International Journal of Sciences & Applied Research

www.ijsar.in

Physico-chemical and functional characteristics of muscle proteins from ribbon fish (*Trichiurus Spp*.) of different weight groups

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Abstract

The fishing operations landing ribbon fish (*Trichiurus lepturus*) constitute diverse length groups ranging from 10 to 120 cm and weighing up to 1000 g. It is largely being utilised for preparation of surimi and whole fish exports by fish processing industry in India. The present work was carried out to study the physicochemical and functional characteristics of muscle proteins of fish with respect to different size/weight groups. These properties of muscle proteins of four different weight groups (up to 100 g (I), 100-200 g (II), 200-400 g (III) and 400-800 g (IV) of the fish are analysed. Solubility of sarcoplasmic protein (SPP) was higher in group IV. The myofibrillar protein (MFP) concentration was significantly higher in group III and IV. The concentration of reactive sulphydryl group and Ca^{++}ATPase activity of MFP were higher in group III and IV than in I and II.

High emulsion activity index (EAI) was noticed for group III and IV muscle proteins. EAI of SPP and MFP decreased with increase in protein concentration in all the group of fishes. MFP from group III and IV showed better emulsion stability (ES) and the ES showed concentration-dependent increase in all groups for both SPP and MFP. Foam expansion (FE) and foam volume stability (FVS) was higher in group III and IV in MFP fraction but FE and FVS of all groups did not show any significant difference in SPP fraction. Viscosity of MFP from group III and IV was significantly higher but Viscosity of SPP did not show any significant difference. Water holding capacity (WHC) and gel strength (GS) were higher for group III and IV. Electrophoresis of SPP and MFP of all the groups showed difference in band pattern indicating various proteins expressed during various stages of growth. Extraction of SPP showed 3 bands in group I, II, III and IV of fishes. Band intensity of myosin and actin were found to be the highest in the case of group III and IV. With respect to various weight groups of ribbon fish, group III and IV had significantly better physico-chemical and functional characteristics than group I and group II fish.

Keywords: Ribbon fish, weight groups, Functional properties.

Introduction

Fish proteins constitute a diverse and complex collection of biological macromolecules. Although contributing to nutritional quality, fish proteins also act as integral component, by virtue of their diverse functional properties. Protein stability is a condition which permits protein molecules to achieve and maintain their native conformation but not to an extent that would preclude conformational adjustments necessary for biological functions (Beektel and Schellman, 1987). Ribbonfish was chosen for the present study because it is a fish of low economic value and contributes to 7.5% of total Indian marine landings (CMFRI 2003). Commercial fishery for Ribbon fish contributed various size range of *Trichiurus spp.* in the trawler catches are 15-124 cm, 36-124 cm in gill net and 22-84 cm in boat seines (CMFRI, 2002-2003). Ribbon fish is being considered as an alternative source for surimi production and value addition. As the fish catch for Ribbon fish includes fish of different size/weight groups and there is no information as to the optimum size of fish for utilization in surimi preparation. This information will help in understanding the functional characteristics of fish muscle protein as a function of size variation.

Materials and methods

Fresh Ribbon fish (*Trichiurus spp*) were procured from Mirkarwada fish landing centre of Ratnagiri. Fishes of four different weight groups viz., up to 100 g (group I), 100-200 g (group II), 200-400 g (group III), 400-800 g (group IV), were selected for the study based on their most frequent sizes of landings. The samples were transported to the laboratory with ice. Immediately on reaching the laboratory, the fishes were thoroughly washed with cold water to remove blood, slime, dirt, etc. The total length and weight of fish sample were recorded and different sizes of average weight viz., 88 g, 164 g, 353 g and 502 g and average length of 48 cm, 60 cm, 73 cm and 99 cm respectively.

