

1 **COMPOSITION, PHYSICOCHEMICAL AND**
2 **RHEOLOGICAL PROPERTIES OF FRESH BIGEYE**
3 **SNAPPER FISH (*PRIACANTHUS HAMRUR*) MINCE**

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25 **Properties of fresh bigeye fish mince**

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ABSTRACT

Composition and properties of fresh bigeye snapper fish (*Priacanthus hamrur*) mince has been investigated. The protein content of fish mince was 16.71g/100 g mince. Amino acid analysis revealed higher proportion of glutamic acid, alanine, lysine and leucine. Fatty acid profile indicated higher proportion of EPA and DHA content. Gel filtration profile of total proteins from fresh bigeye fish mince revealed a major peak (high molecular weight component) and a few minor peaks, which was further confirmed by SDS-PAGE pattern. The differential scanning calorimetry profile of fresh bigeye fish mince revealed transitions at 38.35°C, 47.72°C and 63.02°C indicating denaturation temperature of different protein fractions. Gel forming ability of fresh bigeye fish mince was evaluated by both small strain and large strain tests. The flow behaviour of total protein solution from fresh bigeye fish mince as a function of protein concentration and temperature showed pseudoplastic behavior.

Key words: Bigeye fish mince, amino acid composition, differential scanning calorimetry, rheology

PRACTICAL APPLICATIONS

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52 The properties of fish mince including amino acid and fatty acid profile will be critical in
53 deciding the nature of processed products to be prepared. Due to lesser catch of bigeye
54 snapper in earlier days, this fish was not commonly available and hence it has been
55 regarded as a nonconventional marine fishery resource. Recently it has assumed
56 significance as an important species in the commercial landings. In the present
57 investigation composition, thermal and rheological properties of fresh bigeye snapper
58 have been assessed. High proportion of omega 3 fatty acids shows the nutritional
59 importance of this fish whereas its gel forming ability shows the potential to be utilized
60 as raw material in surimi industry. Establishing thermal denaturation profile,
61 measurement of dynamic viscoelastic behavior and assessing flow behavior of proteins
62 from bigeye snapper will aid in appropriate processing and design in formulating mince
63 based products.

64 **INTRODUCTION**

65 The bigeye snapper (*Priacanthus spp*) fish is regarded as one of the non conventional
66 marine fishery resources, due to its lesser availability in commercial fish landings in
67 earlier days. However, the species recently assumed significance as an important species
68 in the commercial fish landings on quantity basis. Being non conventional resource, the
69 precise catch statistics is not available, however, during the year 2001 the total quantity
70 landed was 103,102 t (FAO 2002). In India, the catches of bigeye snapper (also refereed
71 as ‘Bulls eye’) is showing increasing trend and constitutes 1-3% of total marine landings
72 during 1997-2002 (Sivakami *et al.* 2003). The potential yield in the Indian Exclusive
73 Economic Zone in the depth range of 30 – 150 m is estimated to be 54,800 t
74 (Somavamshi, 2001). The species available in abundance in Indian waters is *Priacanthus*
75 *harmur*. The abundance of bigeye snapper has been reported from South China Sea
76 (Morrissey and Tan 2000).

77 The consumer acceptance of fresh bigeye snapper is limited because of appearance,
78 thick skin and meat color. It is mainly used for the preparation of surimi (separated meat
79 water washed, partially dehydrated, mixed with cryoprotectants, frozen and frozen stored)
80 and surimi based products (Benjakul *et al.* 2002). The textural and sensory characteristics
81 of surimi and surimi based products depend on the ability to form gel. The gel forming
82 ability of bigeye snapper has been rated as high (Morrissey and Tan 2000) and therefore
83 used as raw material for surimi manufacture. Among different *Priacanthus spp*, the gel
84 forming ability of *P. tayenus* was found to be superior (Benjakul *et al.* 2002). The
85 textural properties surimi or fish mince based products are controlled by the internal
86 structure of the gel which is mainly made of myofibrillar proteins (Ko *et al.* 2007).

87 It is important to have details on amino acid profile and fatty acid composition of big
88 eye snapper so as to have complete nutritional details of the prepared products. Further,
89 the literature on rheological properties of bigeye snapper mince and the prepared gel is
90 lacking. Rheology is defined as the study of deformation and flow of matter (Barnes
91 2000). The rheological behavior of fish mince is of special importance when they are
92 used to modify textural attributes. It is also well recognized that rheological properties
93 play a role in process design, evaluation and modeling. These properties are sometimes
94 measured as an indicator of product quality (e.g. indication of total solids or change in
95 molecular size). Rheological data are required for calculation in any process involving
96 fluid flow (e.g. pump sizing, extraction, filtration, extrusion, purification) and play an
97 important role in the analyses of flow conditions in food processes such as pasteurization,
98 evaporation, drying and aseptic processing. Thus rheological measurements can yield
99 information relating both to acceptability of product texture and the chemical mechanism
100 operating under the various processing conditions.

101 With this rationale the present investigation was undertaken to elucidate a) the amino
102 acid and fatty acid profile of fresh big eye snapper (*Priacanthus harmur*), b) the dynamic
103 viscoelastic behavior of fish paste c) rheological characterization of heat set gel c) shear
104 stress sweep (flow properties) of proteins as a function of protein concentration and
105 temperature for predicting the behavior.

106 MATERIAL AND METHODS

107 Material

108 Fresh bigeye fish (*Priacanthus hamrur*) caught by trawl net along the West coast of
109 India, Mangalore, was used for the study. The length of fish used was 25-30 cm,

110 weighing 350-400g. Immediately after harvest, the fish were washed in chilled fresh
111 water and iced in the ratio of 1:1 (fish: ice) and transported to the laboratory for analysis.

112 **Methods**

113 **Proximate composition of bigeye snapper fish mince**

114 Meat was separated manually and macerated well for 5 minutes at 4°C using a pestle
115 and mortar. The macerated meat was used for proximate composition analyses. Moisture,
116 crude protein, fat and ash content in the mince were determined by the AOAC (2006)
117 method. All the analyses were carried out in triplicate.

118 **Non-protein nitrogen content and pH**

119 Non-protein nitrogen (NPN) content of bigeye snapper fish mince was determined by
120 the method as described by Velankar and Govindan (1958), using trichloroacetic acid
121 (TCA) extract and was expressed as mg/100 g of mince. About 3.0 g of mince was
122 macerated with 15 ml of 15ml/100 ml TCA for 5 minutes using a dried pestle and mortar.
123 The homogenate was allowed to stand at 4°C for 30 minutes. The slurry was filtered and
124 made up to 50 ml with distilled water and 5 ml of aliquot was taken for nitrogen
125 estimation using the Kjeldhal method. Analyses were carried out in triplicate.

