater fish collagen and mammalian collagen. In this paper we focused on the effects of temperature and concentration on the rheological behaviors of collagen solution from largefin longbarbel catfish skin.

2. Experimental

2.1. Materials

As described in our previous paper (Zhang *et al*., 2009), type I collagen was derived from the purified skin of largefin longbarbel catfish (*Mystus macropterus*) with pepsin (EC 3.4.23.1, 1:10,000, Sigma Chemical Co.). The solution obtained was dialyzed against 0.1 M acetic acid, after this step it was lyophilized in a freeze dryer (Labconco FreeZone 2.5L, USA). Bovine skin collagen solution was prepared through a process described by Zhang *et al*. (2006). The solution obtained was also dialyzed against 0.1 M acetic acid and lyophilized in a freeze dryer. Both of the freeze-dried fish skin collagen and bovine skin collagen were stored under the same conditions at 4°C within one month before test.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted according to the method of Laemmli (1970), using 7.5% resolving gel and 4% stacking gel. Both collagens (fish skin collagen and bovine skin collagen) displayed two α bands (α1 and α2) and one β band in the electrophoretic patterns, indicating the similar molecular weight (approximately 300 kDa). The enzymes remove only the non-helical ends (telopeptides) of the collagen. The cutting of the telopeptides region would remove most of the intermolecular cross-links of the collagen (Hickman *et al*., 2000).

2.2. Samples preparation

For the dynamic viscoelastic measurements, fish skin collagen solutions (FSCSs) with concentrations of 1.0, 1.25, 1.5 and 2.0% (w/w) were prepared by dissolving the freeze-dried fish collagen in 0.5 M acetic acid. All solutions were centrifuged at 9000 × *g* for 15 min to remove entrapped air-bubbles.

For the surface morphology measurements, fish skin collagen solutions and bovine skin collagen solutions were prepared by dissolving the freeze-dried collagens into 0.5 M acetic acid. Concentration used for both collagen solutions was 8 μg/ml.

2.3. Investigation on the rheological properties of fish skin collagen

Oscillatory rheological experiments were performed by the method of Lai *et al*. (2008) with slightly modification. The samples were tested on a Rheometer System Gemini 200 (Malvern Instruments, UK). For precise control of sample temperature, a Peltier temperature controller, the range of which was from −30 to 200°C with an accuracy of ±0.1°C, was used.

Effects of temperature on the viscoelastic behaviors of 1.5% FSCS were studied by both dynamic temperature sweeps and dynamic frequency sweeps. The dynamic temperature sweeps for 1.5% collagen solution were conducted within the linear range at a constant strain of 5% and a given frequency of 1 Hz and the solution was heated from 20 to 32.5°C at a rate of 0.5°C/min. The dynamic frequency sweeps for collagen solutions at different temperatures were induced from 0.1 to 10 Hz at a constant strain of 5%. At the designated temperatures (17.5, 20, 22.5, 25, 27.5, and 30°C), each sample was equilibrated for 20 min before measurement. Dynamic frequency sweeps for collagen solutions with different concentrations (1.0, 1.25, 1.5 and 2.0%) were performed from 0.1 to 10 Hz at 20°C at a constant strain of 5%. The storage modulus (G'), loss modulus (G''), complex viscosity (η^*) and the loss tangent (tan $\delta = G''/G'$) were recorded. Duplicate experiments gave the quite similar results and the data reported represent mean values from two replicates.

2.4. Atomic force microscopy

Droplets of collagen solutions (6 μl) were evaporated on mica and dried at room temperature (~20°C) for two days. The surface morphology was examined on the drying samples by Atomic force microscopy (AFM) measurements. The AFM (SHIMADZU SPM 9600, Japan) with a pinpoint (NSG 11, Russia) was operated in the dynamic mode at room temperature (~20°C). Each sample was scanned with a scan rate of 2 Hz.

3. Results and discussion

3.1. The effect of temperature on dynamic rheological properties

Fig. 1 shows how the η^* and tan δ values of 1.5% FSCS

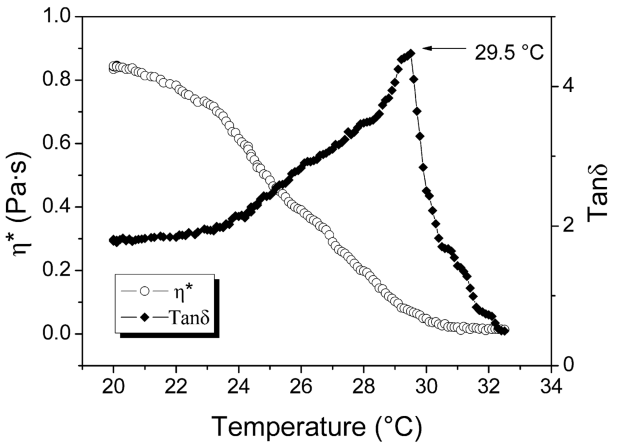

Fig. 1. Temperature dependence of η^* and tan δ for 1.5% fish skin collagen solution.

change as the temperature is increased from 20 to 32.5°C. The η^* values of FSCS decreased with the rising temperature. This phenomenon might attribute to the fact that the energy for heat motion of polypeptide chains increased and thus the resistance to segment motion became weaker as the temperature increased. Within the temperature range from 24 to 30°C, a sudden decrease of η^* in magnitudes and a concomitant rapid increase in tan δ were observed. These obvious changes reflected the collapse of the collagen triple helix to a random coil, that is, the breakage of bonds that stabilizes the secondary structure of collagen (Pietrucha, 2005). The temperature where tan δ reached the peak value could be taken as the denaturation temperature (T_d) under dynamic rheological measurement. Therefore, T_d of fish skin collagen was observed to be 29.5°C (±0.3°C S.D.), which was slightly lower than that measured by DSC (31.6°C). It seemed that the stability of collagen triple helix would be influenced by mechanical treatment. This result indicated that changing the temperature could be an effective treatment for adjusting collagen viscosity for casting and filling.