Fishes were de-skinned and filleted. The fillets were minced in a kitchen mixer/grinder and boneless meat was used for experiments. The temperature of mince was maintained at 2-4^{0}C throughout the experiment**.** Proximate composition of ribbon fish meat was estimated as per the Association of Official Analytical Chemists (AOAC, 1990). The Extraction of muscle protein fraction was estimated according to the method of King and Poulter, (1985). Protein determination of myofibrillar (MFP) and sarcoplasmic (SPP) fractions were estimated according to Biuret method (Raghuramulu et al., 1983). Extraction of actomyosin was followed based on the method described by MacDonald and Lanier (1994). The ATPase assays of actomyosin were estimated according to the method of MacDonald and lanier (1994).The reactive SH group were estimated according to Sedlak and Lindsay (1968). Viscosity of salt soluble and water-soluble protein at different concentrations (2.5, 5.0 and 10 mg/ml) were determined with a viscometer (Model DV II + Pro, Brookfield) at shear rate 100 rpm. As described by Mohan *et al.*(2006). The Foam ability of the protein was determined by the method of Wild and Clark, 1996.The heat-induced gels were prepared from MFP concentrate according to Lan *et al* (1995). Water holding capacity (WHC) of mince was carried out by the method of Kocher and Foegeding (1993). The SDS-PAGE of MFP and SPP fractions was determined by the method of Laemmli (1970).

Statistical analysis of data generated was done by the method of Snedecor and

Cochran (1967).The significant results are mentioned as P <0.01 and P <0.05.

Results and discussion

The proximate composition of four (I, II, III, IV) groups of ribbon fish were observed as follows: Moisture - 74.513%, 74.143%, 75.123% and 75.157% respectively, Protein -14.87%, 15.893%, 17.203% and 17.64 % respectively, Fat - 0.842%, 0.855%, 0.842% and 0.857% respectively, Ash – 2.74%, 2.99%, 3.75% and 3.66% respectively. (Tab.1). Dileep *et al.*(2005) The protein content of Ribbon fish MFP in four groups such as I, II, III, and IV was observed to be 54.738 mg/g, 54.999 mg/g, 57.422 mg/g and 55.980mg/g respectively. The protein content of Ribbon fish MFP from groups III and IV had higher as compared to groups I and II (Fig. 1). The protein content of SPP in four groups such as I, II, III, and IV was observed to be 54.447, 54.493, 54.516 and54.554mg/g respectively. The protein content of Ribbon fish SPP from groups III and IV had higher as compared to groups I and II (Fig.2). Ramchandran *et al*. (2006) reported the values of extractability of SPP and MFP fraction for barracuda (*Sphyraena jello*) were 107 and 158.40 mg/g for group I and 167.18 and 203.08 mg/g for group II fishes, respectively. Mohan *et al*. (2006) reported values for the protein content of SPP and MFP as 47.6 and 76.5 mg/g, respectively for Rohu.

The Ca^{++}ATPase activity of MFP fraction of Ribbon fish from different weight groups was group I-0.137, II-0.149, III-0.166, IV-0.180µmole/mg/min. The Ca^{++} ATPase activity of Ribbon fish from groups III and IV fishes had higher as compared to groups I and II. (Fig.3) Ramchandran *et al*., (2006) reported values for Ca^{++} ATPase activities of the MFP were 0.29 and 0.65 µmole Pi/mg protein/min for groups I and II fishes. The Ca^{++}ATPase activity values were comparable with that of actomyosin reported for Barracuda (*Sphyraena jello*). Ca^{++} ATP ase activity indicates the integrity and functionality of the myosin molecule in the actomyosin complex. The mature fishes have higher relative percentage of myosin in there actomyosin (Montecchia *et al*. 1997). Therefore, the higher Ca^{++} ATPase activity in group III and IV fishes could be attributed to the higher percentage of myosin in the actomyosin of this fishes.

The concentration of reactive sulphydryl groups of MFP fraction of Ribbon fish from different weight groups was for group I, II, III, IV 19.212, 19.978, 20.568, 20.774 µmole SH/g protein respectively. The reactive SH groups of Ribbon fish from groups III and IV fishes had higher as compared to groups I and II (Fig.4).

The pH of fish muscle of four groups of Ribbon fish such as up to I, II, III and IV was observed to be 6.53, 6.53, 6.57 and 6.57 respectively. Dileep *et al.* (2005) reported nearly similar value of 700-800 g of Ribbon fish (*Trichiurus spp*) fish for the pH 6.92.

Ramchandran *et al*. (2006) reported nearly similar values of 700-800 g of Ribbon fish (*Trichiurus spp*) fish for the moisture, crude protein, fat and ash content in Ribbon fish meat as 78 %, 17.9 %, 1.18 % and 3.08 % respectively.

