Rheological and functional properties of gelatin from the skin of Bigeye snapper (*Priacanthus hamrur*) fish: Influence of gelatin on the gel forming ability of fish mince

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Abstract

The rheological and functional properties of gelatin from the skin of bigeye snapper (*Priacanthus hamrur*) fish were assessed. The protein content of dried gelatin was 94.6% and moisture content was 4.2 %. The amino acid profile of gelatin revealed high proportion of glycine and imino acids. The bloom strength of solidified gelatin was 108 g. The average molecular weight of fish skin gelatin was 282 kDa as determined by gel filtration technique. The emulsion capacity (EC) of gelatin at a concentration of 0.05% (w/v) was 1.91ml oil /mg protein and with increase in concentration, the EC values decreased. The gelling and melting temperatures of gelatin were 10^{o} and 16.8^{o}C respectively as obtained by small deformation measurements. The flow behavior of gelatin solution as a function of concentration and temperature revealed non-Newtonian behavior with pseudoplastic phenomenon. The Casson and Herschel-Bulkley models were suitable to study the flow behavior. The yield stress was maximum at 10^{o}C with the concentration of 30 mg / ml. Thermal gelation behavior of threadfin bream (*Nemipterus japonicus*) mince in presence of different concentration of gelatin was assessed. Gelatin at a concentration of 0.5% yielded higher storage modulus (G') value than control. Frequency sweep of heat set gel with gelatin revealed strong network formation.

Key words: **gelatin, fish skin, rheology, functional properties**

1. Introduction

Gelatin is a polypeptide derived by hydrolytic degradation of collagen, the principle component of animal connective tissue. Gelatin is considered a highly digestible dietary food ideal as a complement in certain types of diet. Gelatin has found application in food, photographic, cosmetic and pharmaceutical industries over the years. Recently, its use is expanding to new applications such as colloid stabilizer, foaming agent and emulsifier (McClements, 2005 and Surh, Decker and McClements, 2006)

The source and type of collagen will influence the properties of the resulting gelatins. The main raw material for gelatin production is skin and bones from bovine and porcine source. With the outbreak of Bovine Spongiform Encephalopathy (BSE) in bovine animals, there has been an interest in the gelatin production from non-bovine source (Badii & Howell, 2006). Fish processing waste can form an ideal raw material for gelatin preparation as fish skin and bone is a major by-product of the fish-processing industry (Haug, Draget & Smidsrod, 2004). The utilization of fish skin for the production of gelatin could add value to the processing waste. (Kristinsson & Rasco, 2000 and Sato, Katayama, Sawabe & Saeki, 2003).

The quality of food grade gelatin depends largely on its thermal and rheological properties (Gimenez, Gomez-Guillen & Montero, 2005). The gel strength, viscosity, setting behavior and melting point of gelatin depends on their molecular weight distribution and the amino acid composition (Johnston-Banks, 1990). Appropriate rheological properties are required for many applications and are related to their chemical

characteristics. Competitive gelling agents like starch, alginate, pectin, agar, carrageenan etc. are all carbohydrates from vegetable sources, but their gels lack the melt in the mouth and elastic properties of gelatin gels.

A number of studies have been devoted to the processing and functional properties of fish gelatin. The gelatins were prepared either from skin, bone or cartilage and mantle of squid. The species of fish that were used for gelatin production were: lumpfish (Osborne, Voight & Hall, 1990), tilapia (Grossman & Bergman, 1992 and Jamilah & Harvinder, 2002), conger eel and arrow squid (mollusk) (Kim & Cho, 1996), shark (Yoshimura et al., 2000), megrim (Montero &Gomez-Guillen, 2000), cod (Gomez-Guillen, Turney, Fernandez-Diaz, Olmo, Lizarbe & Montero, 2002), nile perch (Muyonga, Cole & Duodu, 2004), shark cartilage (Cho et al., 2004), pollock (Zhou & Regenstein, 2004), yellow fin tuna (Cho, Gu & Kim, 2005), skate (Cho, Jahncke, Chin & Eun, 2006), catfish (Yang, Wang, Jiang, Oh, Herring & Zhou, 2007), sin croaker and shortfin scad (Cheow, Norizah, Kyaw and Howell, 2007). The available literature suggests that rheological properties of gelatins from fish skin have not been fully evaluated with reference to flow behavior. It is well recognized that rheological properties play a vital role in process design, evaluation and modeling. These properties are sometimes measured as an indicator of product quality. Rheological data are required for calculation in any process involving fluid flow and play an important role in the analysis of flow conditions in different food processing operations. The use of gelatin from fish skin in different product formulation calls for deeper understanding of various rheological properties with reference to concentration and temperature.

The application of non-meat proteins as functional ingredients to improve the gelling ability of myofibrillar proteins have been assessed (Ensor, Mandigo, Calkins & Quint ,1987, Alvarez, Smith, Morgan & Booren, 1990, Slavin, 1991, Atughonu, Zayas, Herald & Harbers ,1998, Chin, Keeton, Miller, Longnecker & Lamkey, 2000 and Hsu & Lung-Yueh Sun, 2006). Many carbohydrate hydrocolloids are widely used in a variety of comminuted meat products for their ability to enhance gelling character and retain water and to provide a desirable texture (Barbut & Mittal, 1996, Gomez-Guillen, Solas, Borderias & Montero, 1996 and Montero, Hurtado & Perez-Mateos, 2000). However, very limited research has been undertaken on protein biopolymer like gelatin obtained from fish and their behavior in fish gelled products.

In the present investigation, gelatin from the skin of bigeye snapper (*Priacanthus hamrur*) fish was prepared and its properties were assessed. The skin of the bigeye snapper is relatively thick and can form an ideal raw material for gelatin production. The objectives of the present study was to prepare gelatin from the skin of bigeye snapper and characterize for physico-chemical and rheological properties. Further, gelatin at various concentrations was incorporated to mince from threadfin bream (*Nemipterus japonicus*) fish and gelling characteristics were assessed by small deformation test.

2. Material and methods

2.1. *Gelatin preparation*

Skin of fresh bigeye snapper (*Priacanthus hamrur)* was used for gelatin preparation according to the method as described by Badii and Howell (2006). The fish was caught by trawl net during the month of October along the coast of Mangalore, West

coast of India. The habitat temperature where the fish was harvested ranged from 26^{o}-27^{o}C.

2.2. *Proximate composition*

The moisture, ash and fat content of fresh fish skin and extracted gelatin was determined according to the method as described by AOAC (2006). The crude protein content was determined by estimating its total nitrogen content by Kjeldahl method (AOAC, 2006). A factor of 5.8 was used to convert the nitrogen value to protein. The yield of gelatin was calculated based on wet weight of fresh skin using the following formula

$$\text{Yield of gelatin (\%)} = \frac{\text{Weight of freeze dried gelatin} \times 100}{\text{Wet weight of fresh skin}}$$

2.3. *Amino acid analysis*

Amino acid composition of gelatin was determined after derivatization with phenylisothiocyanate (PITC) according to the Waters Pico-Tag method as described by Bidlingmeyer, Cohen and Tarvin (1987) using the Waters Pico-Tag HPLC amino acid analyzer (Water Model 712 WISP, Waters, Watford, Herts, UK).

2.4. *Nitrogen solubility index (NSI)*

The gelatin samples were separately dissolved in distilled water in the ratio of 1:100 (gelatin: distilled water). The pH of the gelatin solution was adjusted ranging from 2.0 to 10.0 with either 1.0 M HCl or 1.0 M NaOH using a pH meter (Systronics, Model 361, Ahemdabad, India). The samples were centrifuged at 9000 x g using high-speed refrigerated centrifuge (IEC B-22, International Equipment Company, Needham Heights,

USA) for 15 minutes at 4^{o}C. The final pH of the supernatant was measured. Nitrogen content of clear supernatant was determined by Kjeldahl method and was expressed as percentage of total nitrogen in the gelatin sample taking into account of the volume of acid / alkali consumed at each pH level. NSI was obtained by plotting pH values verses percentage of total nitrogen in the sample solubilized at each pH values measured.

2.5. *Solubility of proteins as a function of sodium chloride concentration*

Gelatin samples were separately dissolved in phosphate buffer pH 7.5, 50 mM containing different concentration of sodium chloride (0 - 2.0 M). The ratio of gelatin to buffer used was 1: 100 (gelatin: buffer). The gelatin solution was centrifuged at 9000 × g for 15 min at 4ºC. The total nitrogen content of the clear supernatant was determined by Kjeldahl method. Nitrogen value obtained was multiplied by a factor of 5.8 to obtain the protein content and was expressed as the percentage of total gelatin. A plot of protein solubility as percentage of total protein in the gelatin sample versus molar concentration of sodium chloride was obtained.

2.6. *Molecular weight analysis by Gel filtration technique*

The molecular weight analysis of gelatin was carried out by gel filtration technique using a Sepharose- 6B gel packed in a column of 1.5 x 80 cm (dia x height). The eluant used was phosphate buffer (50 mM, pH 7.5) containing 0.3 M sodium chloride. The flow rate was adjusted to 25 ml / hr. Total bed volume of the column was 150 ml and the void volume determined by using blue dextran (2 mg / ml) was found to be 60 ml. Standard molecular weight markers supplied by Sigma Chemicals (Bovine serum albumin (66 kDa) 10 mg / ml, β-Amylase (200 kDa) 4 mg / ml, Thyroglobulin

(669 kDa) 8 mg / ml) were loaded separately. Gelatin was loaded to the column at a concentration of 10 mg / ml. Fractions of 3.0 ml were collected manually in a series of test tubes. The protein concentration of the fractions was determined by taking absorbance at 280 nm (Systronics UV-VIS Spectrophotometer 119, Ahmedabad, India). Peak elution volume was determined for the protein samples. A calibration curve was obtained by plotting log Molecular weight *vs* peak elution volume. The average molecular weight of gelatin was determined from the standard curve.

2.7. *Sodium Dodecyl Sulphate - Poly Acrylamide Gel Electrophoresis (SDS-PAGE)*

The SDS-PAGE of gelatin was carried out under reduced condition according to the method as described by Laemmli (1970). Electrophoresis was carried out using poly acrylamide gel slabs of 10 × 8 cm (length × width) in a vertical slab gel electrophoresis apparatus (SE 250 Hoefer –Pharmacia Biotech Inc. San Francisco, Calif., U.S.A.). The concentration of acrylamide was 10% and the thickness of the gel was 0.75 mm. The gel was stained in Coomassie Brilliant blue-R-250. The molecular weight of the protein bands obtained in the sample was approximated by measuring the relative mobility of the standard protein molecular weight markers (high molecular weight markers from Sigma, St.Louis, MO, USA).