Under dynamic rheological measurement, the T_d value of FSCS was lower than that of BSCS (32.6±0.1°C S.D.) (Lai *et al.*, 2008). As reported in our previous paper (Zhang *et al.*, 2009), the amino acid composition of collagen from skin of largefin longbarbel catfish was different from that of bovine skin collagen. Therefore, in the acetic acid solution with the same pH (~2.5), the electrostatic repelling force amongst fish skin collagen chains might be different from that amongst bovine skin collagen chains, which led to the weaker stability for fish skin collagen helices.

Additionally, the general amino acid sequence in the type I collagen chain is the repeating Gly-X-Y, where X is often proline and Y is often hydroxyproline. Two different explanations were proposed to explain the stabilizing effect of the Hyp-OH group. The first explanation is a stabilizing effect of water bridges between the Hyp-OH groups and backbone carbonyl groups. Later on, questions were raised about whether these indirect hydrogen bond networks provide stabilizing energy to the peptide (Ramachandran *et al.*, 1973). The second and now more generally accepted explanation is that the stabilization is achieved from the inductive effect of the Hyp-OH group (Jenkins and Raines, 2002). It seems that the Hyp-OH groups play an important role in the stabilization of collagen molecules. For type I collagen from largefin longbarbel catfish skin, the hydroxyproline content was 74 residues/1000 residues, which was lower compared to that of bovine skin collagen (94 residues/1000 residues) (Zhang *et al.*, 2009). The lower content of the Hyp-OH groups led to a relatively lower T_d value for fish skin collagen.

As a function of oscillation frequency (0.1~10 Hz), the viscoelastic behavior of the η^* values of 1.5% FSCS was further studied. As can be seen from Fig. 2a, at T<25°C, the collagen solution exhibited a shear-thinning flow behavior as η^* decreased with the rising frequency. However, the solution behaved without regularity at T>27.5°C. The irregular behavior might reflect the thermal denaturation of collagen. The η^* value of FSCS was lower than that of BSCS. For instance, at 25°C and 10 Hz, η^* was approximately 3.0 Pa·s for 1.5% BSCS (Fig. 2b) whereas only 0.2037 Pa·s for 1.5% FSCS. Sai and Babu (2001) suggested that viscosity of collagen was contributed by inter- and intramolecular hydrogen bonding. In our study, the different viscoelastic behavior of η^* values between the two collagens might be caused by the different amino acid composition including the hydroxyproline content. As mentioned, this difference would influence inter- and intramolecular interactions, and finally influence the viscosity.

The loss tangent (tan δ) crossed the threshold from solid-like to liquid-like behavior (tan δ= 1) (Al-Ruqaie *et al.*, 1997). The smaller the value of tan δ, the more rubbery or elastomeric was the behavior of the material (Korhonen *et al.*, 2001). Fig. 2c shows the loss tangent as a function of oscillation frequency (0.1~10 Hz). The tan δ of 1.5% FSCS increased with the increase of temperature within the temperature range from 17.5 to 25°C. In addition, tan δ> 1 was obtained when the testing temperature was below 25°C, which indicated that 1.5% FSCS exhibited a liquid-like behavior but not a solid-like behavior. Also, the tan δ curves of FSCS were observed without regularity above 27.5°C.

The technique of dynamic oscillation was useful in resolving the structural properties of materials into a solid and a liquid-like response (G' and G'', respectively) (Kasapis and Mitchell, 2001). The value of G' reflects energy stored elastically in the system whereas the value of G'' reflects energy dissipated as a characteristic of the viscous properties (Yang and Kaufman, 2009). According to the relative magnitude of G' and G'', a logarithmic plot of G'