126 The pH of bigeye snapper fish mince samples was measured using a pH meter
127 (Systronic 324 pH meter, Ahemdabad, India). Five grams of mince was macerated for 5
128 minutes with 45 ml distilled water and the pH was measured.

129 **Amino acid composition**

130 Amino acid composition of fresh bigeye snapper fish mince was determined after
131 derivatization with phenylisothiocyanate (PITC), according to the Waters Pico-Tag
132 method as described by Bidlingmeyer and others (1984) using the Waters Pico-Tag

133 HPLC amino acid analyzer (Water Model 712 WISP, Waters, Watford, Herts., UK). The
134 amino acid content was expressed as percentage of total amino acids.

135 **Fatty acid composition**

136 Fatty acid composition of fat extracted from bigeye snapper fish mince was
137 determined. Transesterification was performed according to Schmarr, Gross, and
138 Shabamoto (1996). The prepared fatty acid methyl esters (1 µl) were then injected into a
139 Varian gas chromatograph, (Series 3600, Walton-on-Thames, UK) with a hydrogen flame
140 ionization detector using helium as a carrier gas. An initial temperature of 180°C for 4
141 minutes was raised to 250°C at a rate of 4°C/minute. Chromatography of standard
142 reference fatty acids were also carried out to identify the individual fatty acids.

143 **Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

144 Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) was
145 performed as described by Laemmli (1970). The concentration of acrylamide was
146 10ml/100 ml of double distilled water. The thickness of the gel was 0.75mm.
147 Electrophoresis was carried out at a constant current mode in a vertical slab gel
148 electrophoresis apparatus (Hoefer –Pharmacia Biotech Inc., SE –50, San Francisco,
149 Calif., U.S.A.). A standard marker protein mixture of high range molecular weight
150 obtained from Sigma Chemicals, St Louis, MO, USA, was loaded into a separate well.
151 The molecular weight of the protein bands obtained in the sample was approximated by
152 measuring the relative mobility of the standard protein molecular weight markers.

153 **Gel filtration profile**

154 Gel filtration profile of total proteins extracted using extraction buffer (EB) was
155 carried out on a Sepharose 6B gel packed in a column of 1.5 x 80 cm (diameter x height)

156 at ambient temperature (27°C). The eluant used was EB. Total bed volume of the column
157 was 150 ml and the void volume determined by using blue dextran was found to be 50
158 ml. A protein concentration of 4 mg/ml was loaded on to the column and elution was
159 carried out at a flow rate of 30 ml/h. Fractions (3 ml) were collected manually and the
160 concentration of the eluant was determined by measuring the absorbance at 280 nm (A_{280})
161 using a spectrophotometer (Bausch and Lomb, Model 21-UVD, Austin, Texas, USA). A
162 plot of A_{280} nm against elution volume was obtained to get gel filtration profile.

163 **Differential Scanning Calorimetry (DSC)**

164 The thermodynamic parameters of the bigeye snapper fish mince were examined
165 using a DSC VII calorimeter (Setaram, Lyon, France). Fish mince was prepared by
166 macerating 5 g of meat for 5 minutes using a pestle and mortar. Water was kept as
167 reference for all the samples. Heating rate employed was 0.5°C/min from 10°C to 90°C.
168 Heat absorbed or released by the sample results in either endothermic or exothermic
169 peaks as function of temperature. From the thermogram, parameters such as T_o (onset
170 temperature) and T_m (denaturation temperature) for fish mince were obtained. The energy
171 required to denature the sample, the enthalpy change (ΔH), was measured by integrating
172 the area under the peak using the Setaram DSC software.

173 **Preparation of heat induced gel**

174 The bigeye fish meat (100g) was ground manually with 2.5g of Sodium chloride in a
175 pre-chilled pestle and mortar for 10 min. The resulting paste was packed in krehalon
176 (krehalon is the trade mark of Kureha Chemical Industry Co., Ltd., Japan) casing (260
177 mm long, 48 mm diameter and 200 gauge thickness) without air pockets, using a hand
178 stuffer. Prior to stuffing of paste into the casing, one end of the casing was sealed and

179 after stuffing the other end was sealed. The sealing was done with aluminium wire using
180 a clipping machine. The sealed casings were heated at $90^{\circ} \pm 2^{\circ}\text{C}$ for 45 min in a water
181 bath (Haake, model K10, Germany) and cooled in chilled water ($5\text{-}6^{\circ}\text{C}$) for 20 min. The
182 gel was kept in a refrigerator ($6\text{-}8^{\circ}\text{C}$) overnight.

183 **Gel strength measurement (Large strain test)**

184 The gel strength measurement was carried out using an Okada gellometer (Saitama
185 Keki Seisa Kuso Co. Ltd., Tokyo, Japan) as described by Suzuki (1981) after keeping at
186 ambient temperature for 1hr. Analysis was carried out in triplicate.

187 **Dynamic viscoelastic behaviour**

188 Dynamic viscoelastic behaviour (DVB) of fresh bigeye fish mince in the temperature
189 range of $30^{\circ}\text{-}90^{\circ}\text{C}$ was measured using a Carri Med Controlled Stress Rheometer (CSR-
190 500, Carri-Med, Surrey, UK) under oscillatory mode, using a 4 cm parallel plate
191 measuring geometry. Fish meat devoid of connective tissue, fins and scales was
192 macerated well using pestle and mortar. About 4 g of macerated mince was mixed with
193 sodium chloride at a concentration of 2.5 % of mince (w/w) and macerated thoroughly to
194 get a fine ground paste. The fish paste obtained was used for DVB measurement. The gap
195 between measuring geometry and peltier plate was adjusted to $2000\ \mu\text{m}$ at 80°C manually
196 using the micrometer provided at the base of the rheometer. The applied stress of 500 Pa
197 was within the viscoelastic region. The linear viscoelastic region was determined by a
198 torque sweep with a frequency of 1 Hz. Measurements were made by applying a small
199 displacement amplitude oscillation (0.0005 Rad) with a frequency of 1 Hz. A heating rate
200 of $1^{\circ}\text{C}/\text{min}$ was achieved through peltier plate of rheometer. Applied stress was compared
201 with the resultant strain. The results of such measurements were expressed as the storage

202 modulus (G') and loss modulus (G''). An average of three replicates was used for plotting
203 the results.

204 The gels obtained after temperature sweep were subjected to frequency sweep as well
205 as torque sweep at 30°C. Frequency was varied from 0.5 Hz to 5.5 Hz. Storage and loss
206 modulus (G' and G'') were obtained as a function of frequency. The slope of the
207 regression of $\log G'$ and $\log G''$ with change in frequency were obtained in order to
208 assess the viscoelastic nature of the sample. In the case of torque the critical stress values
209 at which there was a sudden drop in G' values were noted which is also accompanied
210 with a sharp rise in $\tan \delta$ values.

