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Physico-chemical and Functional Properties of Myofibrillar Proteins of Fishes from Different Habitats

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The conformational and functional characteristics of myofibrillar proteins of fishes from different habitats were investigated. Surface hydrophobicity and Ca^{2+}ATPase activity of the MFP were higher in *Mugil cephalus* compared with the other fishes studied. Reactive sulphydryl groups were higher in *Hypophthalmichthys molitrix*. Lowest surface hydrophobicity (6.60) was recorded in MFP extracted from *Oreochromis mossambicus*. Solubility of MFP was higher for *Lutjanus argentimaculatus*. Solubility correlated well with the concentration of reactive sulphydryl groups and the surface hydrophobicity of the proteins. Viscosity was higher in *H. molitrix* and goes well with the reactive sulphydryl groups. Foam expansion and emulsion activity index was high in *H. molitrix* but foam volume stability and emulsion stability were lower in the MFP of this fish compared with the other fishes. Foam stability, emulsion stability and gel strength were higher for *Lethrinus lentjan*. Protein conformation was found to have profound effect on the functional properties.

Keywords : **Surface hydrophobicity, Emulsion activity, Foam expansion, Reactive sulphydryl groups, Solubility.**

Fish proteins are unique in nature and exhibit high degree of functional properties. The functional properties of meat depend mainly on myofibrillar proteins (Goll *et al.*, 1997) and are related to the composition and structure of proteins and their interaction with other substances present in the food (Colmenero & Borderias, 1993). The functional properties of myofibrillar proteins are important in determining the quality of the product (Roura & Crupkin, 1995). Functional properties of frozen and ice stored fish muscle proteins are a widely explored area (Mohan *et al.*, 2006, Parthiban *et al.*, 2005, Benjakul *et al.*, 2003, Sarma *et al.*, 1999). Storage in iced and frozen conditions, drying etc brings about changes in the muscle proteins. The native characteristics of proteins in fish muscles can be better understood when investigated with out much delay after the catch. Knowledge of the native conformation can help to predict the changes in muscle during processing and preservation. This study envisages to explore the conformation and functional properties of fresh fish from different habitats in order to understand the effect of species and habitat on functional properties of proteins.

Materials and methods

The marine fishes Pig faced bream (*Lethrinus lentjan* (Lacepede, 1802)) and Yellow snapper (*Lutjanus argentinaculatus* (Forskal, 1775)) and the brackish water fishes Tilapia ((*Oreochromis mossambicus* (Peters, 1852)) and Mullet (*Mugil cephalus* Linnaeus, 1758) were procured form landing centers. Fresh water fishes Silver carp (*Hypophthalmichthys molirtix* (Valenciennes, 1844)) and Common carp (*Cyprinus carpio* Linnaeus, 1758) were collected from a fresh water farm. Marine fishes had an average length and weight of 50cm and 1800g, brackish water fishes 36 cm and 500 g, and fresh water fishes 35cm and 470g respectively. The samples were transported to the laboratory in ice. Immediately on reaching the lab, the fishes were thoroughly washed with cold water to remove blood, slime, dirt etc.

Three fishes from each species, in early stages of post rigor condition were eviscerated, de-skinned and filleted. The fillets were minced and boneless meat was used for experiments. Temperature was maintained at 2 - 4°C throughout the experiment. Moisture, total protein, crude fat and ash content of the fishes were analysed according to the methods of AOAC (1990). The sarcoplasmic proteins (SPP) was extracted by using the procedure of Sankar and Ramachandran (2001) and myofibrillar proteins by the method of King and Poulter, (1985). Concentrations of proteins in extracted solutions were estimated by Biuret reaction (Gornall *et al*, 1949) using alkaline copper sulphate reagent and measuring the colour developed at 540nm using a spectrophotometer (Spectronic 20 Genesis, Rochester, NY, USA). Bovine serum albumin was used as standard. Ca^{2+} ATPase activity of myofibrillar proteins was assayed according to the method of Jiang *et al.*, (1987). Inorganic phosphate liberated was estimated using the method of Fiske & Subbarow (1925).