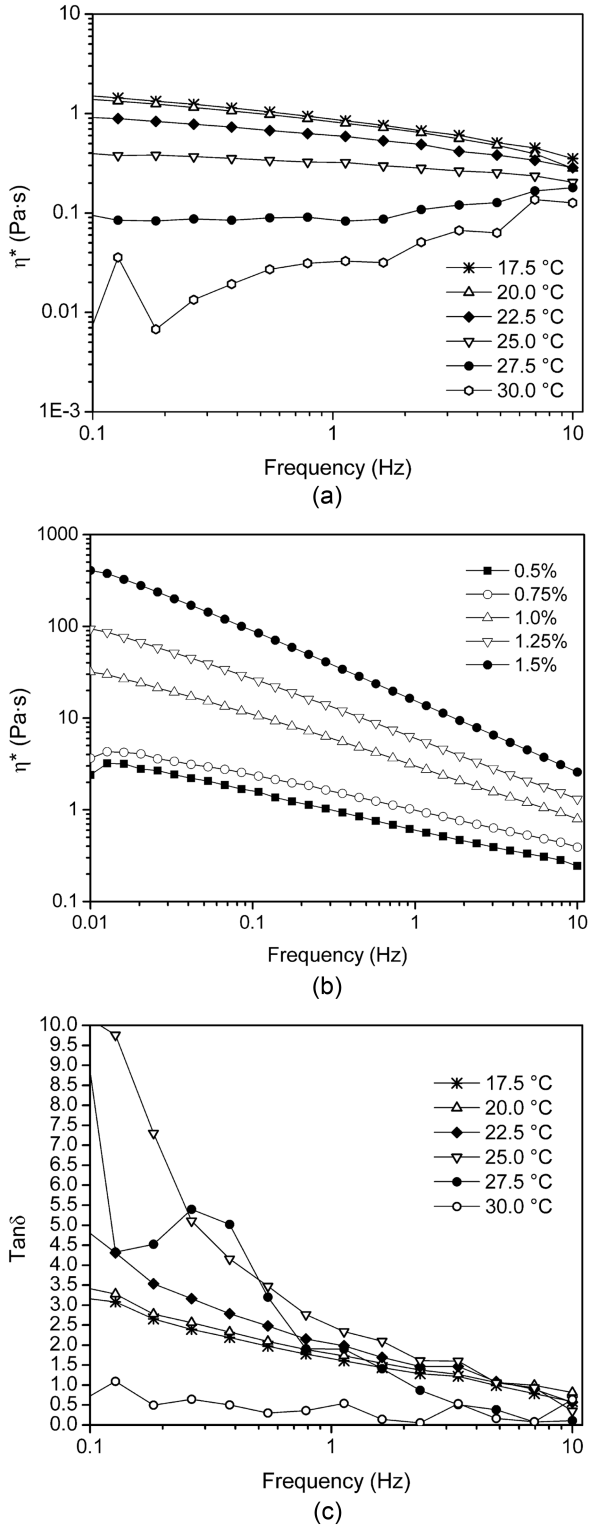

Fig. 2. The complex viscosity η^* (a) and loss tangent (tan δ) (c) for 1.5% fish skin collagen solution at different temperatures. Fig. 2b (Lai *et al.*, 2008) was the complex viscosity η^* of bovine skin collagen solution with different concentrations (w/w) at 25°C, and only the data of 1.5% bovine skin collagen solution was used to compare with that of 1.5% fish skin collagen solution.

and G'' as a function of frequency could be divided into three zones in this order: terminal, plateau and transition zone (Ferry, 1989). For conventional polymers with flexible molecular chains, such behavior is thought to arise from the influences of molecular weight of polymers or entanglements between macromolecules (Hsieh *et al.*, 1999). Fig. 3 presents the linear viscoelastic behavior of G', G'' of 1.5% FSCS at different temperatures as a function of oscillation frequency (0.1~10 Hz). According to the mentioned three-zone model, FSCS behaved as if it is in the terminal zone even though at a high frequency level. For all temperatures below 25°C, initially $G''>G'$, both moduli were very small and increased rapidly with the increase of frequency, but this occurred for G' at a greater rate than for G''. The crossover frequency data where G' was equal to G'' was obtained and found to be somewhat temperature dependent. For the temperatures of 17.5, 20, 22.5 and 25°C, the crossover frequencies of FSCS were 4.72, 5.57, 5.93 and 6.00 Hz, respectively. The rising crossover frequency was indicative of a faster relaxation characteristic (shorter relaxation time) for FSCS when the testing temperature was higher. There were two sets of relaxation characteristics with respect to short-range (between entanglements) and long-range (beyond entanglements) configurational rearrangements, which were thought to be relevant with an entanglement network. The long-range relaxation occurred at the time scale of terminal zone and short-range relaxation occurred at the time scale of transition zone (Hsieh *et al.*, 1999). Thus, the changeover from terminal zone to plateau zone occurred at higher frequencies as the testing temperature increased due to the decreasing number of entanglements, which strengthened the flexibility of collagen chains in solution. Being the same to η^* or tan δ, the G' and G'' curves of FSCS were observed without regularity at T>27.5°C.

BSCS had the viscoelastic behaviors of G', G'' similar to those of FSCS at different temperatures as a function of oscillation frequency. However, under the same testing conditions, the crossover frequencies of BSCS were extremely lower than those of FSCS. At the temperature of 25°C, the crossover frequency data of 1.0% BSCS was 0.046 Hz whereas 5.93 Hz for 1.5% FSCS. This finding suggested that the entanglements between bovine skin collagen molecules were more than those between fish skin collagen molecules even though the latter had a relatively higher concentration. Moreover, the G' value was also lower for FSCS. At 25°C and 10 Hz, the G' value of 1.5% BSCS was more than 100 Pa (Fig. 3g), while the G' value of 1.5% FSCS was approximately 10 Pa (Fig. 3d). Some earlier rheometric studies were performed on the collagen-derived material known as fish gelatin and mammalian gelatin and the lower G' for fish gelatin was explained by the lower content of imino acids (Haug *et al.*, 2004; *Joly-Duhamel et al., 2002*). For fish skin collagen, the lower