2.8. *Emulsion Capacity (EC)*

The emulsion capacity of gelatin solution at different concentrations (0.05, 0.1 and 0.2 % w/v) was determined by the method as detailed by Swift, Lockett and Fryer (1961) with slight modification. Dry gelatin was dissolved in phosphate buffer (50 mM) containing 0.3 M sodium chloride (pH 7.5). About 25 ml of gelatin solution at different

concentrations were mixed thoroughly with 75 ml of refined sunflower oil (ITC, Mumbai, India) at 9000 rpm for 10 seconds using Ultra Turrax homogenizer (Ultra-Turrax, T 25, Janke & Kunkel GMBH & Co. KG Staufen, Germany). Homogenization was continued at a high speed (23,000 rpm) with addition of oil at a rate of 0.5-0.6 ml/ sec until phase inversion was observed visually. Protein content of the gelatin solutions was determined by Kjeldahl method. Emulsion capacity was calculated after considering the initial volume of oil added and expressed as ml oil / mg protein. The EC was expressed as ml oil / mg protein and the average of four replicates were taken as the emulsion capacity of the sample.

2.9. *Setting index of gelatin*

Different concentrations of gelatin *viz*. 10, 20, 30 and 40 mg / ml was prepared in test tubes of dimension 2 x 15 cm (dia x height) and kept at 5^{o}C for different durations. The volume of gelatin solution used was 20 ml. At periodic intervals of 5 min, visual observation was made for solidification process. The solidification process was given index depending on the extent of solidification. A maximum index of '100' was taken as complete solid and '0' as complete liquid. A plot of time in minutes and setting index was obtained.

2.10. *Determination of bloom strength of gelatin gels*

Bloom value was determined using a TA-XT2 Texture analyzer (Stable Microsystems, Godalming, UK) according to the method described by Stable Micro System. Gelatin at a concentration of 6.67% (w/v) was used for bloom strength measurements. The solution was stirred with a glass rod, covered and allowed to stand at

198 room temperature (not more than 22^{o}C) for 3 hr. The mixture was heated in a water bath
199 at less than 60^{o}C and stirred on a magnetic stirrer for 15 min to dissolve the gelatin
200 completely. Gelatin solution was immediately poured into standard bloom jar
201 (SCHOTTGLAS. Mainz. Bloom test vessel. Product No. 2112501) of dimension 6 x 8
202 cm (bottom dia x height), over which a cover was placed. After 2 min, bloom jars were
203 kept in a water bath (10^{o}C) overnight (17 hr), and immediately tested using Stable
204 Microsystems TA-XT2 texture analyzer. The bloom jar was placed centrally under the
205 standard probe and the penetration test was commenced. The diameter of the plunger for
206 bloom strength measurement was 1.27 cm flat-faced cylindrical probe. After a trigger
207 force of 4 g was attained the probe proceeded to penetrate into the gel to a depth of 4 mm.
208 At this depth, the maximum force reading (the resistance to penetration) was obtained
209 and translated as the Bloom strength (g) of the gel. The method described corresponds to
210 the British standard method for sampling and testing gelatins (BSI 757, 1975).

211 2.11. *Dynamic viscoelastic behaviour (DVB)*

212 Dynamic viscoelastic behaviour of gelatin samples was measured using a
213 Controlled Stress Rheometer (CSR- 500, CarriMed, Dorking, Surrey, U.K) under
214 Oscillatory mode. The measuring geometry used was 4 cm parallel plate and the gap was
215 set to 500 microns at 25^{o}C manually using the micrometer provided with the instrument.
216 The viscoelastic properties of gelatin solutions (6% w/v) were measured in the
217 temperature range of 5-20^{o}C and 20-5^{o}C, with heating / cooling rate at 0.5^{o}C / minute.
218 The frequency of oscillation was 1 Hz, and the oscillating stress of 5.0 Pa was applied.
219 The measurements were made by applying a constant displacement amplitude oscillation

of 0.0005 Rad. The elastic modulus (G′), viscous modulus (G″) and Tan δ (G”/ G’) values were measured as a function of temperature.

2.12. *Frequency sweep of gelatin*

The gelatin gel (6.67% w/v) obtained after overnight maturation at 5^{o}C was subjected for frequency sweep at 5^{o}C. Frequency was varied from 0.2 Hz to 5.2 Hz. Storage and loss modulus (G’ and G’’) was obtained as a function of frequency. The slope of the regression of G’ and G’’ (on log scale) with change in frequency were obtained in order to assess the viscoelastic nature of the sample.

2.13. *Flow behavior of gelatin (Shear rate sweep)*

The flow properties of gelatin were measured as a function of temperature using Controlled Stress Rheometer (CSR Carri-Med model CSL 500, Dorking, Surrey, UK) with flow package software. Shear stress weep of gelatin solutions (10, 20 and 30 mg / ml) was carried out at 30^{o}, 40^{o}, 50^{o} and 60^{o}C separately. At a concentration of 30 mg / ml shear stress sweep has been carried out at 10^{o}C. The sample was equilibrated for 15 min before the shearing experiment was started. The measuring geometry used was 4 cm cone and plate with truncation of 59 microns. The range of stress applied varied between 2 to 5 Pa depending on the angular velocity in the preshear experiment. The ascent and descent time were 2 min each. A flow curve was obtained by plotting log viscosity *vs* log shear rate values. Different rheological flow models based on shear stress-shear rate data were tested using the software provided with rheometer. The best-fit model selected on the basis of standard error was found to be Casson and Herschel-Bulkley model.

The Casson model was applied using the following equation

$$\tau^{1/2} = \tau_o^{1/2} + (k\gamma)^{1/2} \quad \text{---------} \quad 1$$

The Herschel-Bulkley model as represented in the following equation was used.

$$\tau = \tau_o + k\gamma^n \quad \text{- - - - - -} \quad 2$$

where, τ is the shear stress (Pa), τ_o and are the yield stress (Pa), γ is the shear rate (s^{-1}), k is the consistency coefficient (Pa s^n) and n is the flow behavior index (dimensionless). The consistency coefficient (k) and flow behavior index (n) of gelatin solution was determined by the flow software provided with the equipment.

2.14. *Dynamic viscoelastic properties of fish mince mixed with different concentrations of gelatin*.

Fresh threadfin bream (*Nemipterus japonicus*) was used to prepare the mince. After removal of head, gut and skin, the meat from the whole body (edible part only) was separated manually and used for the study. The manually separated meat (devoid of connective tissue) was macerated using pre-chilled pestle and mortar for 5 min. The minced meat obtained was used for dynamic viscoelastic behavior measurements without and with different concentrations of gelatin. The fish mince was mixed with gelatin at different concentrations (0, 0.1, 0.2, 0.5, 1, 5 and 10% (w/w)) separately and macerated well using pestle and mortar. Further, sodium chloride at a concentration of 2.5 % (w/w) was added and macerated for 4 minutes under chilled condition (4^o -6^oC) to get the sol. The final moisture content in the samples without and with gelatin at different concentrations was maintained at 78%. The sol obtained was subjected for dynamic viscoelastic behavior study using Controlled Stress Rheometer. The measuring geometry

used was 4 cm parallel plate and the gap was set to 2000 microns at 80^{o}C manually using the micrometer provided with the instrument. The viscoelastic properties of mixture of fish mince and gelatin were measured in the temperature range of 25^{o} - 90^{o}C with a heating rate of 1^{o}C / minute. The frequency of oscillation was 1 Hz, and the oscillating stress of 500 Pa was applied. The measurements were made by applying a constant displacement amplitude oscillation of 0.0005 Rad. The elastic modulus (G′), viscous modulus (G″) and Tan δ (G"/ G') values were measured as a function of temperature. The fish mince-gelatin sol as well as gel obtained after heating of the sample was subjected for frequency sweep analysis at 30^{o}C.

3. Results and discussion

The proximate composition of fresh fish skin indicated a protein content of 25.19% and moisture content of 52.79 %. The fat content of fish skin was less than 2% (Table 1A).

The yield of gelatin from the whole skin was 4 % (Table 1B). The gelatin yields obtained for tilapia and hake was in the range of 5-8% (Jamilah & Harvinder, 2002 and Gomez-Guillen et al., 2002). Leaching of collagen in the skin and degradation of gelatin during extraction are the probable reasons for lower yield (Jamilah & Harvinder, 2002 and Kolodziejska, Kaczorowski, Piotrowska & Sadowska, 2004).

The protein content of freeze-dried gelatin was 94.6% and more than 90% of protein was soluble in phosphate buffer (50 mM) containing 0.3 M sodium chloride (pH: 7.5) (Table 1B). After freeze-drying, the moisture content of the gelatin was reduced to 4.2%. The pH of gelatin (1% solution) from the skin of bigeye snapper fish was 6.44.

Gudmundsson and Hafsteinsson (1997) reported that pH of gelatin from cod skins varied between 2.7 and 3.9, while Grossman and Bergman (1992) reported a value of 3.77 for gelatin from tilapia. The pH of gelatin in the present study was higher than reported values as the gelatin was subjected to deionisation by amberlite resin after extraction.

Amino acid composition of gelatin prepared from skin of bigeye snapper fish showed high proportion of glycine (21.86%) and alanine (11.85%) (Table 2). However, the quantity was less than that of gelatin from tilapia, horse mackerel and cod (Grossman & Bergman, 1992, Badii & Howell, 2006 and Gomez-Guillen et al., 2002). Glycine is the most dominant amino acid in gelatin (Arnesen and Gildberg, 2002). Furthermore, the amino acid composition in the present study showed low contents of methionine, cysteine and tyrosine that are characteristic to all gelatins. The imino acids (Pro + H Pro) content of the gelatin in the present study was 14.43%, which was lesser than gelatin prepared from tilapia, cod, nile perch and skate skin (Grossman &Bergman, 1992, Gomez-Guillen et al., 2002, Muyonga et al., 2004 and Cho et al., 2006). The stability of collagen and gelatin is proportional to their imino acid and glycine content (Lehninger, Nelson & Cox, 1993). Gelatin with high levels of imino acids tends to have high gel strength and melting point (Haugh et al., 2004 and Muyonga et al., 2004), as imino acids are important in the renaturation of gelatin subunits during gelling (Johnston-Banks, 1990). For close packing of the triple helix, glycine molecules are required to occupy every third position (Te Nijenhuis, 1977). The amino acids like tryptophan and cysteine are normally absent in a conventional gelatin. However, presence of cysteine at low concentration in gelatin from

307 skin of horse mackerel, sin croaker and shortfin scad have been reported (Badii &Howell
308 2006 and Cheow et al., 2007).