Table 1: Proximate composition of Different weight groups of Ribbon fish.

Groups / Parameters	I (Up to 100 g)	II (100-200 g)	III (200-400 g)	IV (400-800 g)
Moisture	74.513%	74.143%	75.123%	75.157%
Protein	14.87%	15.893%	17.203%	17.64%
Fat	0.842%	0.855%	0.842%	0.857%
Ash	2.74%	2.99%	3.75%	3.66%

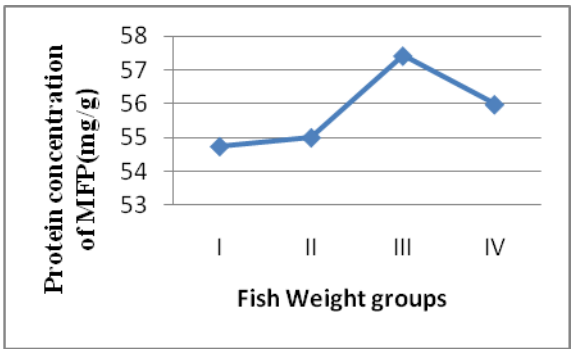

Fig. 1 Protein concentration of MFP (mg/g) of Ribbon fish weight groups

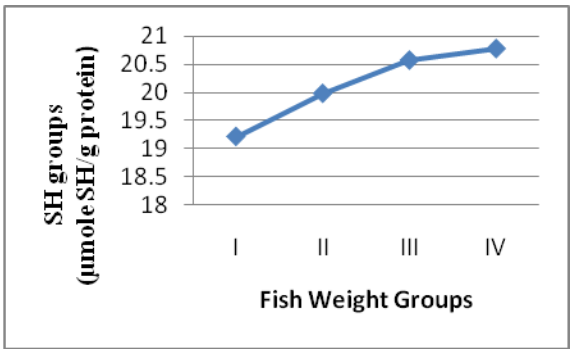

Fig. 4 Reactive Sulphydryl groups (SH) of MFP of Ribbon fish weight groups

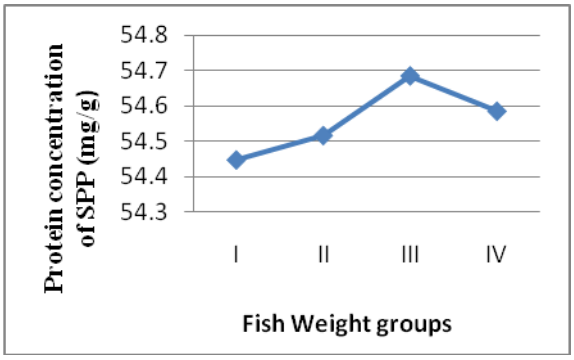

Fig. 2 Protein concentration of SPP (mg/g) of Ribbon fish weight groups

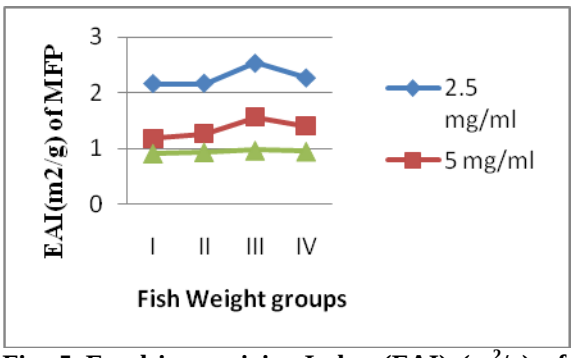