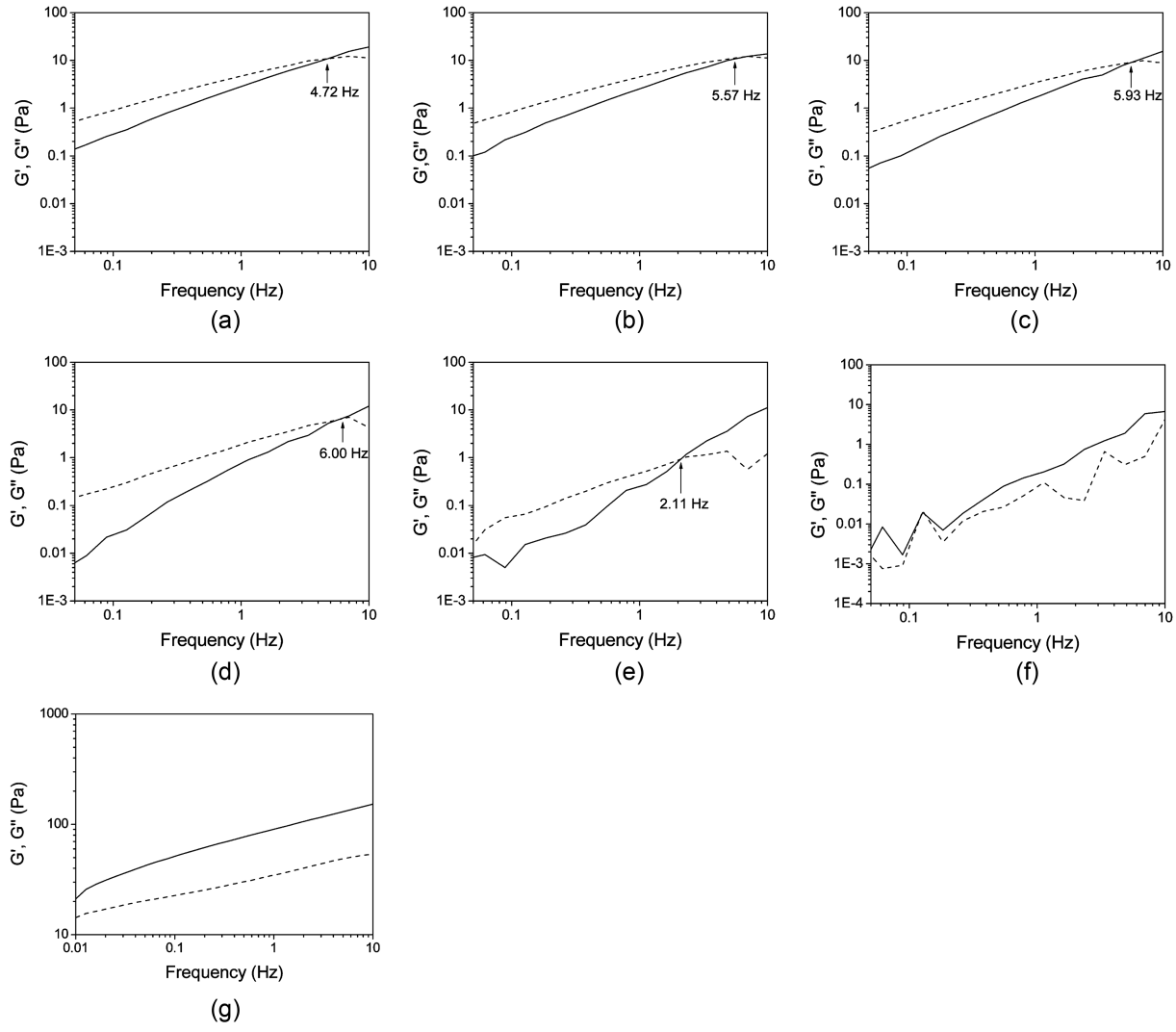

Fig. 3. Dynamic modulus G' (——) and G'' (------) of 1.5% fish skin collagen solution at different temperatures (a) 17.5°C, (b) 20°C, (c) 22.5°C, (d) 25°C , (e) 27.5°C and (f) 30°C. Fig. 3g (Lai *et al.*, 2008) was the dynamic modulus G' (——) and G'' (------) of 1.5% bovine skin collagen solution at 25°C.

content of Hyp-OH groups resulted in a weaker entanglement effect between molecules, giving a weaker elastic effect and a lower G' value for FSCS.

3.2. The effect of concentration on dynamic rheological properties

In order to gain a better understanding of the rheological properties of FSCS, solutions with different concentrations were tested. The typical viscoelastic behavior of the η^* value of FSCS with different concentrations at 20°C as a function of oscillation frequency (0.1~10 Hz) is shown in Fig. 4a. It appeared that all the collagen solutions exhibited a shear-thinning flow behavior as η^* decreased gradually with the rising frequency even though the 1.0% collagen solution behaved somewhat irregular.

The typical viscoelastic behavior of the tan δ of FSCS with different concentrations at 20°C as a function of oscillation frequency (0.1~10 Hz) is shown in Fig. 4b. Below the denaturation temperature, the tan δ of tested collagen solutions decreased with the increase of concentration. However, the tan δ of fish skin collagen remained at a high level (tan $\delta > 1$) even though collagen concentration reached 2.0%. It seemed that fish skin collagen with a relatively high concentration still possessed a relative liquid-like network structure. By contrast, for 1.5% BSCS, a nearly constant tan δ value at a low level with obscure frequency dependence was observed, showing a relative solid-like network structure (Fig. 4c). These results were attributed to the fact that BSCS behaved much more rubbery while FSCS had a much more viscous behavior and the viscoelastic behaviors of the two collagens seemed extremely different.