309 Nitrogen solubility index of gelatin revealed minimum solubility of 64.1% at pH
310 6.44 (Figure 1A). On either side of this pH, solubility increased and maximum solubility
311 of 95.8% at pH 2.42 was recorded. The minimum solubility at pH 6.44 which may be
312 nearer to its isoelectric point is due to higher electrostatic interaction because of net
313 charges of the molecules being equal (Hall, 1996). Gelatin is an amphoteric protein with
314 isoelectric point between 5 and 9 depending on raw material and method of manufacture.
315 (Johnston-Banks, 1990 and Poppe, 1997). At pH values below and above isoelectric point
316 proteins tend to carry charges, thereby enhancing hydration (Kinsella, 1984).

317 The solubility profile of gelatin as a function of sodium chloride concentration
318 indicated a maximum solubility at 0.3 M concentration (Figure 1B). Nearly 65% of total
319 protein was found to be soluble in 0 M concentration. With increase in sodium chloride
320 concentration the solubility of gelatin increased up to 0.3 M, beyond which solubility
321 decreased. Generally solubility increases with increase in salt concentration upto certain
322 level (salting 'in') and with further increase in salt concentration, the solubility decreases
323 (salting 'out') (Damodaran & Kinsella, 1982). At 0.3 M concentration, nearly 93% of
324 gelatin was found to be soluble. Hence, 0.3 M sodium chloride concentration was used
325 for solubilization of gelatin to study gel filtration profile and emulsion capacity.
326 However, for determining bloom strength, setting index and flow properties of gelatin
327 distilled water was used as solvent.

The gel filtration profile of gelatin indicated a single peak eluting at an elution volume of 89 ml (Figure 2). The gel filtration profile of gelatin also confirms the homogeneity of protein molecule. The molecular weight of gelatin as revealed by gel filtration technique (using standard molecular weight markers) was 282 kDa (Figure 2-Inset). The molecular weight by gel filtration technique relates to undissociated molecule, while by SDS-PAGE gives under reduced conditions. SDS-PAGE pattern of gelatin showed two intense bands at molecular weight region of 97-205 kDa (Figure 3). The band closer to 97 kDa is the ∞-chain and the band closer to 205 kDa is β-chain component. The average molecular weight of one ∞-chain is reported between 95 and 100 kDa (Veis, 1964, Piez, 1968 and Norland, 1990). The bands corresponding to 95 kDa and 200 kDa in the SDS-PAGE of gelatin represents the collagen α- chains and β components (Gomez- Guillen et al., 2002). Hence, the molecular weight of gelatin from the skin of bigeye snapper can be approximated to ≈ 290 kDa which is in accordance with the molecular weight obtained by gel filtration technique. In the present study the average molecular weight of gelatin by SDS-PAGE is an approximation as distribution of subunits is not clear. This is possibly due to higher concentration of polyacrylamide used in the experiment. The appearance of low molecular weight components in the pattern may be indicative of some hydrolysis of gelatin during extraction process.

The emulsifying capacity of gelatin was found to be decreasing with increase in concentration of gelatin (Table 3). Emulsifiers are surface-active materials that adsorb to interfaces and facilitate the production of small droplets by lowering the interfacial tension during homogenization (Walstra, 2003). The higher value of EC recorded for

lower concentration may be due to higher degree of unfolding of polypeptides during the shearing involved in the emulsifying process (Kinsella, 1976 and Borderias, Colmenero & Tejada, 1985). The EC of gelatin obtained in the present study was higher than that of ribbon fish meat, oil sardine and threadfin bream meat (Dileep, Shamasundar, Binsi, Badii & Howell, 2005, Karthikeyan, Shamasundar, Sijo Mathew, Ramesh Kumar & Prakash, 2004 and Karthikeyan, Dileep & Shamasundar, 2006). There is a growing trend within the food industry to replace synthetic emulsifiers with more natural ones (Garti, 1999). Proteins extracted from a variety of natural sources can be used as emulsifiers in foods because of their ability to facilitate the formation, improve the stability, and produce desirable physicochemical properties in oil-in-water (O/W) emulsions, e.g. soy, whey, casein, fish, meat and plant proteins (Dickinson, 2003 and McClements, 2004). Our results indicate that oil-in-water emulsions can be prepared using a relatively low concentration of gelatin, 0.05%.

Setting index of gelatin showed the time needed for the gelatin solution to solidify completely at 5°C as a function of concentration. A preliminary analysis of concentration *vs*. setting behavior on visual basis shows that the gelatin from the skin of bigeye snapper fish has the critical concentration of 0.9 % (w/v). It was observed that with increase in concentration of gelatin solution, time taken for complete solidification reduced (Figure 4). Setting index is a macroscopic property wherein, solidification process is observed visually and the methodology is highly subjective. In the present study the setting index indicated the formation of ordered structure is dependent on concentration. Gelatin solution with a concentration of 40 mg / ml took about 2 hr for complete solidification.

Visual observation of gelatin solution at this concentration indicated that strong gels did not form even after gelation periods of up to 1hr and that the samples looked more like highly viscous solutions.

Gel strength is one of the most important functional properties of gelatin and fish gelatin typically has lower gel strength than mammalian gelatin (Gilsenam & Ross Murphy, 2000). According to Holzer (1996), the gel strength of commercial gelatin expressed as bloom value, ranges from 100-300 but gelatin with bloom values of 250-260 are most desired. Bloom strength of gelatin (6.67% w/v in distilled water) prepared in the present investigation recorded a value of 108 g (Table 1B). The bloom strength values of gelatin obtained from skin of hake, cod and megrim varied from 100 g to 210 g (Montero & Gomez-Guillen, 2000, Fernandez-Diaz, Montero & Gomez-Guillen, 2001 and Gundmundsson & Hafsteinsson, 1997). The bloom strength of gelatin from the skin of bigeye snapper fish was slightly on the lower side compared to that of cod and hake. It is likely that relatively limited imino acids and glycine content might have resulted in less organized tripple helical structures and is largely responsible for lower bloom strength. The average molecular weight of gelatin plays an important role in determining the bloom strength but also is dependent on other factors such as the chemical treatment of raw collagen material, type and concentration of the gelatin and the time / temperature history of the sample (Babin & Dickinson, 2001 and Kolodziejska et al., 2004).

The dynamic viscoelastic profile (DVB) of gelatin in the temperature range of 20^{o}-5^{o}C and 5^{o}-20^{o}C is given in Figure 5. The concentration of gelatin used for DVB measurement was 6% (w/v). The storage modulus (G') values were higher than loss

394 modulus (G") values during both gelling and melting. The relatively higher rate of
395 increase of storage modulus values at less than 12.2^{o}C indicates gelation has started at
396 around 12.2^{o}C. This type of behavior was also explained previously for flounder skin
397 gelatin (Fernandez-Diaz, Montero & Gomez-Guillen, 2003). At 10^{o}C, G′ values increased
398 sharply which indicates rapid formation of junction zones and strong reinforcement of the
399 gel network. Hence, the gelling point of gelatin from the skin of bigeye snapper was
400 considered as 10^{o}C (Figure 5A), which was further confirmed from the sharp drop in Tan
401 δ values at 10°C during the cooling process. The gelling temperature value in the present
402 study was less than reported value for gelatin from skin of hake, cod (Gomez-Guillen et
403 al., 2002), nile perch and bovine bone (Muyonga et al., 2004).

404 The melting temperature of gelatin from the skin of bigeye snapper fish was
405 found to be at 16.8^{o}C as indicated by a sudden drop in G' values (Figure 5B). This is
406 higher than the reported value for gelatin from cod, hake (Gomez-Guillen et al., 2002)
407 and flounder skin (Fernandez-Diaz et al., 2003), but lesser than that of skate skin (Cho et
408 al., 2006), Alaska pollack skin and bovine gelatin (Kim, Byun & Lee, 1994, Badii &
409 Howell, 2003 and Haug et al., 2004). The lower gelling and melting temperatures of
410 gelatin from big eye snapper can be related to lower content of imino acids and glycine.
411 The gycine content of gelatin in the present study was low compared to that reported
412 previously for gelatin from various sources (Badii & Howell, 2006 and Cheow et al.,
413 2007). However, it has been pointed out in literature (Gomez-Guillen et al., 2002) that
414 gelatin from different fish species exhibited different viscoelastic properties even though
415 the amino acid composition was similar. They are also affected by the concentration, pH,

molecular weight, relative content of α, β and γ-chain componets, gel maturation time and temperature (Choi & Regenstein, 2000 and Gomez-Guillen et al., 2002).

In order to assess the strength of gel network, frequency sweep of overnight-matured gel at 5^{o}C was carried out from 0.2-5.2 Hz. The G' values were higher than G" values. The slope of G' values as a function of frequency sweep was 0.02 indicating the higher stability of the gel against shearing in the given frequency range (Figure 6). The lower Tan δ values during frequency sweep is indicative of good gel network (Hudson, Daubert & Foegedding, 2000). The frequency sweep curve gives a good rheological description of how the product will behave during storage and application.

The flow profile data of gelatin samples as a function of concentration and temperature indicated non-Newtonian behavior (Figure 7 & 8). The flow profile at a concentration of 10 and 20 mg / ml as a function of temperature revealed almost similar data. Hence, the flow profile of gelatin at a concentration of 10 and 30 mg / ml is depicted in Figure 7. The flow profile of the proteins at specific temperature provides information on resistance to shearing thereby indicating structural impairment, if any. Further, thixotropic shear-thinning / pseudoplastic phenomenon was evident wherein, increase in shear rate decreased the viscosity indicating structural breakdown due to shearing. Irrespective of the concentration of sample, at temperatures of 30^{o}, 40^{o}, 50^{o} and 60^{o}C, shearing caused the impairment of the sample. The flow profile carried out at 10^{o}C at a concentration of 30 mg/ml (Figure 8) showed minimum thixotropic area indicating minimum damage to the structure of gelatin due to shearing. This can be related to network formation at this temperature.

Shear stress–shear rate data of gelatin solutions were tested for various rheological models using the software provided along with the rheometer. Some researchers have reported a clearly Newtonian behavior for gelatin (Marcotte, Hoshahili & Ramaswamy, 2001). In the present study, based on standard error data obtained, it was found that Casson and Herschel-Bulkley models were suitable to predict the flow behavior of gelatin solutions at various concentrations and temperatures. The Casson model was found suitable for a concentration of 10 and 20 mg / ml and Herschel-Bulkley model was suited for a concentration of 30 mg / ml at different temperatures studied (Table 4). The rheological models were tested for up curve data only as full down curve data could not be obtained due to shearing and temperature effect.