Fig. 5 Emulsion activity Index (EAI) (m^2/g) of MFP of Ribbon fish weight groups

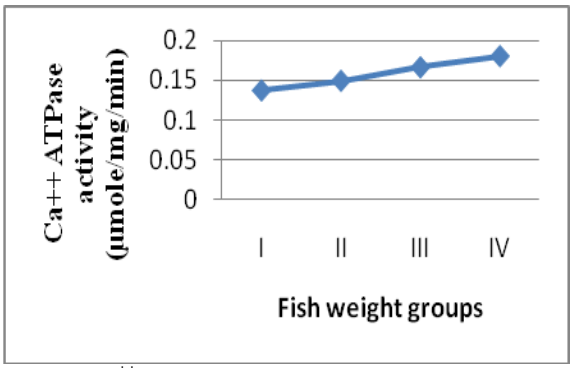

Fig. 3 Ca^{++} ATPase activity (µmole/mg/min) of Ribbon fish weight groups

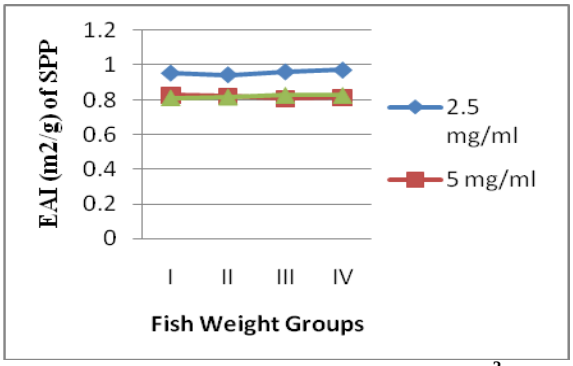

Fig. 6 Emulsion activity Index (EAI) (m^2/g) of SPP of Ribbon fish weight groups

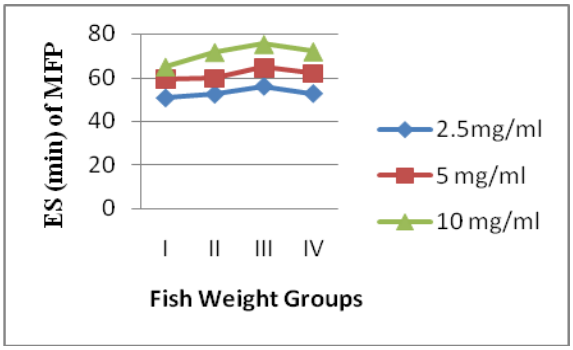

Fig. 7 Emulsion Stability (min) of MFP of Ribbon fish weight groups

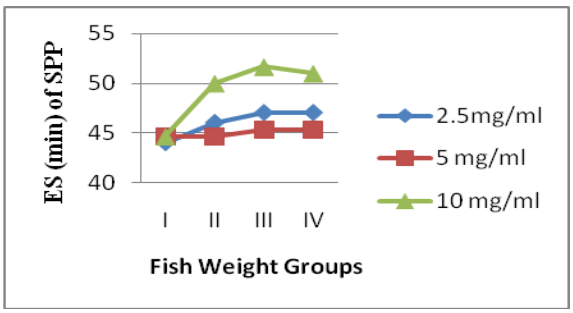

Fig. 8 Emulsion Stability (min) of SPP of Ribbon fish weight groups

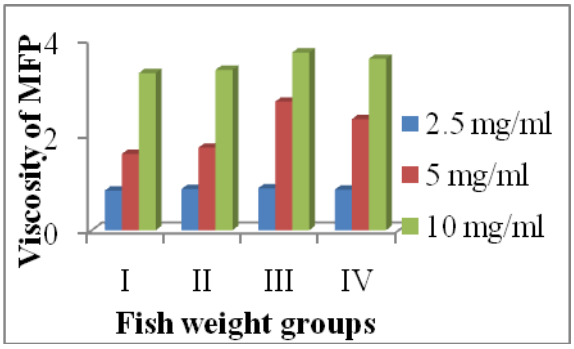

Fig. 9 Viscosity of MFP of Ribbon fish weight groups

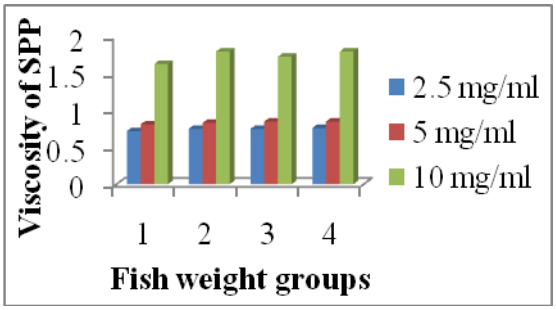

