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

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

INTRODUCTION

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

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

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

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

MATERIAL AND METHODS

Material

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

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

Methods

Proximate composition of bigeye snapper fish mince

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

Non-protein nitrogen content and pH

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

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

Amino acid composition

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

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

Fatty acid composition

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

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

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

Gel filtration profile

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

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

Differential Scanning Calorimetry (DSC)

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

Preparation of heat induced gel

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

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

Gel strength measurement (Large strain test)

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

Dynamic viscoelastic behaviour

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

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

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

Flow profile measurements (shear stress sweep)

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

RESULTS AND DISCUSSION

Proximate composition of bigeye snapper fish mince

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

Aminoacid composition

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

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

Fatty acid composition

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

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

Gel filtration profile

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

3.5. Differential Scanning Calorimetry (DSC)

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

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

Dynamic Viscoelastic Behavior (DVB)

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

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

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

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

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

3.7. Flow profile measurements (shear stress sweep)

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

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

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

4. Conclusion

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

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

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

^aValues in parenthesis denotes standard deviation, n = 3

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

Figure 7

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