Gelatin solution at all concentrations and temperatures exhibited a yield stress value and thereafter showing shear thinning / pseudoplastic behaviour. It has been recognized that pseudoplasticity represents an irreversible structural break down and the decrease in viscosity occurs as a result of molecular alignment that takes place within such substance (Glicksman, 1969 and Rha, 1975). The upswing in viscosity values at low shear rates is indicative of an apparent yield stress (Rao & Tattiyakul, 1999). Yield stress values were found to be decreasing with increase in temperature and were more drastic for 30 mg / ml at 40^{o}C (Table 4). A maximum value of 1.16 Pa was recorded for 30 mg/ml at 10^{o}C. At this temperature the viscosity as well as yield stress values becomes markedly time dependent owing to the degree of cross-links formed during gelling process. Higher value of yield stress at lower temperatures indicated appearance of gel characteristics of gelatin. Yield stress has been recognized as useful property of gums

460 when they are used as binders, because it helps to keep various components of food
461 formulation in place (Rao & Kenny, 1975).

462 In general, the consistency coefficient (k) increased with gelatin concentration
463 (Table 4). At 40^{o}C, the decrease in k value at all concentrations is due to increase in
464 intermolecular distances, because of the thermal expansion caused by the increase in
465 temperature (Constenella, Lozano & Crapiste, 1989). Only a marginal decrease in
466 consistency coefficient was observed with increase in temperature at 10 and 20 mg / ml
467 whereas, the decrease was prominent at 30 mg / ml indicating the lower viscosity at 40^{o}C.
468 The k value at 60^{o}C at 30 mg / ml was higher than at 40^{o}C. This increase in k value is
469 most likely due to complete opening up of polypeptide chain to random chain molecules
470 leading to increased viscosity (Flory and Weaver, 1960). The flow behavior index (n) at
471 10^{o}C for 30 mg / ml was much lower compared to higher temperatures indicating the
472 viscous nature of gelatin at that temperature (Table 4). The farther the flow behavior
473 index from 1, the more the deviation from Newtonian behavior (Lewis, 1990). The food
474 grade gum solutions with a high value of n tend to feel slimy in the mouth. When high
475 viscosity and a good mouth feel characteristics are desired, the choice should be a gum
476 system having a low n value. In the present study, the lower n value of 0.21 at 10^{o}C for
477 gelatin at 30 mg / ml indicates viscous nature and better mouth feel.

478 To study the effect of different concentration of gelatin on the gelation behavior
479 of threadfin bream mince, DVB measurements in the temperature range of 25^{o}-90^{o}C were
480 carried out. Although collagen proteins have been reported to "dilute" the beneficial
481 effects of the myofibrillar proteins on gelling (Whiting, 1989), some studies reported that

collagen proteins help to stabilize batter and may be advantageous in increasing binding properties (Jones, 1984 and Pietrasik, Duda & Lisowska, 1996). In the present study, the addition of gelatin (0.1-1%) to fish mince resulted in higher storage modulus (G') values from the beginning of heating regime indicating the usefulness of incorporation of gelatin to fish mince. The DVB profile of fish mince with 0.1 and 0.2% gelatin did not show significant difference; hence data for 0.2% gelatin is presented. A maximum G' value of 443.7 KPa at 68.3^{o}C was registered in the presence of 0.5% gelatin which was 42% higher than sample without added gelatin (Figure 9). An increase in G' value during thermal gelation process is an indicative of elastic structure development. One of the models proposed for spatial partitioning of a gelling protein and a gelling or non gelling co-ingredient, represents complex gels, which form when interactions among the components lead to their physical association (Ziegler & Foegedding, 1990). In this model a non- gelling component may associate with the primary network in random fashion by means of non-specific interactions. Such interactions may reduce the flexibility of the primary network chains and add to the rigidity of the gel. The addition of gelatin at 5 and 10% to fish mine has reduced the G' values considerably. This reduction in G' values are possibly due to entrapment of water molecule by high concentration of gelatin and making unavailable for protein gelation. The temperature at which sol-gel transition occurred as indicated by Tan δ for fish mince alone was 32.2^{o} and 61.1^{o}C. The transitions at 36.7^{o} and 43.3^{o}C have been attributed to the tail of the myosin molecule and the transition at the higher temperature to that of the head of the myosin molecule (Sano et al., 1988). In the present study, only two transition

504 temperatures have been recorded and it can be attributed to tail and head portion of the
505 myosin molecule. The transition temperature of fish mince with gelatin (up to 1% level)
506 showed similar trend to the fish mince alone. However, with the addition of 10% gelatin,
507 a single transition at 46.8^{o}C was recorded. It is not clear as to why a single transition
508 should occur at higher concentration of gelatin in fish mince.

509 Frequency sweep was carried out for heat set gels for all the concentrations of
510 added gelatin (Figure 10). The slope of G' values *vs* Frequency (on Log-Log scale) was
511 found to be lower in gelatin (up to 1% concentration) added sample than the meat alone,
512 whereas, a higher slope was recoded for addition of gelatin above 1% concentration. The
513 minimum slope of 0.03 for G' values coupled with low and almost constant values of Tan
514 δ indicated the formation of a strong network structure for 0.5% gelatin addition. The
515 lower the Tan δ values, the more solid like the material is (Hudson et al., 2000).

516 The increase in G' values coupled with the unaltered transition temperatures
517 suggest that gelatin is not actively interacting with the three-dimensional gel network.
518 Hence, gelatin can be considered as an inactive binder where there is little or no
519 interaction between filler particles and gel matrix and the modulus decreases with
520 increasing volume fraction of the filler (Lanier, 1991). It looks like gelatin absorbs water
521 and swells in the cavities inside the matrix of gel network formed by the myofibrillar
522 proteins. Similar behavior by carrageenan - meat system has been explained by Montero
523 et al. (2000) and Pietrasik (2003).

524

525

4. Conclusion

The physicochemical and rheological properties of gelatin from skin of bigeye snapper have been evaluated. Bloom strength value of the gelatin was found to be 108 g and solidification of gelatin (as given by setting index) was found to be concentration dependant. The gelling and melting temperatures of gelatin were found to be 10^{o} and 16.8^{o}C respectively as revealed by dynamic rheological testing. Flow profile of gelatin solution as a function of temperature and concentration exhibited pseudoplastic behavior. The Casson and Herschel-Bulkley models were found to be suitable to study the flow behavior and yield stress was dependent on concentration and temperature. The gel forming ability of fish mince could be substantially increased by addition of gelatin at 0.5% level. The study helps in better utilization of processing waste of bigeye snapper and minimizing environmental pollution.

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Table 1A: Proximate composition of skin of Big eye snapper (*Priacanthus hamrur*)

Parameters	*Mean value*
Moisture (%)	52.8 (0.53)
Protein (%) (N x 5.8)	25.2 (0.85)
Fat (%)	1.2 (0.06)
Ash (%)	20.2 (0.64)

Table 1 B: Properties of gelatin from the skin of bigeye snapper (*Priacanthus hamrur*) fish

Parameters	**Mean value**	
Yield (%)	4.0	(0.50)
Moisture (%)	4.2	(0.05)
Protein content (%) (N× 5.8)	94.6	(1.20)
pH (1%)	6.4	(0.07)
Solubility in Phosphate buffer (50 mM) containing 0.3 M NaCl (pH 7.5) (% of total protein)	93.0	(1.09)
Bloom strength	108	(0.04)

Values in parenthesis represent standard deviation, n = 3

Table 2. Aminoacid composition of gelatin from skin of bigeye snapper (*Priacanthus hamrur*) fish (as g amino acid/100g gelatin)

Amino acids	% of aminoacid
Asp	4 .98±0.12
Glu	4 .47±0.19
H.Pro	8.45±0.24
Ser	2.31±0.33
Gly	21.86±0.98
His	2.16±0.10
Arg	5.60±0.83
Thr	2.08±0.41
Ala	11.85±0.82
Pro	5.98±0.64
Met	0.96±0.33
Val	2.18±0.22
Tyr	0.97±0.13
Cys	0.89±0.11
I Leu	1.25±0.19
Leu	2.78±0.27
Phe	3.36±0.36
Lys	3.21±0.33

Values in parenthesis represent standard deviation, n = 3

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Table 3: Emulsion capacity of gelatin from the skin of bigeye snapper (*Priacanthus hamrur*) fish

Concentration of gelatin sample (%)	Emulsion capacity (ml oil/mg protein)
0.05	1.91 (0.09)
0.1	1.03 (0.06)
0.2	0.55 (0.02)

Values in parenthesis represent standard deviation, n = 4

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Table 4. Casson and Herschel – Bulkley model parameters for gelatin solution at different concentrations and temperatures

Concentrations (mg/ml)	Temperature (^oC)	Yield stress (τ_o)	Consistency coeffecient (k)	Flow behaviour index (n)	Regression coeffecient (R^2)
Casson model parameters					
10 mg/ml	30^{o}C	0.10	0.0019	--	0.99
	40^{o}C	0.04	0.0010	--	0.99
	50^{o}C	0.07	0.0016	--	0.99
	60^{o}C	0.08	0.0017	--	0.99
20 mg/ml	30^{o}C	0.17	0.0020	--	0.99
	40^{o}C	0.07	0. 0013	--	0.99
	50^{o}C	0.11	0.0017	--	0.99
	60^{o}C	0.12	0.0019	--	0.99
Herschel-Bulkley model parameters					
30 mg/ml	10^{o}C	1.16	0. 5000	0.21	0.99
	30^{o}C	0.25	0.0184	0.73	0.99
	40^{o}C	0.004	0.0115	0.78	0.99
	50^{o}C	0.14	0. 0217	0.69	0.99
	60^{o}C	0.17	0.0329	0.63	0.98

773

LEGEND TO FIGURES

Figure 1: A Nitrogen solubility index of gelatin from the skin of bigeye snapper with distilled water as solvent in the pH range of 2-10

B Protein solubility of gelatin as a function of molar concentration of NaCl in phosphate buffer (50 mM, pH 7.5)

Figure 2: Gel filtration profile of gelatin on Sepharose-6B gel
Inset: Molecular weight determination of gelatin by gel filtration technique

Figure 3: SDS-PAGE pattern of gelatin from the skin of bigeye snapper
A - standard G - gelatin

Figure 4: Setting Index of gelatin from the skin of bigeye snapper
—○— 10 mg/ml —□— 20 mg/ml —▲— 30 mg/ml —●— 40 mg/ml