The surface hydrophobicity of the extracted myofibrillar proteins was determined (Kato & Nakai, 1980) using cis-parinaric acid (CPA) as fluorescence probe in a spectroflurophotometer (Shimadzu Model RF-540, Kyoto, Japan). Excitation and emission spectra were measured at 325 and 420 nm respectively. The initial slope (So) of fluorescence intensity against protein concentration was taken as the index of protein hydrophobicity.

Reactive sulphydryl groups of the myofibrillar fractions were estimated according to Sedlak & Lindsay (1968) using 5, 5'dithiobis (2 nitrobenzoic acid) [DTNB, Sigma]. Cysteine hydrochloride was used as standard. The colour developed was measured at 420 nm using spectrophotometer (Spectronic 20 Genesis, Rochester, NY, USA).

Viscosity of salt soluble and water-soluble proteins at 5.0mg/ml concentration was determined according to the method of Mohan *et al* (2006) with a viscometer (Model DV III Ultra, Brookfield, USA) equipped with spindle number 41 at 100 rpm.

The ability of the MFP to form emulsions was estimated as emulsion activity index (EAI) according to the methods of Pearce & Kinsella (1978) as per modification of Cameron *et al.*, (1991). To the protein solution at a concentration 5 mg/ml, oil (sunflower oil) was added in the ratio 3:1, and homogenized at 13000 rpm using an emulsion probe attached to a homogeniser (Polytron Model PT 3000, Kinematica, Switzerland). The turbidity of the diluted emulsion was measured at 500 nm and emulsion activity index (EAI) and emulsion stability (ES) were calculated using the formulae,

$$EAI = 2T/\phi C$$

where T = turbidity=2.3A/l (A is absorbance at 500nm and l is light path in meters) ϕ is oil phase volume and C is concentration of protein before the emulsion is formed. The stability of the emulsion (ES) was calculated on the basis of the time taken for attaining half the initial optical density at 500 nm.

MFP at a concentration of 5 mg/ml was mixed in a mixer grinder for one minute at 8000 rpm. Foamability and foam expansion were determined according to method described by Mohan *et al.*, (2006).

The myofibrillar protein concentrate was prepared by washing fish mince in water for 5 minutes (1:3 w/v) once and strained through a nylon cloth. Heat induced gels were prepared from myofibrillar protein concentrate by grinding with 3% sodium chloride for two minutes using a pre-cooled kitchen mixer (Lan *et al*, 1995). During grinding, the temperature of the gel was kept below 5°C. The paste obtained was stuffed using a laboratory model hand stuffer into polypropylene tubing of 5.0 cm diameter, taking care to eliminate the trapped air. The ends of the tubes were tied and the meat was

cooked by immersing in a water bath at 90ºC for 30 minutes. The gels were cooled in ice and kept at 5ºC overnight.

The strength of heat-induced gel was analysed with the help of a Universal Testing Machine (Shimadzu AG-1 10 kN, Japan) equipped with a 10 mm spherical probe using 5 kg load cell. Specimens of 2.5 cm length x 5 cm diameter were used for measurement. The gel strength was calculated from load and deformation trigger. The highest point of peak force was the load at rupture, in gram, multiplied by deformation trigger, i.e., the distance traveled by the probe.

Statistical analysis of the data was performed using Windows based SPSS (10.0) programme.

Results & Discussion

Table 1 gives the proximate composition of the fishes used for the studies. The total protein varied between 16 and 23%. *O. mossambicus* had the lowest (16.6%) and *L. lentjan* the highest protein content (22.2%). Two fishes had fat content below one percent and one with fat content 8.4%.