211 **Flow profile measurements (shear stress sweep)**

212 The flow properties of protein solutions from fresh bigeye fish mince were measured
213 as a function of temperature using a Controlled Stress Rheometer (CSR Carri-Med model
214 CSL 500, Surrey, UK) with flow software. Extraction buffer was used as the solvent for
215 protein extraction. The concentrations of protein in the samples were 5, 10 and 20 mg/ml
216 and the measurements were made at 30° and 40°C. The sample was equilibrated for 5 min
217 before the shearing experiment was started. The measuring geometry used was a 4 cm
218 cone and plate with a truncation of 59 μ m. The range of stress applied varied between 4 to
219 5 Pa depending on the angular velocity in the preshear experiment. The ascent and
220 descent time were 2 min each. Shear rate sweeps of the protein solutions at 30° and 40°C
221 were performed in triplicate and average values were taken for plotting. A flow curve was
222 obtained by plotting \log_{10} viscosity and \log_{10} shear rate values.

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RESULTS AND DISCUSSION

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226 **Proximate composition of bigeye snapper fish mince**

227 The proximate composition of fresh bigeye fish mince is given in Table 1. Bigeye fish
228 (*Priacanthus hamrur*) can be classified under lean variety fish as the fat content was
229 found to be less than 2g/100 g mince. The moisture content of the meat was around 78.8
230 % with a protein content of 16.7 %. The protein content of most fish averages to 15 – 20
231 % (Garrow and James 1993). The main components of fish are water, protein and lipid,
232 which make up 98 % of total weight of fish muscle. These components play a vital role in
233 the functional property and nutritional value of the fish. The remaining 2% mince
234 constitutes carbohydrate, vitamins and minerals (Ofstad *et al.* 1996). The NPN content of
235 fresh bigeye fish mince was 378 mg %, which was considerably lower than other pelagic
236 species (Table 1). NPN content reported for Bristly sardine (*Opisthonema libertate*),
237 Indian oil sardine (*Sardinella longiceps*) and Monterey sardine (*Sardinops sagax*
238 *caerulea*) varies from 410-460 mg/100 g (Pacheco-Anguilar *et al.*, 2000; Cortez-Ruiz *et*
239 *al.* 2001; Karthikeyan *et al.*, 2004). The NPN content in fish meat is due to
240 trimethylamine oxide and other energy-rich compounds such as creatine phosphate (Ikeda
241 1979). The NPN content of fresh ribbonfish was reported to be 672 mg/ 100 g mince
242 (Dileep *et al.* 2005). The pH of fresh bigeye snapper mince registered a value of 6.52
243 (Table 1), which was comparable to the pH of majority of fishes. The pH values of fresh
244 sardine and mackerel meat was reported to be around 6.5 (Chaijan *et al.*, 2005).

245 **Aminoacid composition**

246 The amino acid composition of bigeye fish mince is given in Table 2. The amino
247 acid profile revealed higher proportion of alanine, glutamic acid, lysine and leucine.

248 Bigeye fish meat was having higher proportion of white / light meat. The red meat of fish
249 has more glycine, leucine, arginine and phenylalanine whereas; white / light meat has
250 more lysine, aspartic acid and glutamic acid (Garrow and James 1993). The major amino
251 acids present in blue whiting and codfish were glutamic acid, aspartic acid, lysine and
252 leucine (Dagbjartsson 1975). Fish contains significant amounts of all essential amino
253 acids, particularly lysine. The lysine content of bigeye fish mince was 9.79 as percentage
254 of amino acids. Fish protein can be used therefore to complement the amino acid pattern
255 and improve the overall protein quality of a mixed diet.

256 **Fatty acid composition**

257 Fatty acid composition of fat extracted from bigeye fish mince is given in Table 3.
258 The fatty acid profile revealed that the major fatty acids present in bigeye fish were
259 heptadecanoic acid (C17: 0) and arachidic acid (C20: 0). The proportion of EPA and
260 DHA was higher compared to many freshwater fishes including Indian major carps,
261 common carp and tilapia (Swapna *et al.* 2010; Jabeen and Chaudhry 2011). The sum of
262 saturates in the fat extracted accounts for 43.92%. Polyenes accounted for 17.56% in
263 bigeye fish mince. The major fatty acid profile of bigeye fish was different from that of
264 pelagic fatty fish. The major fatty acids in chub mackerel and horse mackerel were found
265 to be palmitic acid, stearic acid and oleic acid followed by EPA and DHA (Celik 2008).
266 The major fatty acid present in sardine oil was palmitic acid (18%) followed by oleic
267 (14.67%), Eicosa pentaenoic acid (EPA) (14.08%) and Decosa hexaenoic acid (DHA)
268 (11.63%) (Bandarra *et al.* 1997). The EPA and DHA content of bigeye fish were 6.99 and
269 8.96% respectively, which were comparable with that of fatty fishes (Nayak *et al.* 2003;
270 Sahena *et al.* 2009). Among the Poly Unsaturated Fatty Acids (PUFA) in mackerel and

271 horse mackerel DHA was found to be predominant. This may be due to the type of food
272 intake available to these species (Bandarra *et al.* 2001). The EPA and DHA content of
273 Atlantic mackerel were nearly 6.23 and 14.19% respectively (Leu *et al.* 1981; Soriguer *et*
274 *al.* 1996). Fatty fish like mackerel contains a high percentage of PUFA, where the sum of
275 saturates will be usually less than 30% (Leu *et al.* 1981).

276 **Gel filtration profile**

277 Gel filtration profile of total proteins from fresh bigeye fish mince showed a major
278 peak eluting at an elution volume of 69 ml, indicating the high molecular weight fraction
279 (Figure 1). The high molecular weight fraction is actomyosin complex. In our earlier
280 studies this fraction corresponding to actomyosin was eluted at an elution volume of 59
281 ml (Dileep *et al.* 2005; Binsi *et al.* 2006). SDS-PAGE pattern of total proteins from
282 bigeye fish mince showed multiple bands in the molecular weight range of 205 to 6.5
283 KD. The bands in the molecular weight range revealed higher concentrations of 205 KD,
284 97 KD, 66 KD and 45 KD proteins (Figure 2). Chapleau *et al.* (2003) reported the bands
285 corresponding to 200 KD, 95 KD and 45 KD as myosin heavy chain, α -actinin and actin
286 respectively. The band at 66 KD probably may be a sarcoplasmic protein.