Figure 5: Changes in storage (G'), loss modulus (G") and Tan δ values of gelatin A- cooling from 20-5^{0}C, B- heating from 5-20^{0}C
(G' —●— G" —○— Tan δ —△—)

Figure 6: Frequency sweep of gelatin gel at 5^{o}C (G' —●— G"—○— Tan δ —△—)

Figure 7: Flow curves (shear rate sweep) of gelatin solution at 10 and 30 mg/ml as a function of temperature
A- at 30^{o}C, B- at 40^{o}C, C- at 50^{o}C, D-at 60^{o}C
10 mg / ml : —○— upcurve
—○— downcurve
30 mg / ml : —■— upcurve
—■— downcurve

Figure 8: Flow curves (shear rate sweep) of gelatin solution at 30 mg/ml at 10^{o}C
Upcurve ——— Downcurve ▬▬

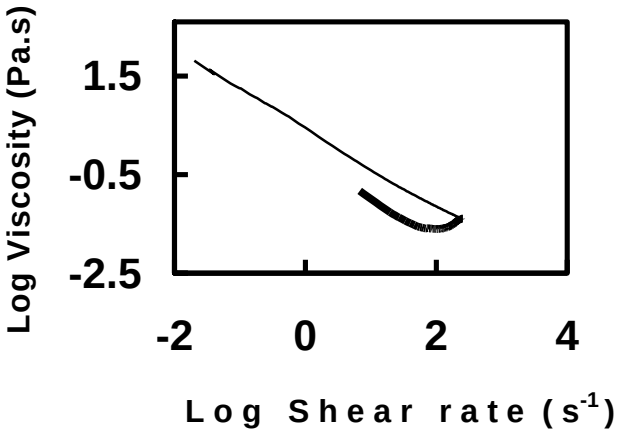
Figure 9: Changes in storage (G'), loss modulus (G") and Tan δ values of fish mince with various concentration of gelatin during heating from 25^o-90^{o}C
A- Control, B- 0.2%, C- 0.5%, D-1%, E-5%, F-10%
(G' —●— G" —○— Tan δ—△—)

Figure 10: Frequency sweep of heat set gel of fish mince with different concentration of gelatin at 30^{o}C
A- Control, B- 0.2%, C- 0.5%, D-1%, E-5%, F-10%
(G' —●— G" —○— Tan δ —△—)

Rheological and functional properties of gelatin from the skin of Bigeye snapper (*Priacanthus hamrur*) fish: Influence of gelatin on the gel forming ability of fish mince

P.K. Binsi[1], B.A. Shamasundar[1,] *, A.O. Dileep[1], F. Badii[2] and N.K. Howell[2]

The functional and rheological properties of gelatin from skin of bigeye snapper (*Priacanthus hamrur*) fish have been assessed. The flow behavior of gelatin solution at different concentrations and temperatures was studied using controlled stress rheometer. The addition of gelatin to fish mince could enhance the elastic component of the heat set gel.



Flow behavior of gelatin from the skin of bigeye snapper fish at 10^{o}C

778

779

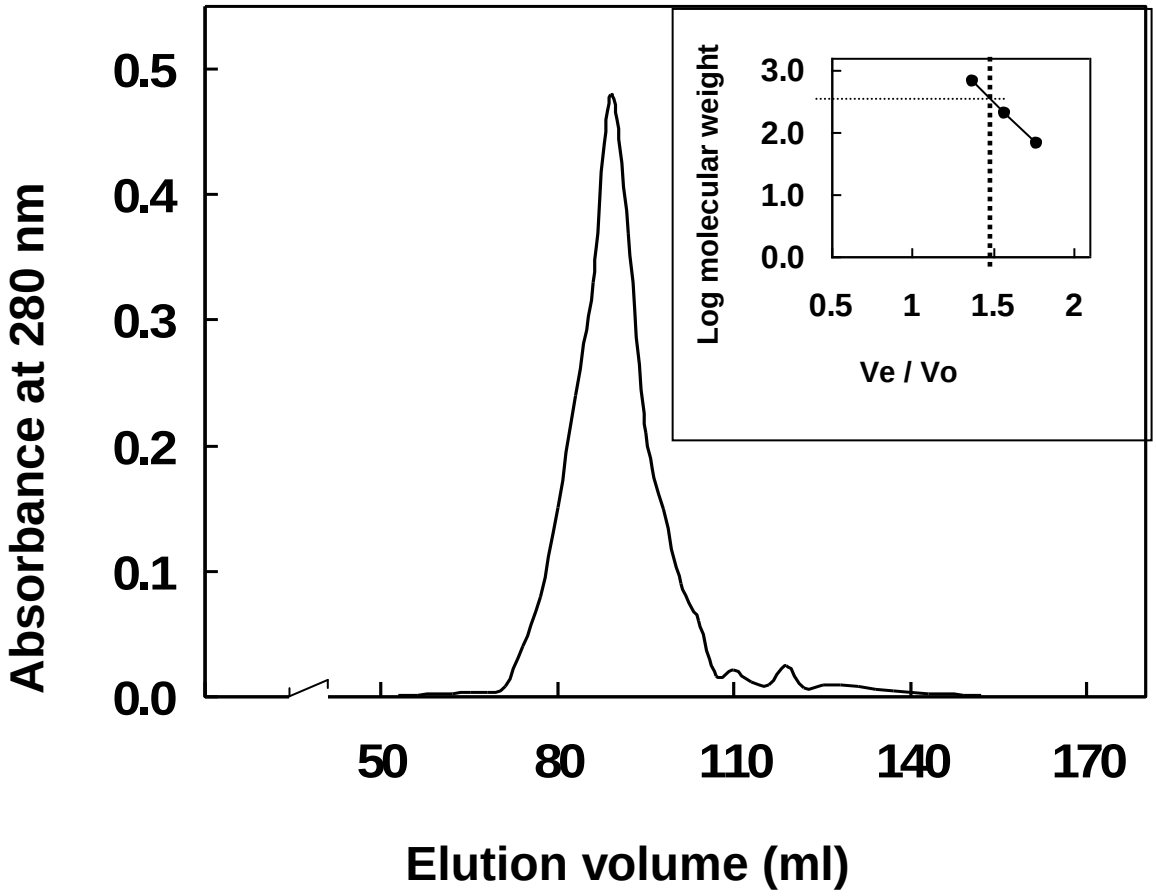

Absorbance at 280 nm
0.5
0.4
0.3
0.2
0.1
0.0
50
80
110
140
170
Elution volume (ml)
Log molecular weight
3.0
2.0
1.0
0.0
0.5
1
1.5
2
Ve / Vo

Figure 2

780

Rheological and functional properties of gelatin from the skin of Bigeye snapper (*Priacanthus hamrur*) fish: Influence of gelatin on the gel forming ability of fish mince

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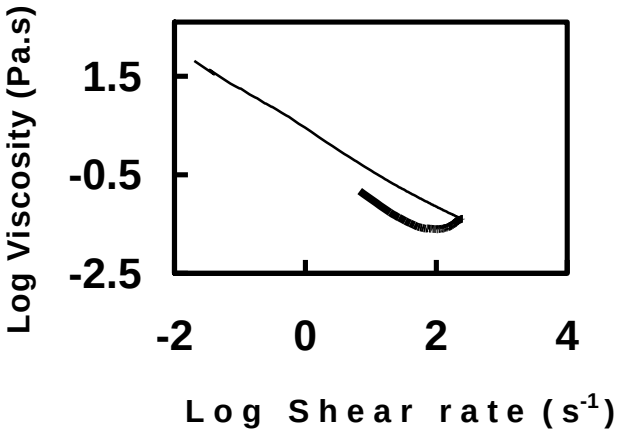