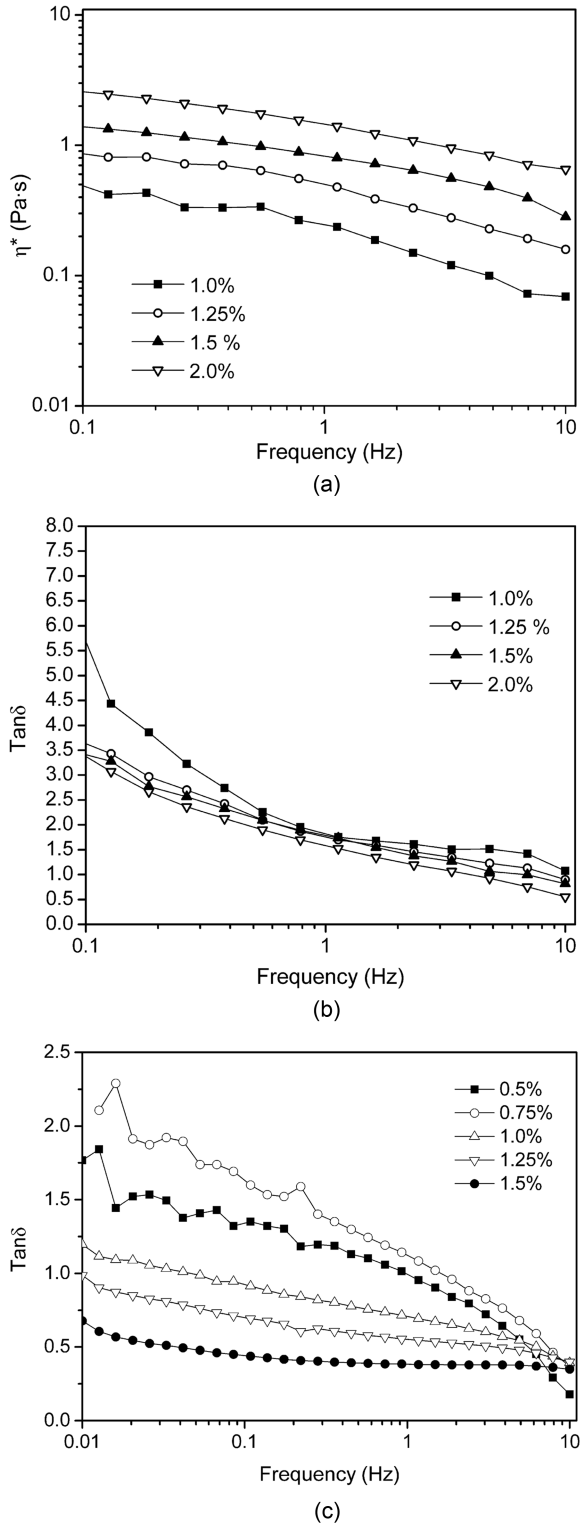

Fig. 4. The complex viscosity η^* (a) and loss tangent (tan δ) (b) of fish skin collagen solutions with different concentrations (w/w) at 20°C. Fig. 4 (c) (Lai *et al*., 2008) was the loss tangent (tan δ) of bovine skin collagen solutions with different concentrations (w/w) at 25°C, and only the data of 1.5% bovine skin collagen solution was used to compare with that of 1.5% fish skin collagen solution.

Fig. 5 presents the linear viscoelastic behavior of G', G'' of FSCS with different concentrations at 20°C as a function of oscillation frequency (0.1~10 Hz). According to the three-zone model, FSCS with different concentrations behaved as if it is in the terminal zone even though at a high frequency level, which was different from the behavior of BSCS. As can be seen from Fig. 3g, the dynamic rheological response of 1.5% BSCS behaved as it was entirely in plateau zone which represented the effect of entanglements (Lai *et al*., 2008). For all selected concentrations at 20°C, both G' and G'' increased with rising frequency. For the concentration of 1.0, 1.25, 1.5 and 2.0%, the crossover frequency data occurred at >10, 8.32, 5.57 and 3.96 Hz, respectively. The decreasing crossover frequency indicated slower relaxation characteristics (long relaxation time) for FSCS with higher concentration. The behavior for collagen with relatively low concentration (*e.g*., 1.0%) was inclined to exhibit faster relaxation characteristics (short relaxation times) as flexible chains. M. Raspanti *et al*. (2007) reported that the aggregation of collagen molecules depended on the initial concentration of collagen. As the concentration increased, the interactions between collagen chains might be stronger due to the aggregation. Finally, the collagen molecules entangled gradually and their motion was constrained. For this reason, FSCS exhibited slower relaxation characteristics (long relaxation time) and the decreased crossover frequencies were observed when the concentration was increased from 1.0 to 2.0%.

In the terminal zone, homo-polymers or Newtonian fluids show the characteristic slopes of 2 for G' and 1 for G'' versus frequency on logarithmic plot (Hsieh *et al*., 1999). As the results from Fig. 5, the slopes of log G' and log G'' versus log ω, decreasing with the increase of collagen concentration, were in the small ranges from 1.29 (±0.05 S.D.) to 1.03 (±0.04 S.D.) and 0.99 (±0.04 S.D.) to 0.65 (±0.02 S.D.), respectively (data not shown), indicating the similar network structure of the samples. However, compared to the corresponding slopes of no more than 0.8 and 0.6 for BSCS (Lai *et al*., 2008), both the slope values of FSCS were greater. These results indicated a weaker linked network for fish skin collagen in solution. The corresponding values of the typical slope for homo-polymers or Newtonian uids are 2 and 1, respectively. Therefore, fish skin collagen exhibited a non-Newtonian behavior, which might be influenced by its long chains and high molecular weight.