The solubility of SPP among the different fishes ranged from 32.65 to 72.61 mg/g (Table 2) with the highest in the *L. argentimaculatus*. In the case of MFP the highest solubility was observed for *L. argentimaculatus* (189mg/g) and lowest for *H. molitrix* (81mg/g). Solubility characteristics of proteins are affected by its amino acid composition on the protein surface and the thermodynamics of its interaction with solvent (Damodaran, 1997). Solubility of MFP shows highly significant correlation (Table 4) with the conformation of the protein (Fig 1) particularly an inverse relation with reactive SH groups. Many earlier works have reported similar relation of surface hydrophobic groups with solubility (Mohan *et al*, 2006; Sankar & Ramachandran, 2005; Sano *et al*, 1994).

Ca^{2+} ATPase activity of the MFP showed values in the range of 0.3 to 0.6 μmoles Pi/ mg protein /minute (Table 2). *M. cephalus* had the highest Ca^{2+} ATPase activity of 0.59 μmoles Pi /mg protein /minute. MFP from brackish water fishes showed a higher ATPase activity compared to fishes from other habitats in their native conformation (Sankar, 2008). Fishes inhabiting colder waters have higher ATPase activity (Johnston, et. al., 1973). This indicates that the integrity of myosin head could be related to the temperature of water inhabited by fishes. The fresh water fish *C. carpio* had the lowest activity among the fishes. Ca^{2+} ATPase is localized in the myosin head region and plays a significant role in the muscle contraction. Any alteration in the structural integrity of myosin affects the activity of enzyme and is widely used as the measure of myosin integrity (Roura *et al.*, 1990, Paredi *et al.*, 1996, Benjakul *et al.*, 1997, Nagai *et al.*, 1999).

The concentration of reactive sulphydryl groups ranged from 23.5μ moles SH/g

Table 1: The proximate composition (%) of fishes from different habitats

Fishes	Moisture	Total protein	Fat	Ash
L. lentjan	74.7 ± 3.41	22.2 ± 0.20	0.3 ± 0.00	1.2 ± 0.02
L. argentimaculatus	75.5 ± 0.25	20.5 ± 4.71	1.5 ± 0.00	1.2 ± 0.02
O. mossambicus	79.9 ± 0.40	16.6 ± 0.21	0.4 ± 0.00	1.2 ± 0.01
M. cephalus	76.7 ± 0.33	20.1 ± 0.15	1.7 ± 0.00	1.5 ± 0.03
H. molitrix	78.4 ± 0.15	20.3 ± 0.17	1.6 ± 0.00	1.4 ± 0.01
C. carpio	72.5 ± 0.40	19.5 ± 0.10	8.4 ± 0.02	1.2 ± 0.02

All values are expressed as mean ± SD; n = 3

Table 2: Solubility and Ca^{2+} ATPase activity of proteins of fishes from different habitat.

Fishes	Sarcoplasmic protein solubility (mg/g tissue)	Myofibrillar protein solubility (mg/g tissue)	Ca^{2+} ATPase (mmoles Pi/mg protein/min)
L. lentjan	48.19 ± 11.2^{ab}	106.51 ± 4.4^{b}	0.34 ± 0.012^{b}
L. argentimaculatus	72.61 ± 14.7^{bc}	189.36 ± 3.4^{e}	0.33 ± 0.031^{ab}
O. mossambicus	32.65 ± 6.8^{a}	152.34 ± 4.7^{d}	0.41 ± 0.005^{c}
M. cephalus	53.29 ± 0.7^{bc}	116.85 ± 0.9^{c}	0.59 ± 0.018^{d}
H. molitrix	46.65 ± 11.3^{a}	80.91 ± 4.2^{a}	0.41 ± 0.016^{c}
C. carpio	66.67 ± 12.8^{bc}	152.54 ± 2.9^{d}	0.28 ± 0.051^{a}

All values are expressed as mean ± SD; *n* = 3, p d" 0.05, values in the same column bearing unlike letters differ significantly.