Flow behavior of gelatin from the skin of bigeye snapper fish at 10°C

781

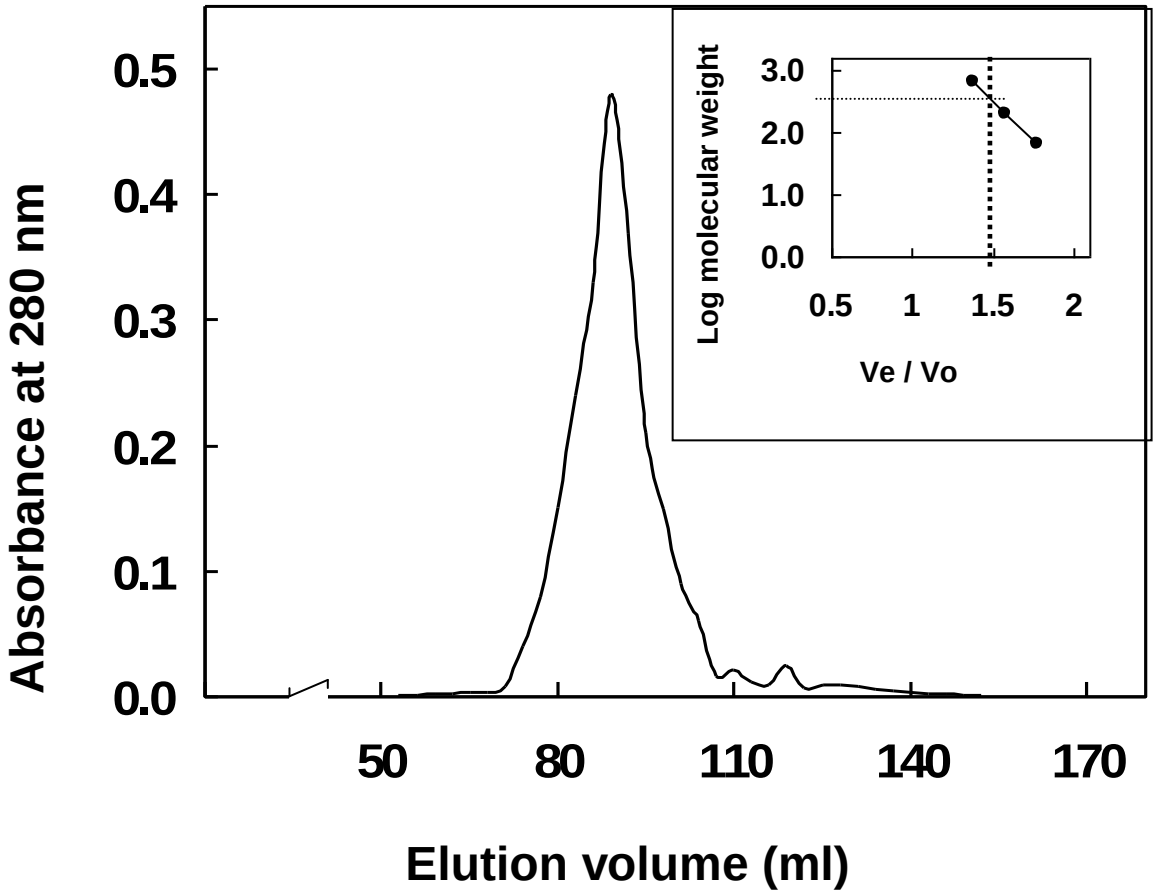
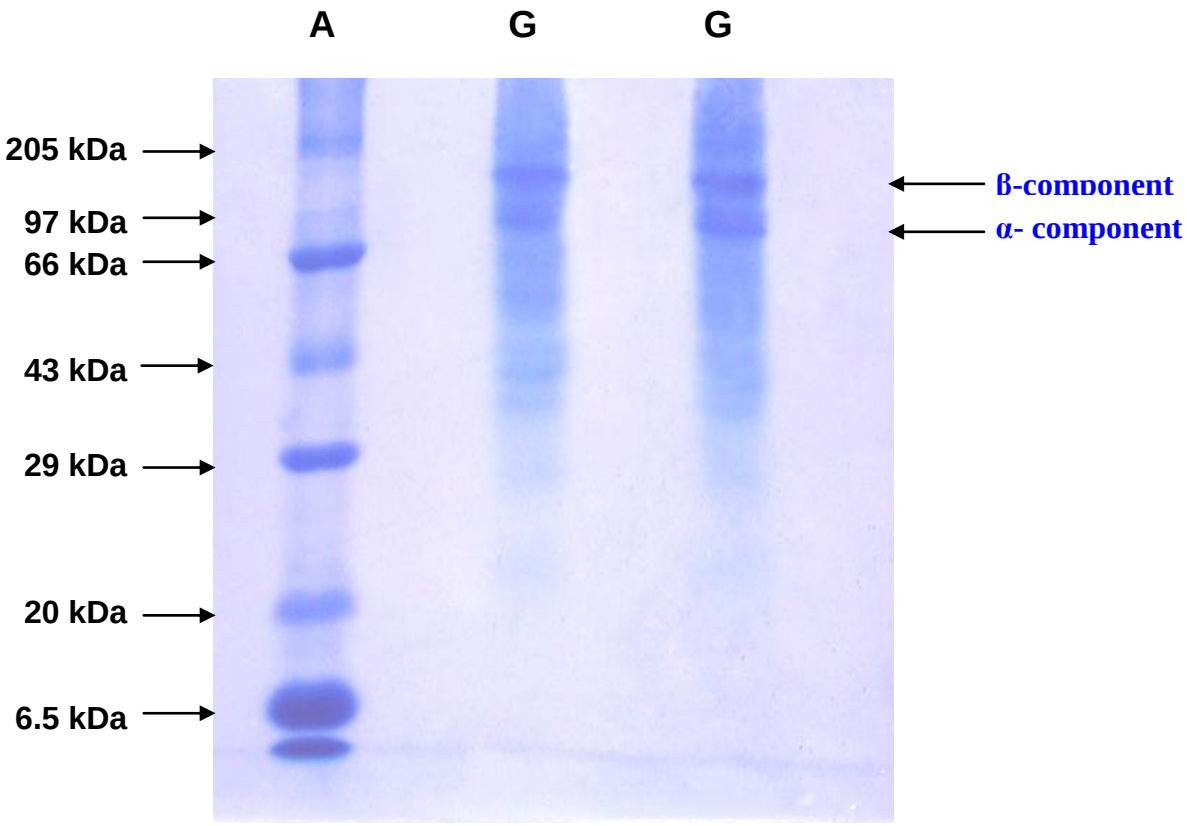


Figure 2

782
783
784



A - Standard G - Gelatin

Figure 3

785
786
787

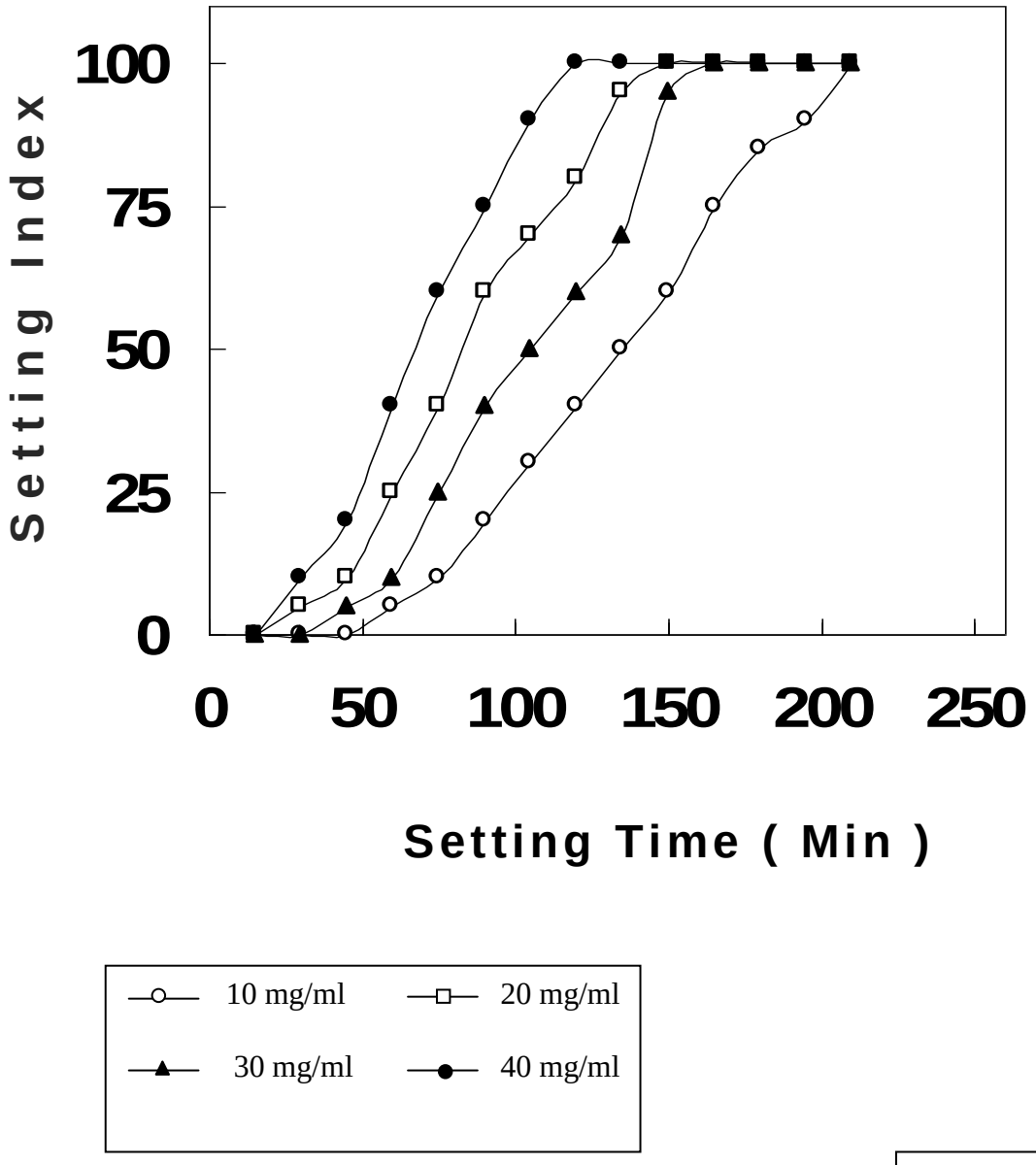

Setting Index
100
75
50
25
0
0
50
100
150
200
250
Setting Time (Min)
10 mg/ml
20 mg/ml
30 mg/ml
40 mg/ml

Figure 4

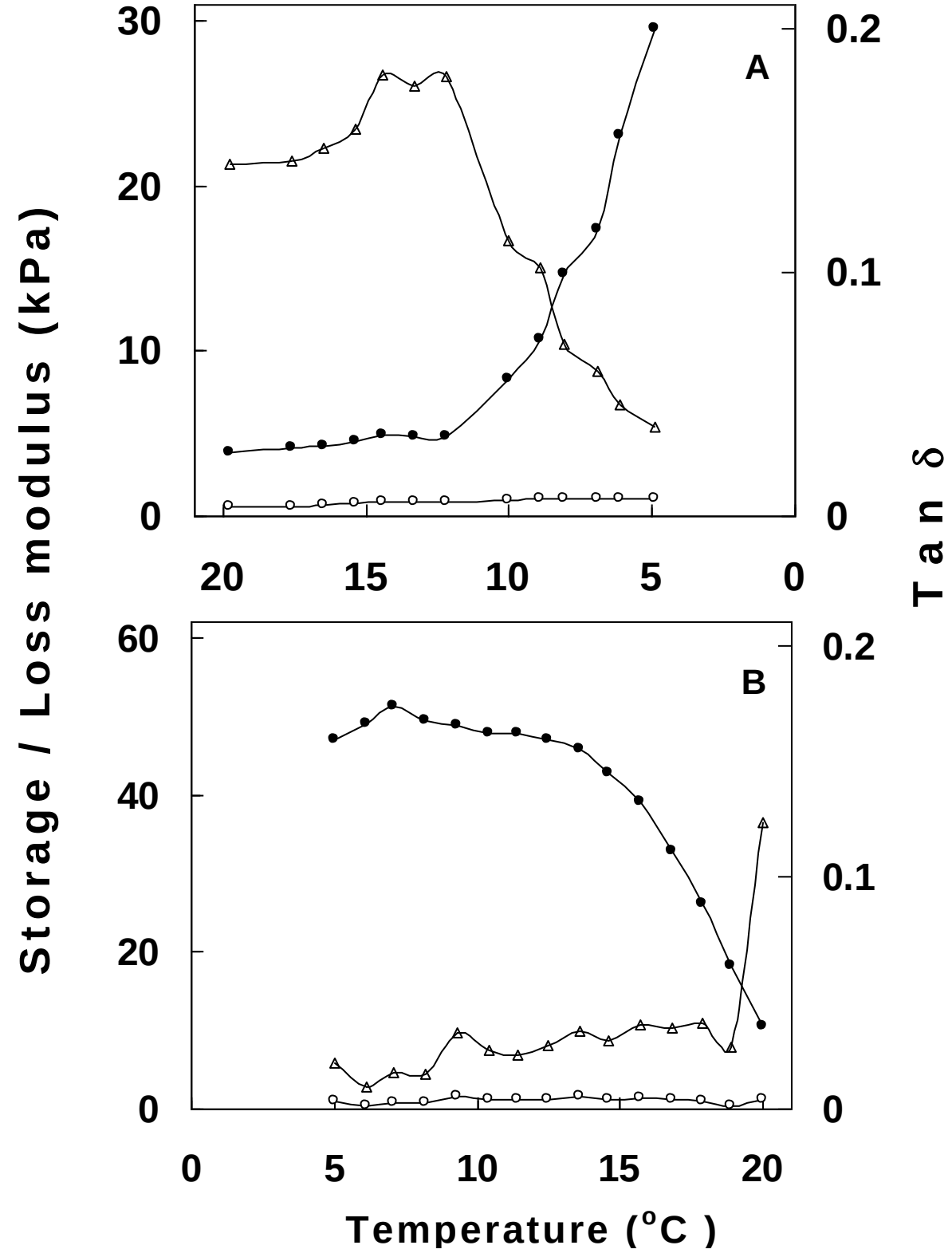

Storage / Loss modulus (kPa)
Tan δ
A
B
30
20
10
0
0.2
0.1
0
20
15
10
5
0
60
40
20
0
0.2
0.1
0
0
5
10
15
20
Temperature (°C)
G'
G "
Tanδ