3.3. The time-temperature superposition (TTS)

The master curve was assumed to represent polymer behavior over a wide frequency or time range, allowing prediction of long-term behavior from short-term tests (Heymans, 2003). Based on the principle of "Time-Temperature Superposition", the master curve was obtained by selecting a reference curve at the desired temperature (T_0), and then shifting the data at other temperatures (T) to the

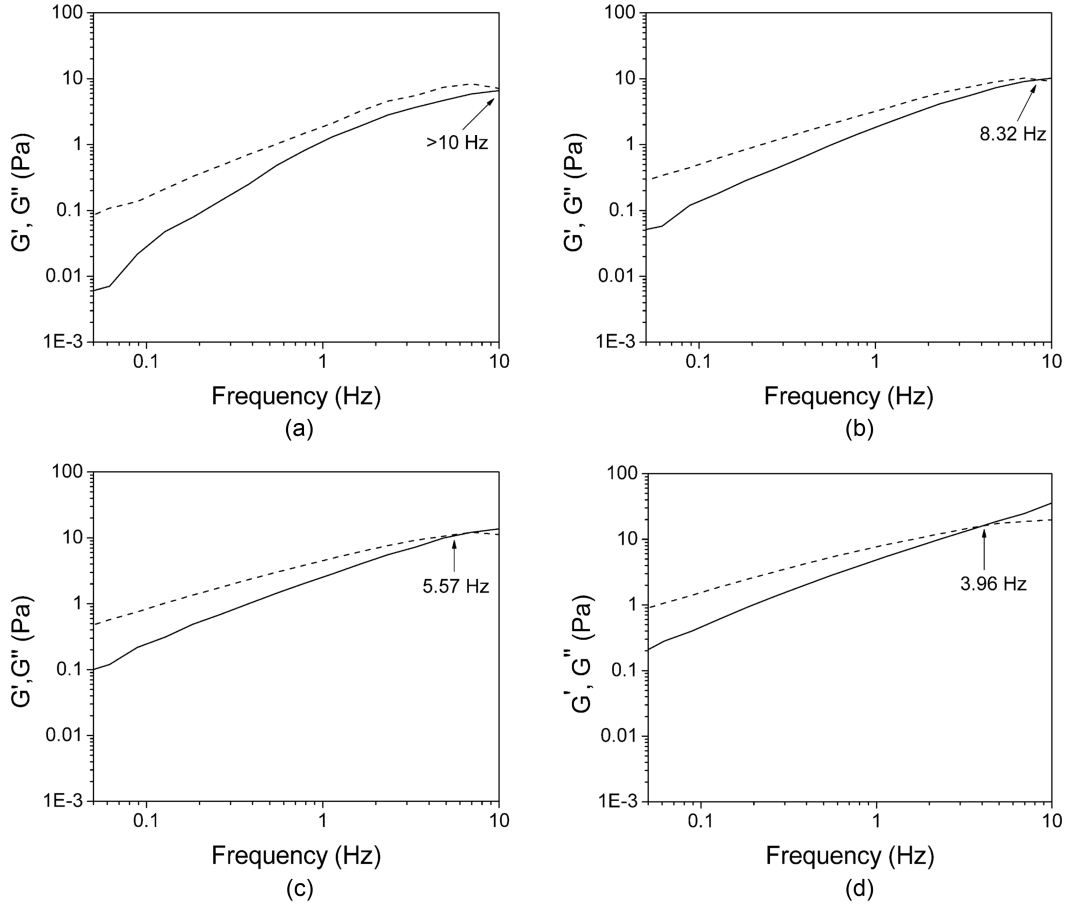

Fig. 5. Dynamic modulus G' (——) and G'' (------) of fish skin collagen solutions at 25°C with different concentrations (a) 1.0%, (b) 1.25%, (c) 1.5% and (d) 2.0%.

reference temperature curve (by horizontally shifting along the frequency axis and then overlapping on the curves at the reference temperatures). The shift distance along the frequency axis is called the frequency-temperature shift factor (Vaidyanathan *et al*., 2003). The time-temperature superposition curve is constructed by empirical shifts of data and implemented in Fig. 6, with 22.5°C as the reference temperature. Obviously, the curve exhibited a remarkable transition from the terminal zone to the plateau zone with a wide frequency roughly range from 10^{-1} Hz to $10^{1.5}$ Hz. However, as shown earlier by Lai *et al*. (2008), the frequency data obtained at 27.5°C did not superimpose well. Moreover, the frequency data obtained at 30°C failed to superpose, which reflected the different relaxation times involved in denaturation and phase transition. The activation energy was calculated by the Arrhenius equation:

$$\ln\alpha_T = \frac{E}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right) \quad (1)$$

in which R is the universal gas constant (8.314 J mol^{-1} K^{-1}), α_T is the shift factor, and E is identified as the activation energy for the jump of a molecule from one equilibrium

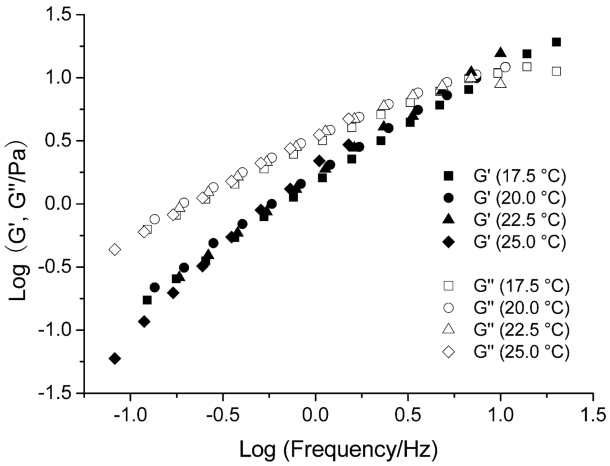

Fig. 6. Master curve of dynamic modulus (G' and G'') for 1.5% fish skin collagen solution at the reference temperature of 22.5°C.

position in the solution to the next (Monkos, 1996). For collagen molecules, E was associated with the relaxation transition. The activation energy of 149.6 KJ mol^{-1} for 1.5% FSCS was calculated from the linear regression plotting of