287 *3.5. Differential Scanning Calorimetry (DSC)*

288 The thermogram of the bigeye fish mince (Figure 3) showed three endothermic
289 transitions. The first and second transitions were at T_m 38.35°C and 47.72°C and the third
290 transition was at T_m 63.02°C. The first transition was due to the denaturation of myosin,
291 the second transition was assigned to water-soluble sarcoplasmic proteins and the third
292 transition was due to the denaturation of actin (Badii and Howell 2002). Yongsawatdigul
293 and Park (2003) explained that the transition at 36.5°C was due to structural changes of

294 threadfin bream myosin, while transition at 76.2 °C was due to actin (Yongsawatdigul
295 and Park, 2003). Previous researches indicate that the denaturation temperature of myosin
296 was slightly higher than the present study. Thermal transition of fresh goatfish
297 actomyosin exhibited two major peaks with the maximum transition temperatures of
298 myosin and actin as 47.4°C and 63.5°C respectively (Yarnpakdee *et al.* 2009). Wright and
299 others (1977) reported that actomyosin of rabbit exhibited three transitions, with myosin
300 transitions at 51.5, 60 °C and actin transition at 73 °C. This lower transition temperature
301 of bigeye fish myosin indicated its lower thermal stability as compared with rabbit.
302 Thermal energy absorbed by the protein will cause denaturation and give rise to
303 endothermic effect (Tanford 1968). The enthalpy change value for bigeye fish mince due
304 to denaturation of myosin was 0.3252J/g. The enthalpy values are slightly lower than that
305 of cod stored at -30°C (0.67 J/g) (Badii and Howell 2002). DSC is a very useful means of
306 studying the thermal properties of muscle protein and thermal denaturation (Ahmed *et al.*
307 2009).

308 **Dynamic Viscoelastic Behavior (DVB)**

309 The dynamic rheological test or small strain or gel rigidity test has been used widely
310 to study the heat-induced gelation of myofibrillar proteins (Visessanguan *et al.* 2000).
311 Venugopal *et al.* (2002) suggested that the changes in storage modulus (G') can be used
312 to monitor protein gelation. Since G' increase is a measure of recovered energy per cycle
313 of sinusoidal shear deformation, increase in G' indicates rigidity of the sample which is
314 associated with formation of elastic gel network (Dileep *et al.* 2005). The dynamic
315 viscoelastic behavior (DVB) of fresh bigeye fish indicated good gel forming ability as
316 revealed by storage modulus (G') values (Figure 4). The maximum G' value of 378.6 KPa

317 was attained at 63.6°C and two transitions were evident at 43.3°C and 63.6°C
318 respectively. This further confirms the transition temperatures of different fractions
319 obtained from DSC. The loss modulus (G'') value also increased during heating;
320 however, the magnitude was less than that of G' values. This is an indication of the
321 formation of a viscoelastic gel network. The maximum rate of increase in G' values were
322 found in the temperature range of 43.3° to 56.7°C. The maximum rate of increase in G'
323 value below 60°C is likely to involve protein unfolding and formation of disulfide and
324 hydrophobic interaction (Niwa 1992).

325 The gelation process was also monitored by measuring changes in stress-strain phase
326 angle during oscillatory test and indicates the temperature at which transition from sol-gel
327 took place. The $\tan \delta$ values were obtained by taking a ratio of G''/G' during isothermal
328 heating. This transition for fresh bigeye fish meat occurred at 43.3°C and 63.6°C (Figure
329 4). The first transition has been attributed to the tail of myosin molecule and the transition
330 at higher temperature to that of head of myosin molecule (Sano *et al.* 1988; Sano *et al.*
331 1990).

332 The slope of regression of G' values of gel obtained after temperature sweep is given
333 in Figure 5. A lower value of the slope (0.0906) in the frequency sweep can be taken as
334 an index of better gel formation (Dileep *et al.* 2005; Kim *et al.* 2005). This was further
335 confirmed by performing a torque sweep, which indicated a maximum stress tolerance
336 limit of 1259 Pa (Figure 6). At a stress value of 1259 Pa there was a sharp increase in \tan
337 δ values indicating a more viscous nature than elastic. The gel strength of gel as
338 measured by large strain test was 771.4 g (breaking force) at a deformation of 2.1 cm

339 (Table 1). The frequency sweep, torque sweep and large strain test data clearly
340 demonstrates the higher gelling ability of bigeye snapper fish.

341 3.7. Flow profile measurements (shear stress sweep)

342 The flow profile of the myofibrillar proteins at specific temperature provides
343 information on resistance to shearing thereby indicating any structural impairment. Shear
344 stress and shear rate data were determined for total proteins from bigeye fish for protein
345 concentration of 5, 10 and 15 mg/ml at 30°C and 40°C. The flow behavior of total
346 proteins from fresh bigeye fish mince at all the concentrations and temperatures revealed
347 pseudoplastic behavior (Figure 7). A decrease in thixotropic area with increase in protein
348 concentration was evident at both the temperatures studied; however, it was more
349 prominent at 30°C. Minimum thixotropic area was recorded at 30°C for a concentration
350 of 15 mg/ml indicating minimum damage to the structure of protein due to shearing. At
351 40°C higher degree of structure impairment of molecules was evident at all the protein
352 concentrations studied as revealed from larger thixotropic area. The shear stress sweep of
353 acidified shark gel dispersion at a protein concentration of 6.8 mg/ml showed large
354 thixotropic area at 40°, 50° and 60°C (Venugopal *et al.* 2002). In the present study flow
355 measurements could not be carried out at above 50°C, as there was extensive coagulation
356 and difficulty in shearing the sample was experienced.

357 All the samples exhibited shear thinning behavior as indicated by shear stress - shear
358 rate data. The maximum stress applied for shearing the sample was 5Pa. Protein solutions
359 at all concentration and temperatures exhibited a yield stress value and thereafter showing
360 shear thinning / pseudoplastic behavior. It has been recognized that pseudoplasticity
361 represents an irreversible structural breakdown and the decrease in viscosity occurs as a

362 result of molecular alignment (Liu *et al.* 2008). The flow profile at 40°C showed a
363 different behavior (Figure 7B, 7D and 7F) showing an upswing in viscosity at lower shear
364 rate values. This may be due to higher protein-protein interaction at 40°C as also evident
365 from DSC and DVB data. The upswing in viscosity values at low shear rates is also been
366 explained as indicative of an apparent yield stress (Rao and Tattiyakul 1999).