Fig. 10 Viscosity of MFP of Ribbon fish weight groups

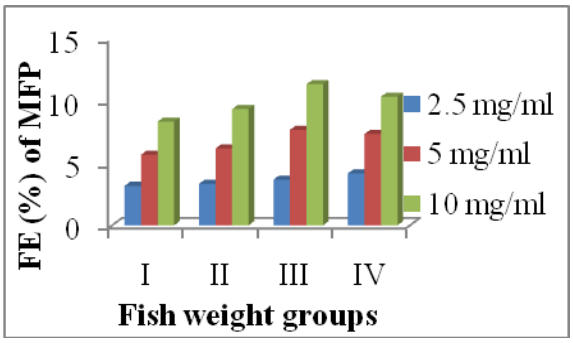

Fig 11 Foam expansion of MFP of Ribbon fish weight groups

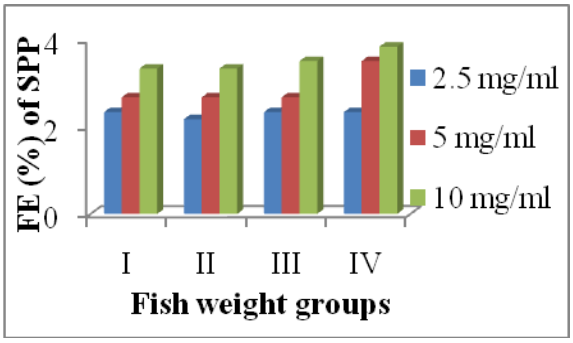

Fig.12 Foam expansion of SPP of Ribbon fish weight groups

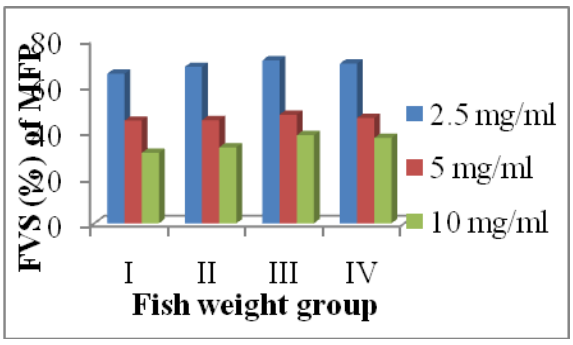

Fig. 13 Foam volume stability of MFP of Ribbon Fish weight groups

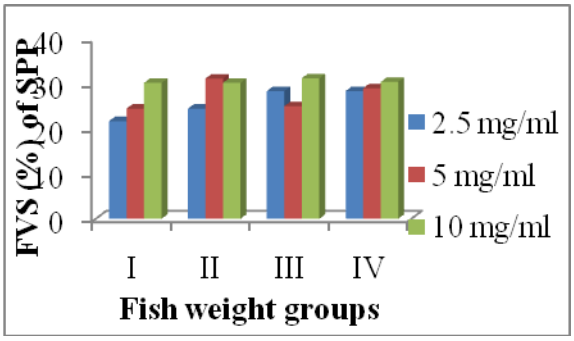

Fig. 14 Foam volume stability of MFP of Ribbon Fish weight groups

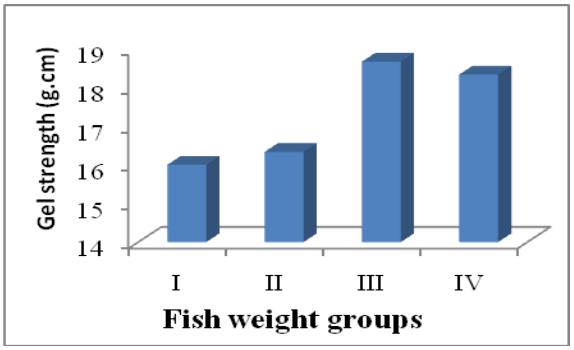

Fig. 15 Gel strength (gf.cm) of Ribbon fish weight groups

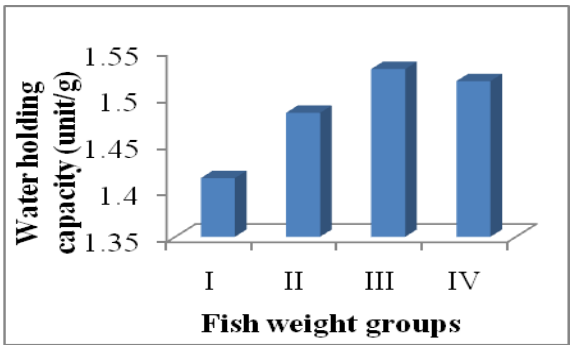

Fig. 16 Water holding capacity (unit/g)