Figure 5

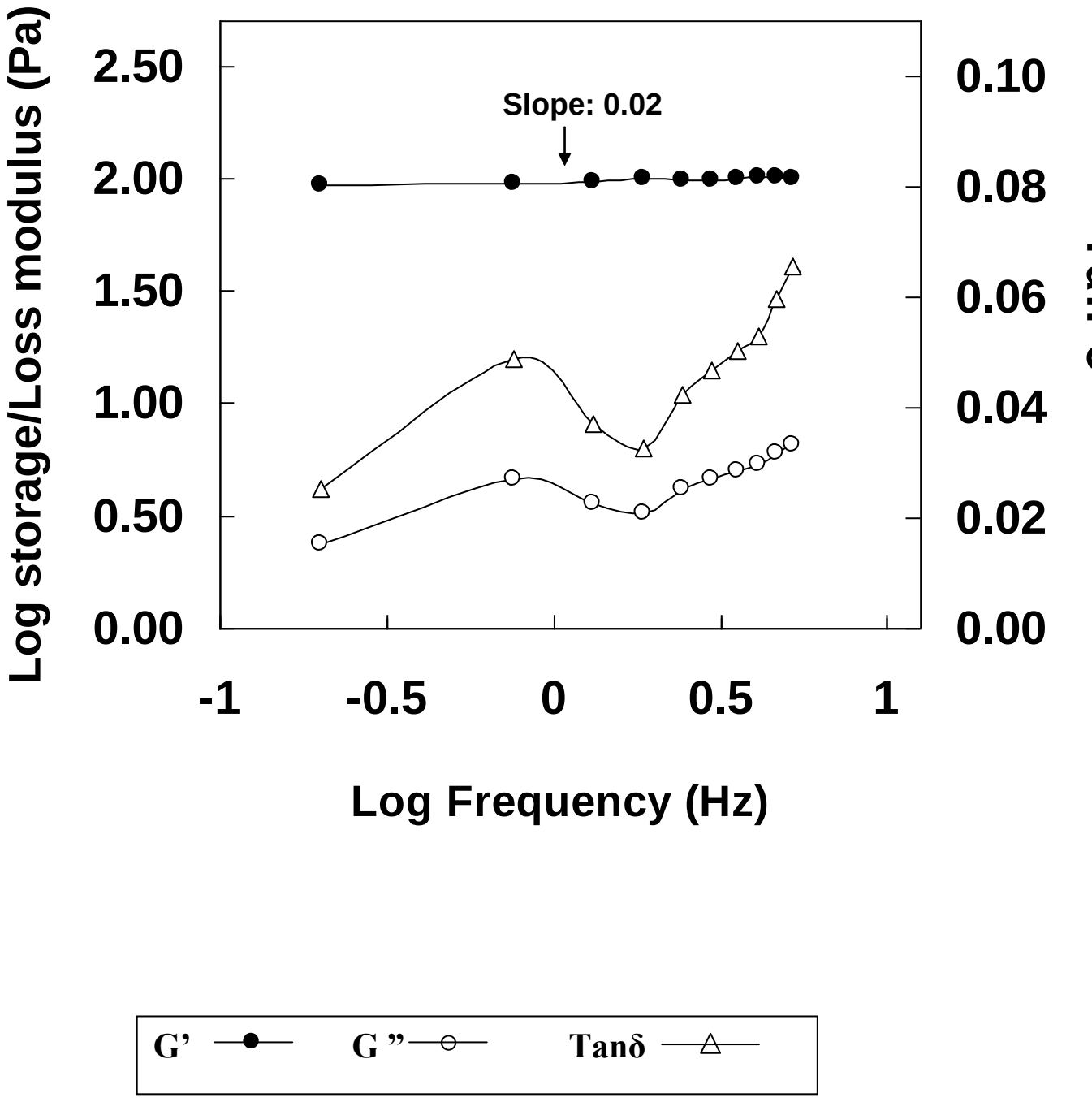


Figure 6

790
791

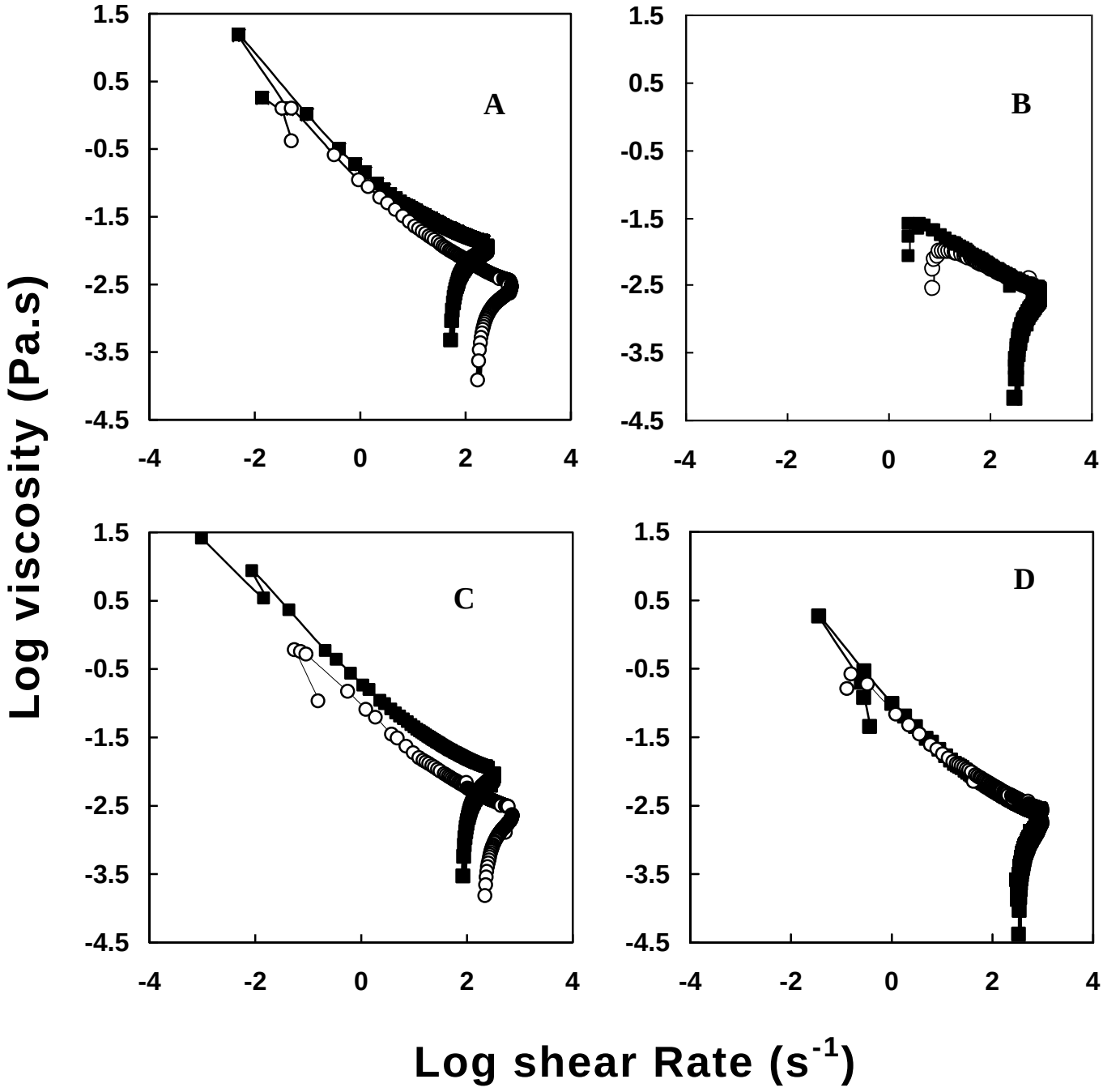


Figure 7

792
793
794

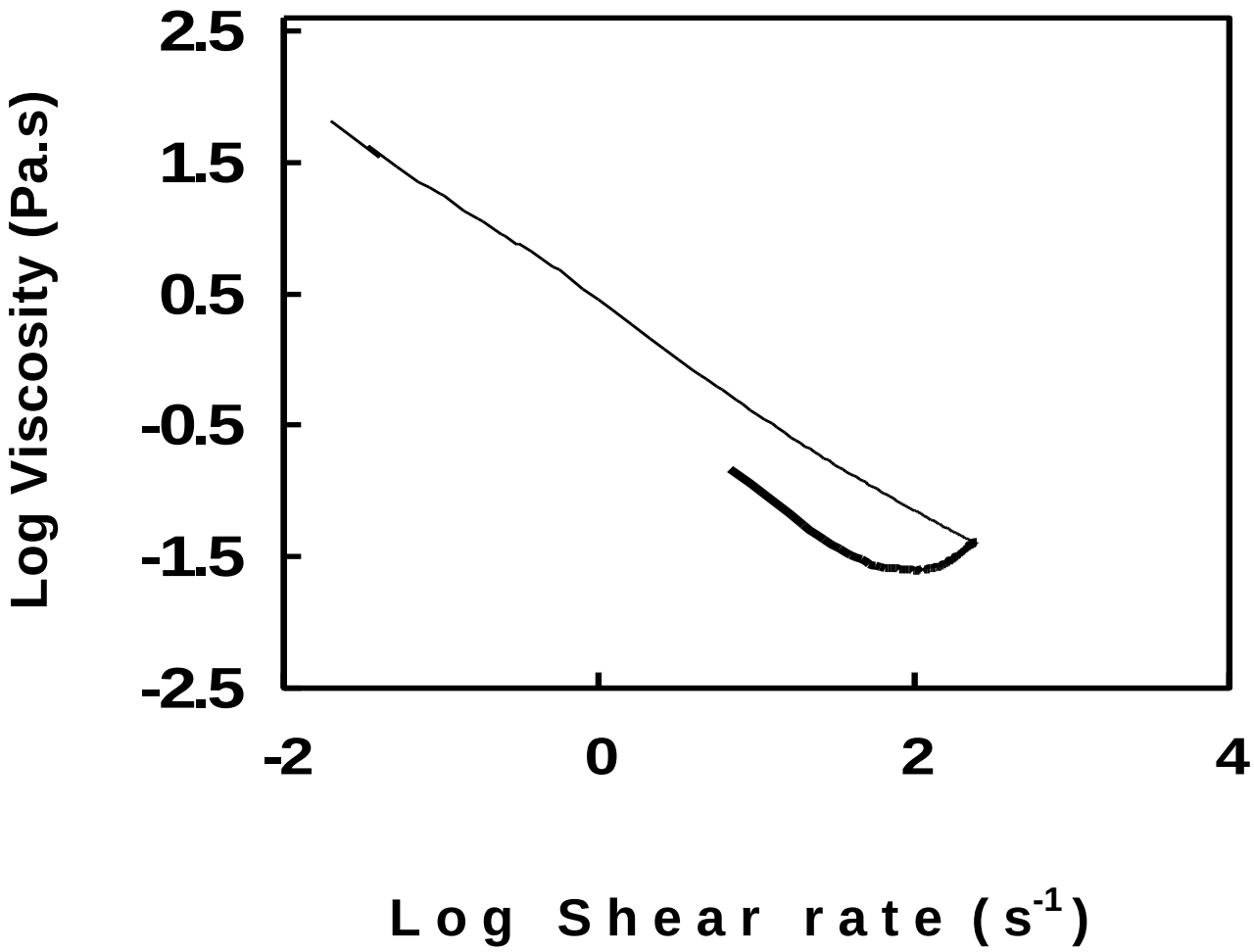


Figure 8

795
796
797

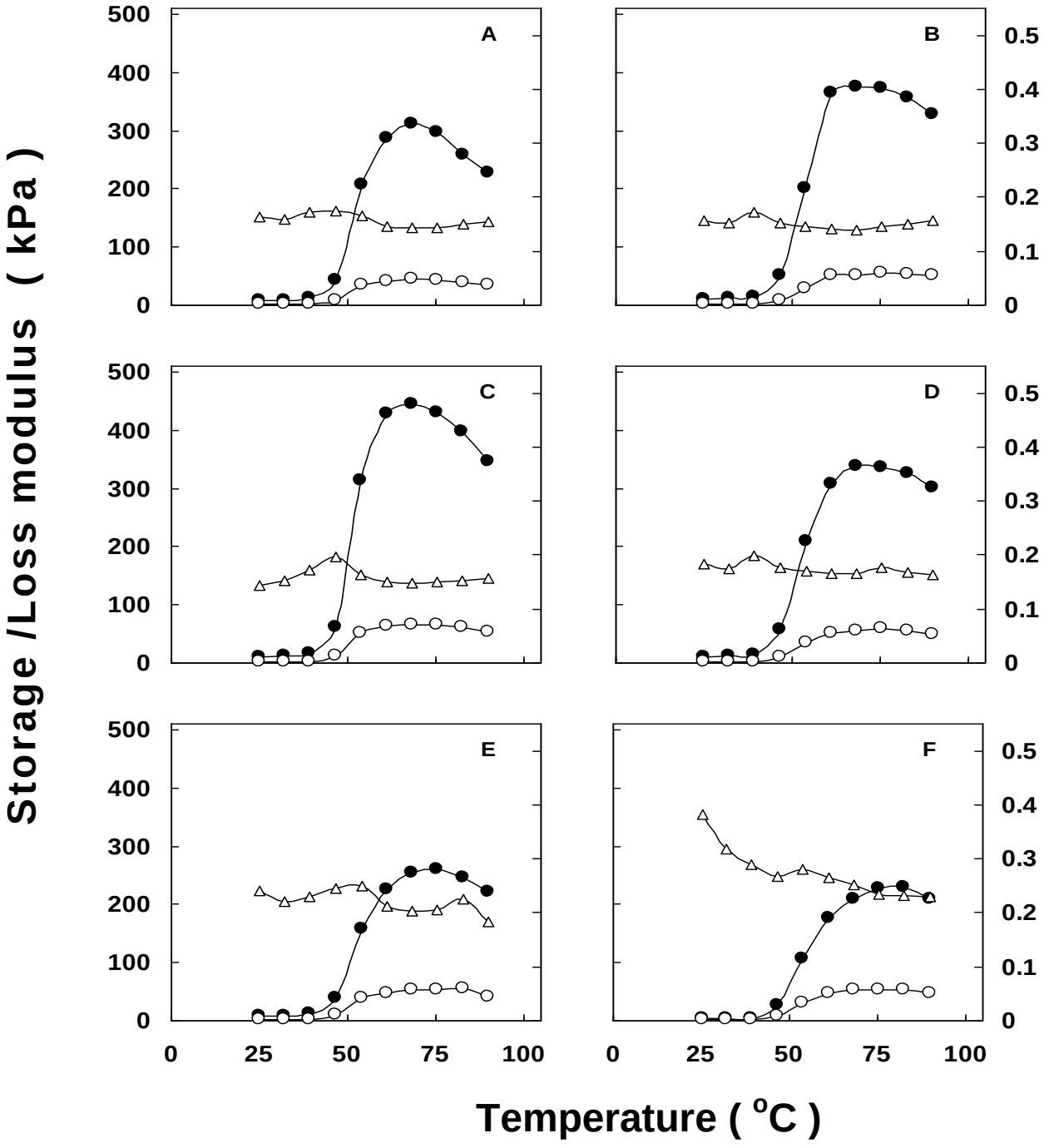

Storage /Loss modulus (kPa)
Tanδ
A
B
C
D
E
F
500
400
300
200
100
0
0.5
0.4
0.3
0.2
0.1