protein to 44.7μ moles SH/g protein among fishes and the highest values were recorded in MFP from silver carp and the lowest in common carp (Fig. 2). No relationship could be established between the habitat of the fish and it's SH content of MFP. It could be seen from Table 4 that species exhibiting higher reactive SH content had lower solubility for their MFP (P>0.01). *H. molitrix* which had the highest reactive SH content (Fig 1) showed the lowest MFP solubility. The SH groups represent the reactivity of the proteins and the content of surface reactive SH groups increased with the unfolding of protein during exposure to extreme conditions (Sankar & Ramachandran, 2005). Reactive SH groups showed a negative correlation (P>0.01) with MFP solubility and ATPase activity and a positive correlation with surface hydrophobicity, viscosity, emulsion activity and stability (P>0.01) and foam expansion and stability (P>0.05) (Table 4). The hydrophobic residues on the protein surface exposed to the solvent would greatly enhance the hydrophobic character or the surface hydrophobicity of the protein and would significantly alter its solubility characteristics. Surface hydrophobicity was higher for MFP from *M. cephalus* (39.80) and lowest in *O. mossambicus* (6.60) among the fishes studied, showing the uniqueness of the protein from these species.

Viscosity was lowest in the MFP of *C. carpio* (3.29cP) and highest in *H. molitrix* (13.39cP) (Fig 2). Viscosity of the MFP of the fishes is seen to increase with increase in reactive sulphydryl content. When proteins unfold, the otherwise buried SH groups are exposed to the surface. The unfolding of proteins brings about changes in the shape and size of the protein, which in turn affects the viscosity. Thus higher content of reactive sulphydryl groups indicates unfolded pro-

Table 3: Emulsion activity index, emulsion stability and gel strength of MFP of fishes from different habitat.

Fishes	Emulsion Activity Index (m^2/g)	Emulsion stability (Minutes)	Gel strength (g.cm)
L. lentjan	3.459 ± 0.17^{a}	128.33 ± 2.88^{c}	504.01 ± 24.3^{d}
L. argentimaculatus	2.193 ± 0.09^{a}	81.66 ± 7.63^{b}	115.35 ± 24.1^{a}
O. mossambicus	2.863 ± 0.09^{a}	53.33 ± 7.6^{a}	281.09 ± 9.6^{c}
M. cephalus	5.221 ± 0.68^{b}	86.67 ± 17.5^{b}	282.72 ± 44.2^{c}
H. molitrix	7.247 ± 1.19^{c}	53.33 ± 7.63^{d}	180.19 ± 23.0^{b}
C. carpio	3.105 ± 0.53^{a}	72.60 ± 17.5^{ab}	166.16 ± 14.3^{b}

All values are expressed as mean ± SD; *n* = 3, p d" 0.05, values in the same column bearing unlike letters differ significantly.

Table 4. Correlation analysis between the various parameters undertaken in the study.

Pearson Correlation	Solubility	S. hydro	Sulphyd	Ca ATP	Viscosit	EAI	ES	FE	FVS	Gel stre
Solubility	1.000	-0.382	-0.660**	0.242	-0.600**	-0.818**	-0.746**	-0.518*	-0.352	-0.438
S.hydrophobicity	-0.382	1.000	0.632**	-0.463	0.350	0.221	0.087	-0.238	0.781**	0.524*
Sulphydryls	-0.660**	0.632**	1.000	-0.757**	0.785**	0.652**	0.615**	0.526*	0.570*	0.272
Ca ATP	0.242	-0.463	-0.757**	1.000	-0.511*	-0.212	-0.262	-0.508*	-0.543*	-0.303
Viscosity	-0.600**	0.350	0.785**	-0.511*	1.000	0.738**	0.895**	0.667**	0.201	-0.127
EIA	-0.818**	0.221	0.652**	-0.212	0.738**	1.000	0.789**	0.569*	0.011	-0.018
ES	-0.746**	0.087	0.615**	-0.262	0.895**	0.789**	1.000	0.773**	0.075	-0.062
FE	-0.518*	-0.238	0.526*	-0.508*	0.667**	0.569*	0.773**	1.000	-0.045	-0.069
FVS	-0.352	0.781**	0.570*	-0.543*	0.201	0.011	0.075	-0.045	1.000	0.752**
Gel strength	-0.438	0.524*	0.272	-0.303	-0.127	-0.018	-0.062	-0.069	0.752**	1.000