In EAI of Ribbon fish MFP from groups III and IV had higher emulsion activity index as compared to groups I and II. The EAI of MFP was observed higher in four groups at concentration of 2.5 mg/ml as compared to concentration of 5 mg/ml and 10 mg/ml. At higher protein concentration the EAI decreased in all groups (Fig.5). In SPP fraction of Ribbon fish from groups IV had higher emulsion activity index as compared to groups such as I, II, and III. The EAI of SPP was observed higher in four groups at concentration of 2.5 as compared to concentration of 5 mg/ml and 10 mg/ml. At higher protein concentration the EAI decreased in all groups (Fig.6). Similarly, Ramchandran *et al*., (2006) reported the emulsion activity index of MFP fraction for barracuda (*Sphyraena jello*) in group I and II fishes SPP showed a slight increase in EAI with increase in concentration. MFP fraction showed higher EAI compared to SPP. At higher protein concentration the EAI decreased in both the groups. Similar observation on the decrease in emulsion capacity with increase in protein concentration has been reported in Rohu (Mohan *et al*., 2006) and chicken muscle proteins (Borderias *et al*., 1985). This might be due to greater degree of unfolding of proteins during the shearing involved in emulsification or saturation of the emulsion system with proteins at higher concentration (Zayas, 1997)

In MFP fraction of Ribbon fish from groups III and IV had higher ES as compared to groups I and II. ES of MFP at concentration of 10 mg/ml in all groups of fishes showed better ES as compared to 5 mg/ml and 2.5 mg/ml ($p<0.05$).In the all groups of MFP fraction showed a slight increase in ES with increase in concentration. (Fig.7). In SPP fraction of Ribbon fish from group IV had higher emulsion stability as compared to groups I, II, and III. The ES of SPP at the concentration of 10 mg/ml was observed higher in groups II, III and IV as compared to group I (Fig. 8). Borderias *et al*. (1985) reported the emulsion capacity (ES) of SPP and MFP of various fish species and found a correlation between the ES and the concentration of soluble proteins. The decreased EC with increase in protein concentration could be due to unfolding of protein during the shearing involved in the emulsification (Zayas, 1997).

In MFP fraction of Ribbon fish from groups III and IV had higher viscosity as compared to groups I and II. The viscosity of MFP at the concentration of 10 mg/ml was observed higher as compared to concentration of 5 mg/ml and 2.5 mg/ml. (Fig.9). In viscosity of Ribbon fish SPP from groups II and IV had higher viscosity

as compared to groups I and III. In viscosity of SPP fraction of Ribbon fish at the concentration of 10 mg/ml was observed higher as compared to concentration of 5 mg/ml and 2.5 mg/ml (Fig.10). Ramchandran *et al*. (2006) reported the result of viscosity for SPP of barracuda from two groups such as 500±10 g (I) and 1500 ±10 g (II) fishes did not show much difference at varying concentration. Viscosity of MFP in group I, at concentration of 5 mg/ml viscosity increased by 2 times and at 10 mg/ml the increase was more than 3 times than that of group II fishes. Montecchia *et al.* (1997) reported the result of viscosity were obtained higher in post spawned and lower
In FVS of MFP at the concentration of 10 mg/ml was observed higher as compared to concentration of 2.5 mg/ml and 5 mg/ml. It could be noted that the FVS of MFP showed a decline with increase in protein concentration (Fig.13). In FVS of Ribbon Ramachandran *et al*. (2006) recorded results of Barracuda with slight variation for foam volume stability, group I fishes showed better foam volume stability than group II fishes for SPP and MFP at all protein concentration. Foam volume stability of MFP shows a decline with increase in protein concentration. The Gel strength of Ribbon fish in four groups such as I, II, III and IV was found to be 16 g.cm, 16.33 g.cm, 18.67 g.cm and 18.33 g.cm respectively. The gel strength of Ribbon fish from groups III and IV had higher as compared to groups I and II. (Fig.15). The water holding capacity of Ribbon fish mince in four groups such as I, II, III and IV was found to be 1.413, 1.483, 1.53 and 1.517 units/g respectively. The water holding capacity of Ribbon fish mince from in pre spawned hake. In FE of MFP from groups III and IV had higher foam expansion as compared to groups I and II. The FE of MFP at the concentration of 10 mg/ml was observed higher as compared to concentration of 5 mg/ml and 2.5 mg/ml. (Fig. 11)