0
0
25
50
75
100
Temperature (°C)
G'
G "
Tanδ

Figure 9

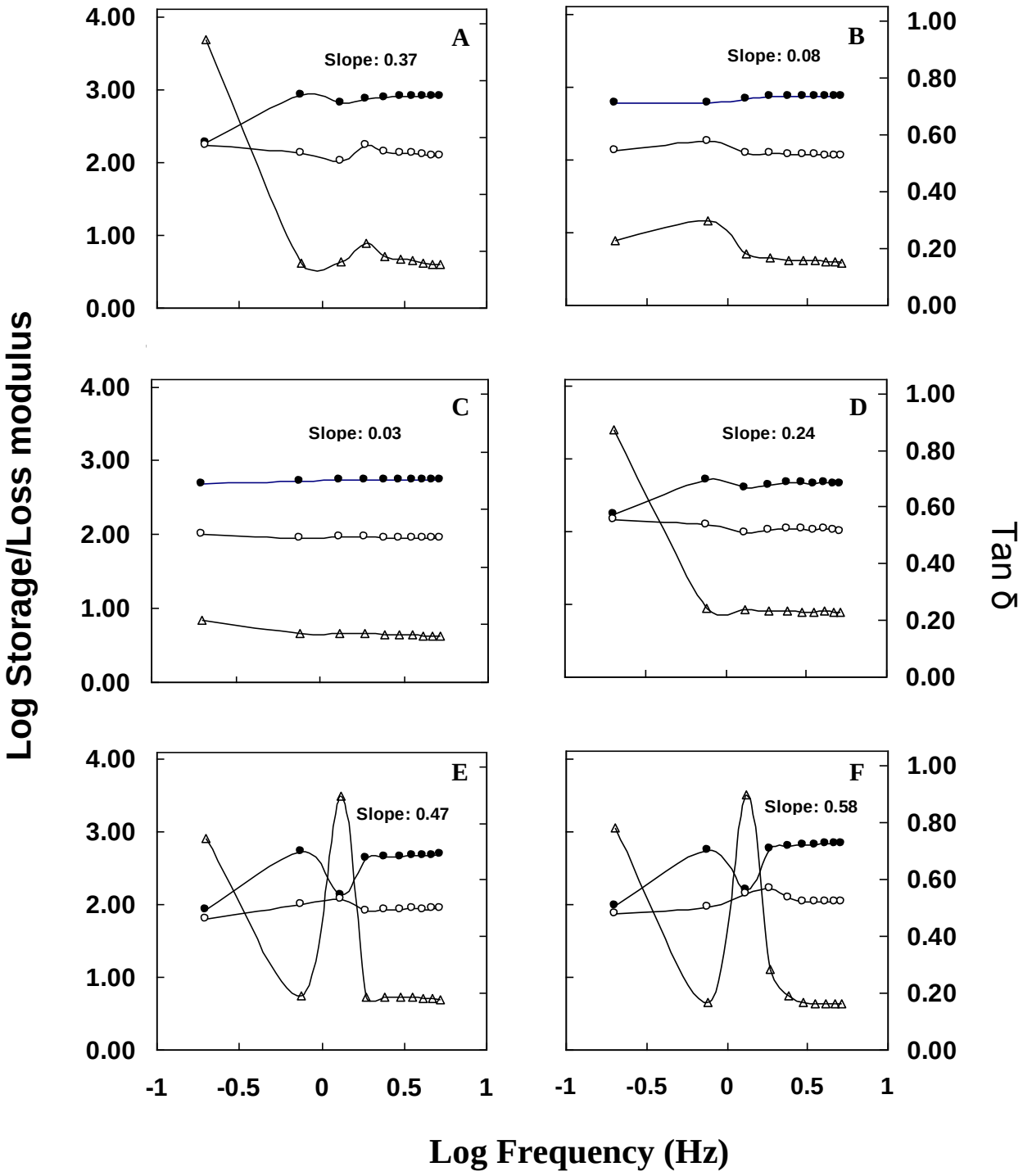

A
Slope: 0.37
B
Slope: 0.08
C
Slope: 0.03
D
Slope: 0.24
E
Slope: 0.47
F
Slope: 0.58
Log Storage/Loss modulus
Tan δ
Log Frequency (Hz)
4.00
3.00
2.00
1.00
0.00
1.00
0.80
0.60
0.40
0.20
0.00
-1
-0.5
0
0.5
1
G'
G "
Tanδ

Figure 10

800