367 **4. Conclusion**

368 With the recent increase in bigeye snapper fish catch in commercial fish landing, it
369 becomes inevitable to improve its utilization for better economic returns. Utilization of
370 fish meat depends on the understanding of its composition and properties of the meat.
371 Higher content of essential amino acids especially lysine along with the presence of
372 higher proportion of EPA and DHA make this fish ideal in nutritional point of view.
373 Results obtained from small and large scale deformation analysis revealed that fresh
374 bigeye fish has good gel forming ability. Therefore bigeye snapper fish can be considered
375 as a suitable species for surimi production and also can be used in many formulated
376 products. Further studies are required to determine the influence of water washing on
377 enhancing the functionality of bigeye snapper fish mince.

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537 **TABLE 1- COMPOSITION OF FRESH BIGEYE FISH (*PRIACANTHUS***
538 ***HAMRUR*) MINCE^a**

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Moisture (g/100 g mince)	78.80 (0.90)
Fat (g/100 g meat)	1.28 (0.18)
Protein (N×6.25) (g/100 g mince)	16.71 (0.36)
Ash (g/100 g mince)	2.94 (0.48)
NPN (mg/100 g mince)	378.60 (12.2)
pH	6.52 (0.08)
Gel strength of the gel	
Breaking force (g)	771.4
Deformation (cm)	2.1

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^aValues in parenthesis denotes standard deviation, n = 3

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TABLE 2- AMINO ACID COMPOSITION OF BIGEYE FISH MINCE^a

Amino acids	Bigeye fish (<i>Priacanthus spp.</i>) Mean \pm S.D
Asp	7.96 \pm 0.50
Glu	11.84 \pm 0.34
h. pro	0.29 \pm 0.10
Ser	4.42 \pm 0.08
Gly	7.51 \pm 0.34
His	0.45 \pm 0.05
Arg	5.79 \pm 0.02
Thr	5.14 \pm 0.16
Ala	13.04 \pm 0.41
Pro	1.80 \pm 0.46
Tyr	3.37 \pm 0.07
Val	6.48 \pm 0.20
Met	1.96 \pm 0.54
Cys	0.53 \pm 0.06
I leu	4.63 \pm 0.02
Leu	8.70 \pm 0.10
Phe	3.30 \pm 0.03
Trp	2.97 \pm 0.45
Lys	9.79 \pm 0.76

^a% Amino acids in fish (S.D – standard deviation, n = 3)

**TABLE 3- FATTY ACID COMPOSITION OF BIGEYE FISH
(*PRIACANTHUS HAMRUR*) MINCE^a**

Fatty acids	Mean ± S.D
C 14:0 (Myristic)	2.38 ± 0.13
C 16:0 (Palmitic)	6.60 ± 1.44
C 16:1 (Palmitoleic)	6.75 ± 1.66
C 17:0 (Heptadecanoic)	12.76 ± 3.65
C 18:0 (Stearic)	5.16 ± 1.68
C 18:1n9c (Oleic)	7.12 ± 1.87
C 18:2n6c (Linoleic)	9.60 ± 2.70
C 18:3n3 (Linolenic)	1.62 ± 0.09
C 20:0 (Arachidic)	12.67 ± 1.55
C 20:1n9 (cis-11-Eicosenoic)	ND
C 20:3n3 (cis-11, 14, 17-Eicosatrienoic)	ND
C 20:5n3 EPA	6.99 ± 1.54
C 22:0 (Behenic)	9.84 ± 1.59
C 22:1n9 Erucic acid/Cetoleic	9.56 ± 2.67
C 22:6n3 DHA	8.96 ± 2.55
Sum of saturates	43.92 ± 3.22
Sum of monoenes	38.52 ± 2.98
Sum of polyenes	17.56 ± 1.99

^a% (14 Fatty acids) (S.D-standard deviation; n = 3; ND- Not determined)

LEGEND TO FIGURES

- FIGURE 1: GEL FILTRATION PROFILE OF TOTAL PROTEINS FROM FRESH BIGEYE SNAPPER FISH (*PRIACANTHUS HAMRUR*) **MINCE**
- FIGURE 2: SDS-PAGE PATTERN OF TOTAL PROTEINS FROM FRESH BIGEYE SNAPPER FISH (*PRIACANTHUS HAMRUR*) **MINCE**. LANE 1: PROTEIN SAMPLE FROM FRESH FISH; LANE 2: DUPLICATE OF LANE 1; LANE 3: STANDARD MARKER
- FIGURE 3: DSC PROFILE OF BIGEYE SNAPPER FISH **MINCE**. THE EXPERIMENT WAS CARRIED OUT AT A HEATING RATE OF $0.5^{\circ}\text{C}/\text{MIN}$.
- FIGURE 4: DYNAMIC VISCOELASTIC BEHAVIOR OF FRESH BIGEYE SNAPPER FISH **MINCE IN THE TEMPERATURE RANGE OF 30° - 90°C** .
- FIGURE 5: FREQUENCY SWEEP OF FRESH BIGEYE SNAPPER FISH **MINCE** (SOL AND GEL) AT 30°C . THE GEL OBTAINED AFTER TEMPERATURE SWEEP IS USED FOR FREQUENCY SWEEP.
- FIGURE 6: TORQUE SWEEP OF **GEL** FROM FRESH BIGEYE SNAPPER FISH **MINCE**. **TORQUE SWEEP WAS CARRIED OUT AT 30°C**
- FIGURE 7: SHEAR STRESS SWEEP OF PROTEIN SOLUTION FROM FRESH BIGEYE SNAPPER FISH **MINCE**. **THE PROTEINS WERE EXTRACTED IN EXTRACTION BUFFER. THE SHEAR STRESS SWEEP WAS CARRIED AS A FUNCTION OF PROTEIN CONCENTRATION AND TEMPERATURE.**
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|-------------------------------------|-------------------------------------|-------------------------------------|
| A: 5 mg/ml at 30°C | B: 5 mg/ml at 40°C | C: 10 mg/ml at 30°C |
| D: 10 mg/ml at 40°C | E: 15 mg/ml at 30°C | F: 15 mg/ml at 40°C |