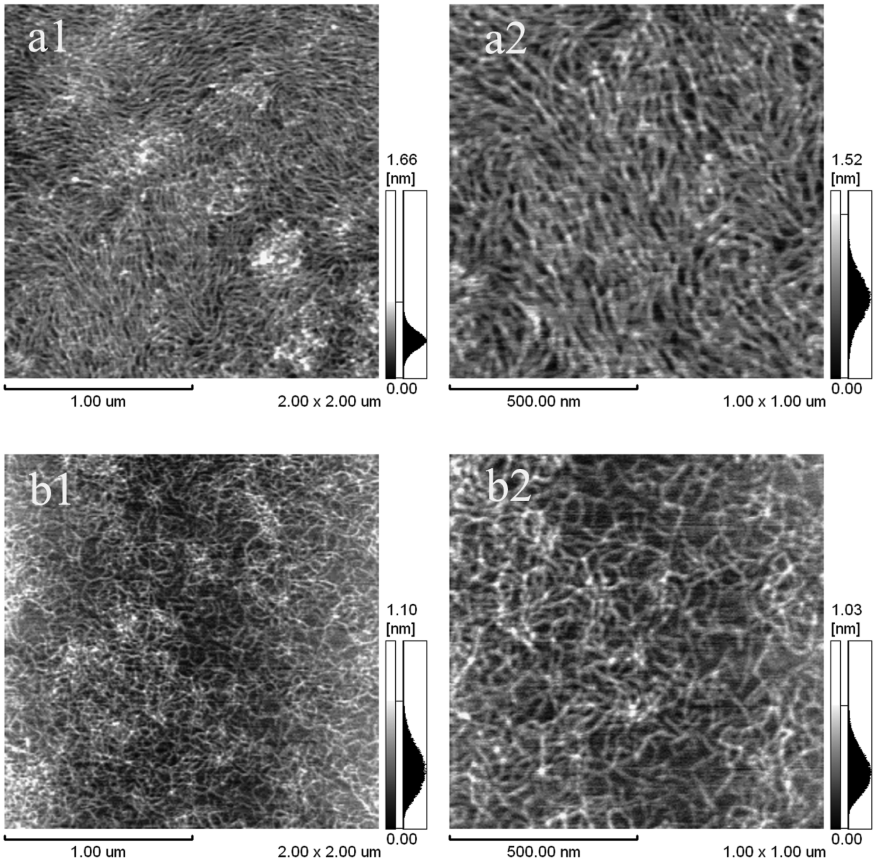

Fig. 7. AFM images of bovine skin collagen (size 2 μm × 2 μm and 1 μm × 1 μm; for a1 and a2, respectively) and fish skin collagen (size 2 μm × 2 μm and 1 μm × 1 μm; for b1 and b2, respectively). Light levels in the images correspond to higher height. Similar images were obtained in different regions of at least three different regions.

ln α_T versus $1/T$ according to this equation. As previously reported by Lai *et al*. (2008), the activation energy of 1.0% BSCS was 161.4 KJ mol^{-1}, which was higher than that of 1.5% FSCS. Considering that the activation energy reflected the need to overcome an energetic barrier of local rearrangements from one state to the other, the lower values of the activation energy for FSCS was in line with the findings that fish skin collagen molecules were easier to motion due to the weaker entanglement network.

3.4. AFM images

AFM could contribute to the understanding of collagen structure and conformation in solution. Fig. 7 shows AFM images of drying samples of bovine skin collagen (Fig. 7 a1 and a2) and fish skin collagen (Fig. 7 b1 and b2). It seemed that collagen molecules assembled on mica, where many fibers lay and were overlapped with each other. The length or diameter of these fibers was greater than those of monomolecular of collagen (1.5 nm × 300 nm). This phenomenon indicated that self-assembly of collagen occurred even though the concentration was at very low level. However, there were still some differences in AFM images between the two collagens observed. The fiber length or diameter of bovine skin collagen was greater than that of fish skin collagen. Furthermore, it seemed that the molecules of bovine skin collagen underwent reorientation while curved fibers were observed for fish skin collagen. It could be assumed that the stronger entanglement effect amongst bovine skin collagen molecules promoted and stabilized the assembly of lateral microfibers, and the longitudinal assembly of microfibers.

4. Conclusions

In conclusion, the features of rheological behaviors of FSCS were different from those of BSCS. The former gave lower modulus, η^* value, relaxation time and activation energy and a higher tan δ value, which indicated that the entanglement effect amongst fish skin collagen molecules was weaker than that amongst bovine skin collagen molecules. The different rheological behaviors might be brought about by the different primary structures between collagens from the skins of largefin longbarbel catfish and bovine. Also, it was suggested by the lower dynamic denaturation temperature (29.5°C) than that of BSCS (32.6°C) that FSCS should be processed under a lower temperature.

FSCS viscosity could be adjusted effectively by increasing the temperature carefully below 29.5°C during the treatment. The surface morphology examined with AFM indicated a weaker entanglement effect amongst fish skin collagen molecules, which was consistent with the results from dynamic viscoelastic measurements.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 20876098/ B060805).

References

Al-Ruqaie, I.M., S. Kasapis, and R. Abeysekera, 1997, Structural properties of pectin-gelatin gels. Part II: effect of sucrose/glucose syrup, *Carbohydr. Polym.* **34**, 309-321.

Amis, E.J., C.J. Carriere, J.D. Ferry, and A. Veis, 1985, Effect of pH on collagen flexibility determined from dilute solution viscoelastic measurements, *Int. J. Biol. Macromol.* **7**, 130-134.

Ferry, J.D. 1989, Viscoelastic Properties of Polymers, Wiley, New York, p. 369.

Friess, W. 1998, Collagen-biomaterial for drug delivery, *Eur. J. Pharm. Biopharm.* **45**, 113-136.

Friess, W. and M. Schlapp, 2001, Effects of processing conditions on the rheological behavior of collagen dispersions, *Eur. J. Pharm. Biopharm.* **51**, 259-265.