In SPP fraction of Ribbon fish from groups IV had higher FE as compared to groups I, II and III.FE of SPP at the concentration of 10 mg/ml was observed higher as compared to concentration of 5 mg/ml and 2.5 mg/ml. (Fig.12). Ramachandran *et al.* (2006) recorded results of Barracuda with slight variation for foam expansion (%), group I fishes showed higher foam expansion for SPP and MFP than group II fishes.

fish SPP at the concentration of 10 mg/ml and 5 mg/ml was observed higher as compared to concentration of 2.5 mg/ml. In all groups of SPP showed a slight increase in foam volume stability with increase in concentration of protein (Fig.14).

group III and IV were slightly higher compared to groups I and II (Fig.16). Ramachandran *et al.*, (2006) recorded results of Barracuda, group I fishes was found better gel strength than group II fishes. Gel strength was observed to be 590.62 and 365.73 g.cm in I and II weight groups of fishes respectively. Mehta *et al*., (2011) reported the values for gel strength of Indian major carps was observed higher in *catla catla* and *Cirrhinus mrigala* (586 and 561 g.cm) and lower in *Labeo rohita* (395g.cm). Ramachandran *et al.,* (2006) recorded results of Barracuda, the water holding capacity for group I fishes were slightly higher compared to that of group II fishes. Water holding capacity was observed to be 2.49 and 1.61 units/g in I and II weight group of fishes respectively.

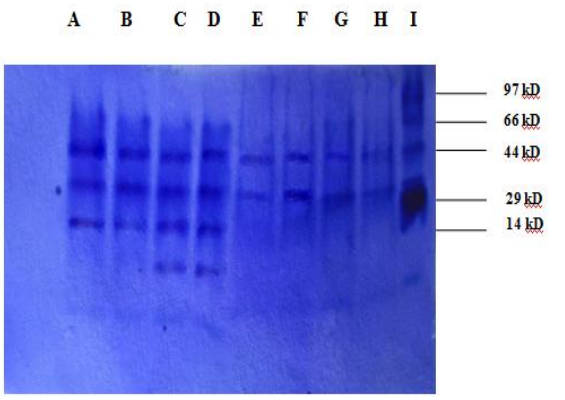

Fig. 17 SDS-PAGE of MFP and SPP of different weight groups of fish

A – SPP fraction of group I, B – SPP fraction of group II, C - SPP fraction of group III, D - SPP fraction of group IV, E – MFP fraction of group I, F - MFP fraction of group II, G – MFP fraction of group III, H - MFP fraction of group IV,I - Protein markers

When comparing the number of bands of proteins separated on PAGE; it is seen that the number of bands are more in SPP fractions owing to the number of soluble proteins appearing in that fraction. The FMP fractions showed the protein bands corresponding to the actin and myosin molecule of that fraction. In the protein bands of ribbon fish of group I, II, III and IV; the SPP showed the bands with a molecular weightrange of 66 kD to 29 kD. In group I and II peak 2 was showing higher intensity followed by peaks 1 and 3 etc (molecular weight 66 kD, 43 kD and 29 kD, respectively). Group III and IV showed 4 peaks (66 kD, 43 kD, 29 kD and 14 kD, respectively) and peaks 2 and 3 showing higher intensity followed by 1, etc. When comparing band pattern of MFP from all the groups showed two bands but two unique bands at relative front (rf) of 0.52 and 0.62 were observed in groups III and IV. The band intensities of myosin and actin separated on SDS-PAGE of MFP from various weight groups of fish. Band intensity of myosin and actin were found to be the highest in the case of group III and IV.

Acknowledgement

The authors are thankful to Associate Dean, College of Fisheries, Ratnagiri for providing necessary facilities during the research work.

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