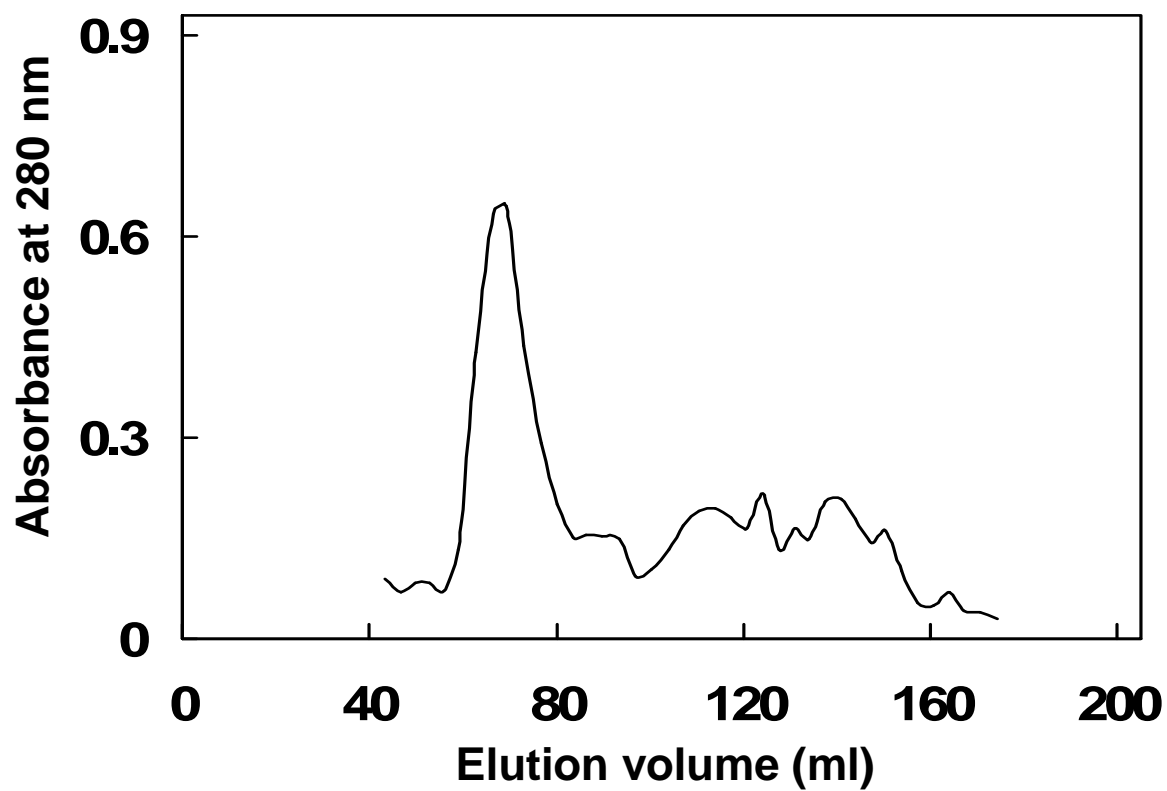


FIGURE 1

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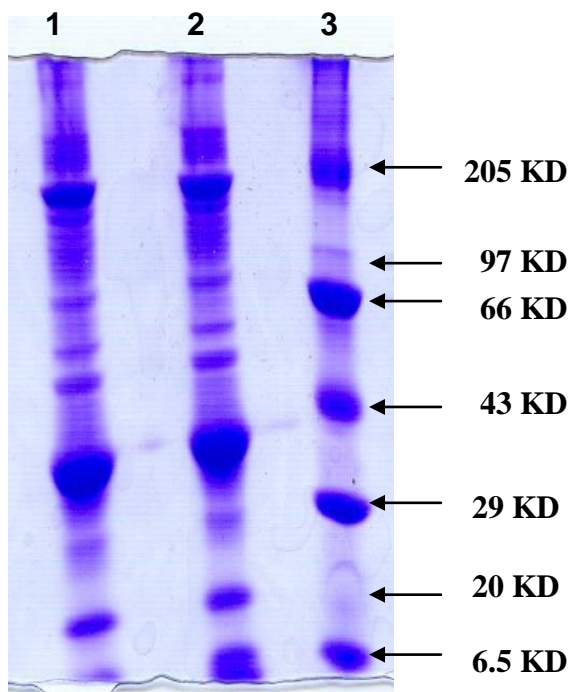


FIGURE 2

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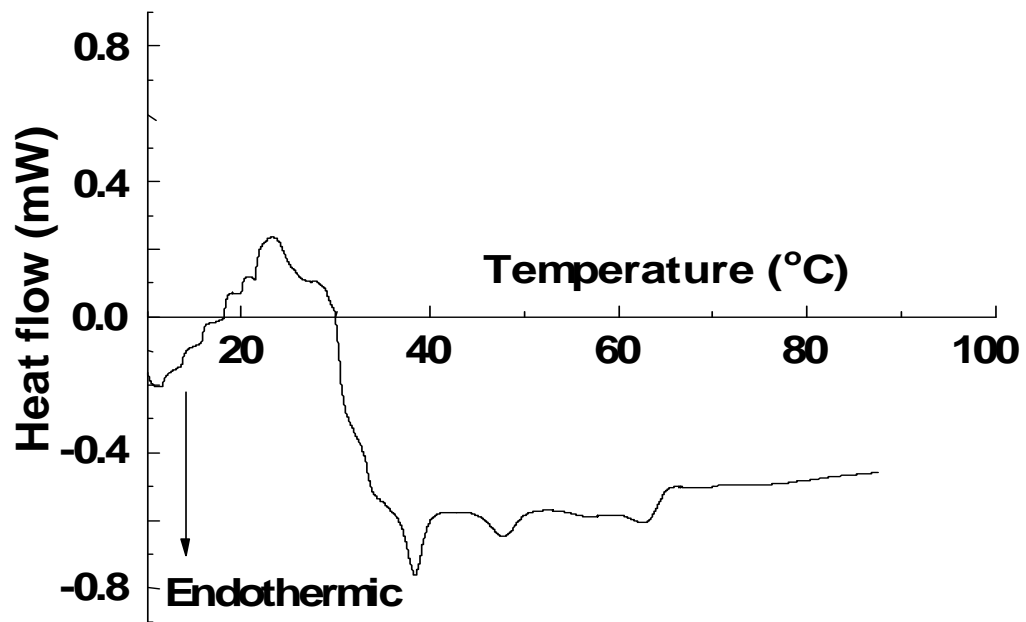