Ford, C.N., D.M. Bless, and J.M. Loftus, 1992, Role of injectable collagen in the treatment of glottic insufficiency: a study of 119 patients, *Ann Otol Rhinol Laryngol.* **101**, 237-247.

Forgacs, G., S.A. Newman, B. Hinner, C.W. Maier, and E. Sackmann, 2003, Assembly of collagen matrices as a phase transition revealed by structural and rheologic studies, *Biophys. J.* **84**, 1272-1280.

Haug, I.J., K.I. Draget, and O. Smidsrød, 2004, Physical and rheological properties of fish gelatin compared to mammalian gelatin, *Food Hydrocolloid.* **18**, 203-213.

Heymans, N. 2003, Constitutive equations for polymer viscoelasticity derived from hierarchical models in cases of failure of time-temperature superposition, *Signal Process.* **83**, 2345-2357.

Hickman, D., T. J. Sims, C. A. Miles, A. J. Bailey, M. de Mari, and M. Koopmans, 2000, Isinglass/collagen: Denaturation and functionality, *J. Biotech.* **79**, 245-257.

Hsieh, T.T., C. Tiu, G.P. Simon, and R.Y. Wu, 1999, Rheology and miscibility of thermotropic liquid crystalline polymer blends, *J. Non-Newtonian Fluid Mech.* **86**, 15-35.

Jenkins, C.L. and R.T. Raines, 2002, Insights on the conformational stability of collagen, *Nat. Prod. Rep.* **19**, 49-59.

Joly-Duhamel, C., D. Hellio, A. Ajdari and M. Djabourov, 2002, All gelatin networks: 2. The master curve for elasticity, *Langmuir.* **18**, 7158-7166.

Kasapis, S. and J.R. Mitchell, 2001, Definition of the rheological glass transition temperature in association with the concept of iso-free-volume, *Int. J. Biol. Macromol.* **29**, 315-321.

Korhonen, M., L. Hellen, J. Hirvonen, and J. Yliruusi, 2001, Rheological properties of creams with four different surfactant combinations-effect of storage time and conditions, *Int. J. Pharm.* **221**, 187-196.

Laemmli, U.K. 1970, Cleavage of structural proteins during assembly of head of bacteriophage T4, *Nature.* **277**, 680-685.

Lai, G.L., Y. Li, and G.Y. Li, 2008, Effect of concentration and temperature on the rheological behavior of collagen solution, *Int. J. Biol. Macromol.* **42**, 285-291.

Lai, G.L., Z.L. Du, and G.Y. Li, 2007, The rheological behavior of collagen dispersion/poly(vinyl alcohol) blends, *Korea-Australia Rheol J.* **19**, 81-88.

Monkos, K. 1996, Viscosity of bovine serum albumin aqueous solutions as a function of temperature and concentration, *Int. J. Biol. Macromol.* **18**, 61-68.

Pietrucha, K. 2005, Changes in denaturation and rheological properties of collagen-hyaluronic acid scaffolds as a result of temperature dependencies, *Int. J. Biol. Macromol.* **36**, 299-304.

Ramachandran, G.N. 1988, Stereochemistry of collagen, *Int. J. Peptide Protein Res.* **31**, 1-16.

Ramachandran, G.N., M. Bansal, and R.S. Bhatnagar, 1973, A hypothesis on the role of hydroxyprohne in stabilizing collagen structure, *Biochim. Biophys. Acta.* **322**, 166-171.

Raspanti, M., M. Viola, M. Sonaggere, M.E. Tira, and R. Tenni, 2007, Collagen bril structure is affected by collagen concentration and decorin, *Biomacromolecules.* **8**, 2087-2091.

Sai, K.P. and M. Babu, 2001, Studies on Rana tigerrina skin collagen, *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.* **128**, 81-90.

Toung, J.S., R.C. Ogle, R.F. Morgan, and W.H. Lindsey, 1999, Insulinlike growth factor 1- and 2-augmented collagen gel repair of facial osseous defects, *Arch. Otolaryngol. Head Neck Surg.* **125**, 451-455

Vaidyanathan, T.K., J. Vaidyanathan, and Z. Cherian, 2003, Extended creep behavior of dental composites using time-temparature superposition principle, *Dent. Mater.* **19**, 46-53.

Woessner Jr, J.F, 1961, The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid, *Arch. Biochem. Biophys.* **93**, 440-447.

Yang, Y.L. and L.J. Kaufman, 2009, Rheology and confocal reflectance microscopy as probes of mechanical properties and structure during collagen and collagen/hyaluronan self-assembly, *Biophys. J.* **96**, 1566-1585.

Yoshimura, K., M. Terashima, D. Hozan, and K. Shirai, 2000, Preparation and dynamic viscoelasticity characterization of alkali-solubilized collagen from shark skin, *J. Agric. Food Chem.* **48**, 685-690.

Yoshimura, K., Y. Chonan, and K. Shirai, 1999, Preparation and dynamic viscoelastic characterization of pepsin-solubilized collagen from shark skin compared with pig skin, *J. Anim. Sci.* **70**, 227-234.

Zhang, M., W.T. Liu, and G.Y. Li, 2009, Isolation and characterization of collagens from the skin of largefin longbarbel catfish (*Mystus macropterus*), *Food Chem.* **115**, 826-831.

Zhang, Z.K., G.Y. Li, and B. Shi, 2006, Physicochemical Properties of Collagen, Gelatin and Collagen Hydrolysate derived from Bovine Limed Split Wastes, *J. Soc. Leather Technol. Chem.* **90**, 23-28.