FIGURE 3

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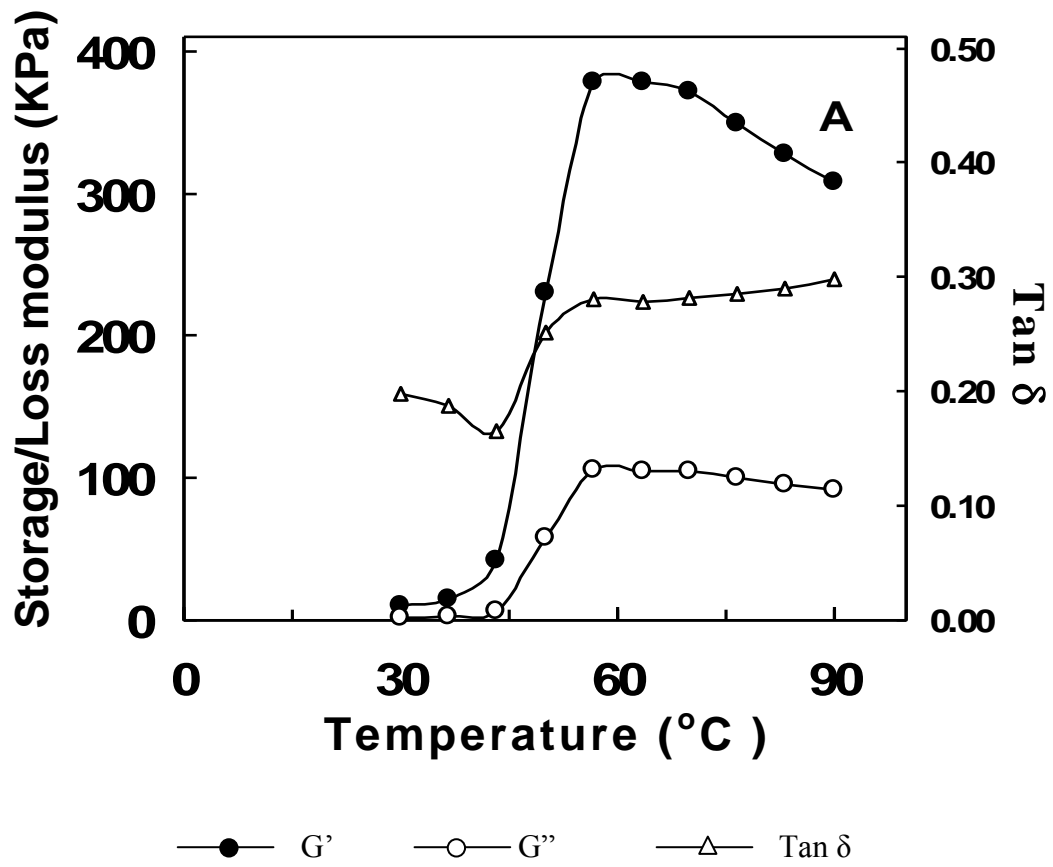
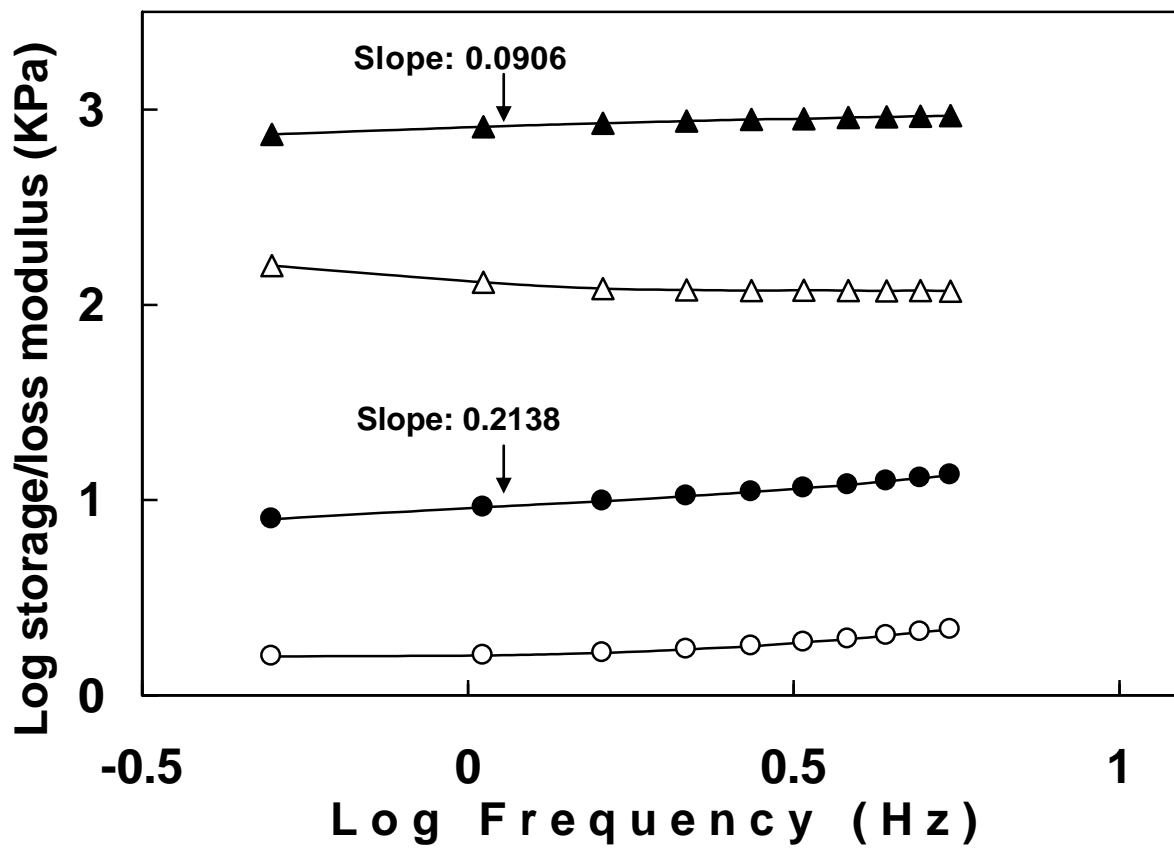


FIGURE 4



Elution volume (ml)

Gel: G' —▲— G'' —△— Sol: G' —●— G'' —○—

Figure 5

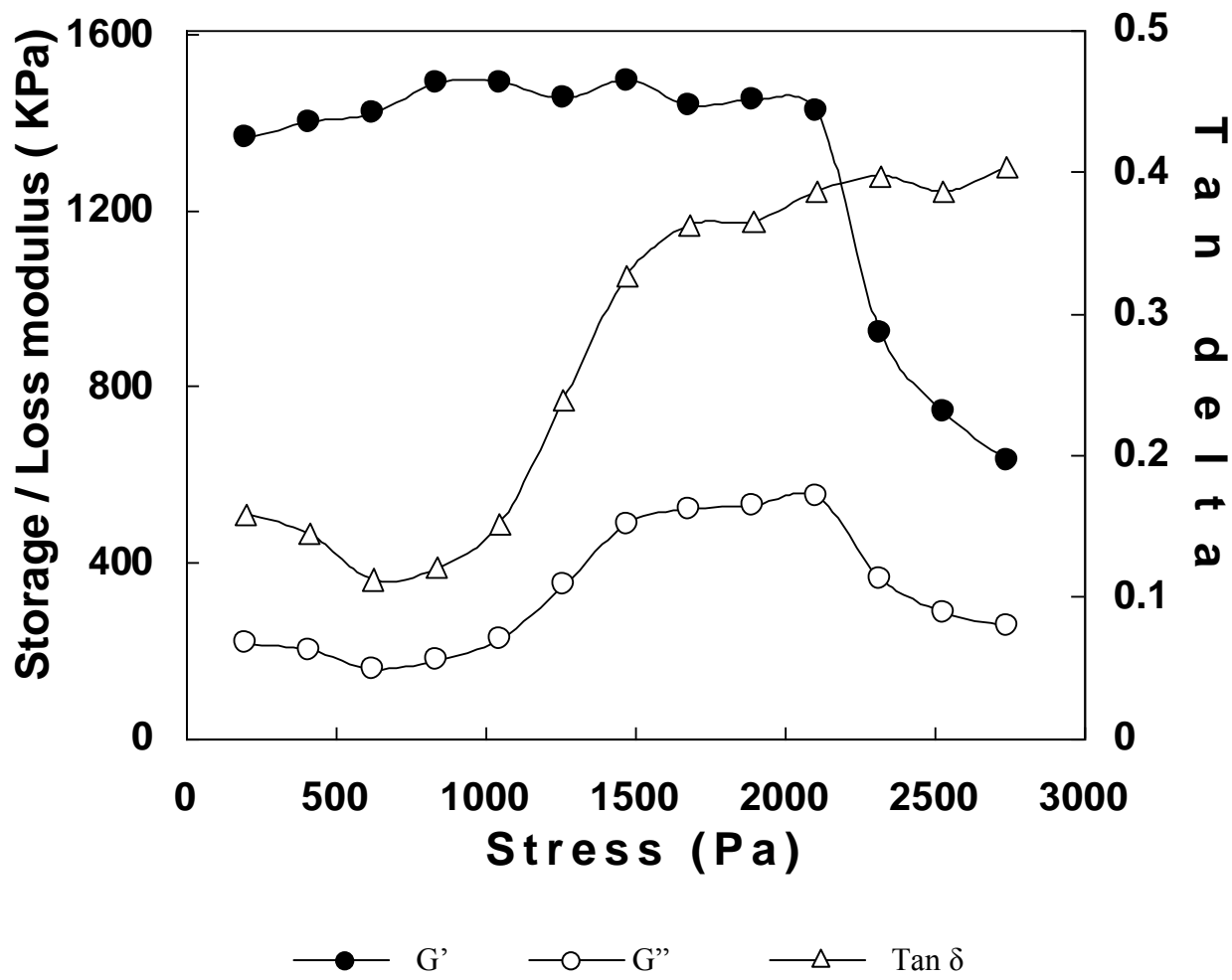
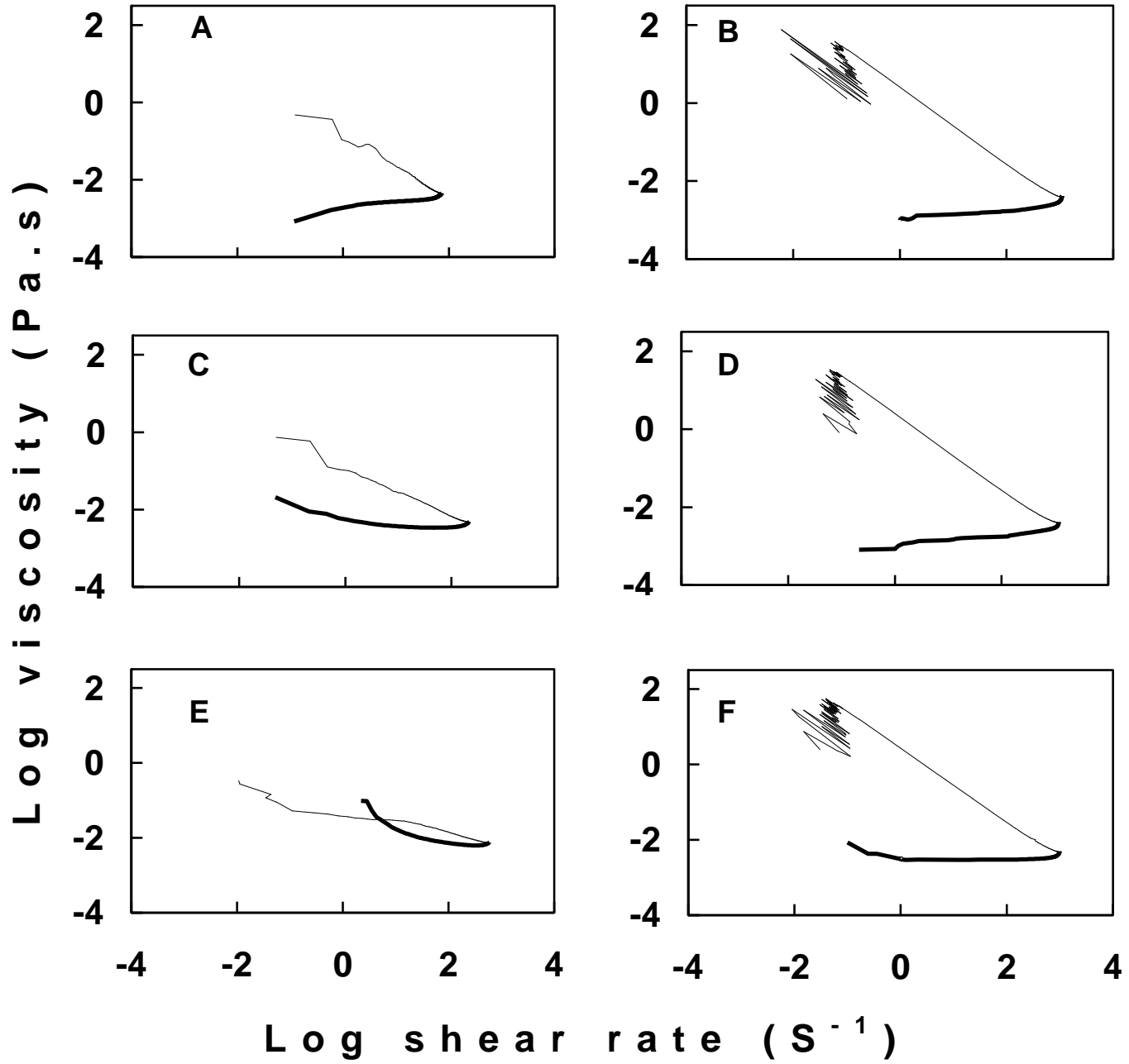


FIGURE 6

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— Up curve

— Down curve

Figure 7

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