**. Correlation is significant at the 0.01 level
*. Correlation is significant at the 0.05 level

tein and thus gives more surface area to the protein to which water molecules can bind and swell. Partial denaturation causes an increase in the hydrodynamic size of the proteins and increases the viscosity (Damodaran, 1997). Mohan *et al* (2006) reported that MFP from rohu showed a viscosity of around 16cP, which is comparable to the viscosity of *H. molitrix* in this study. Intrinsic properties such as molecular size, volume, shape, surface charge and ease of deformation and extrinsic attributes of the protein like pH, temperature, ionic strength, ion type and shear rate can affect viscosity (Xiong, 1997).

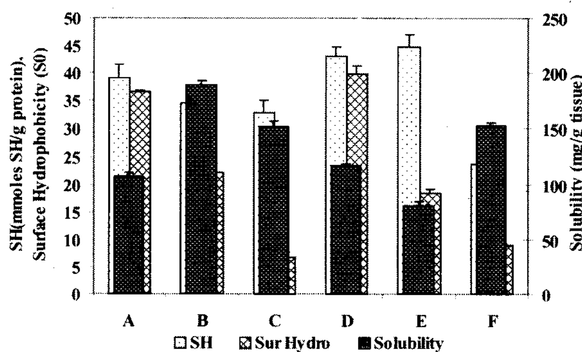

Fig. 1. Relation between Myofibrillar protein solubility, reactive SH groups and surface hydrophobicity of protein from different fishes. Legend A - L. lentjan, B - L. argentimaculatus, C - O. mossambicus, D - M. cephalus, E - H. molitrix, F - C. carpio. Values are mean of triplicate analysis ± S.D.

MFP from *H. molitrix* had better emulsion activity index compared to MFP from other fishes (Table 3). MFP from all other fishes except *M. cephalus* showed similar EAI. Higher EAI in silver carp goes well with the high viscosity and sulphydryl content in this fish. The highly unfolded proteins have the molecular flexibility to be more active in the interface and orient themselves in such a way as to reduce the free energy.

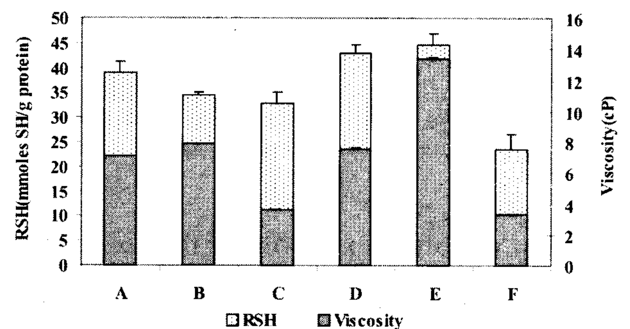

Fig. 2. Relation between viscosity and reactive SH of different fishes. (Legends as in Fig 1)

MFP from *L. lentjan* showed better emulsion stability (Table 3). All the other fishes had emulsion stability in the range of 50-90%. The better emulsion stability of MFP of *L. lentjan* could be due to balance in hydrophobic and hydrophilic groups of the surface amino acids. *H. molitrix which* had higher EAI showed poor ES compared to the

other fishes. Emulsion systems are stabilized through physical entrapment of fat globules with in the protein matrix formed largely via protein-protein interactions followed by the formation of an interfacial protein film that surrounds and stabilizes fat globules (Mohan *et.al*, 2006; Barbut, 1995)

H. molitrix had better foam expansion compared to the other fishes (Fig 3). Unfolding of the protein structure gives the flexibility that the protein needs to orient itself at the interface. Flexible disordered proteins are more surface active (Xiong 1997). The Foam expansion was higher for *H. molitrix* and for all the other fishes the values were below 100%. Higher foam expansion associated with protein from this fish could be related to the higher SH groups and viscosity, which play a prominent role in the surface properties of the protein.

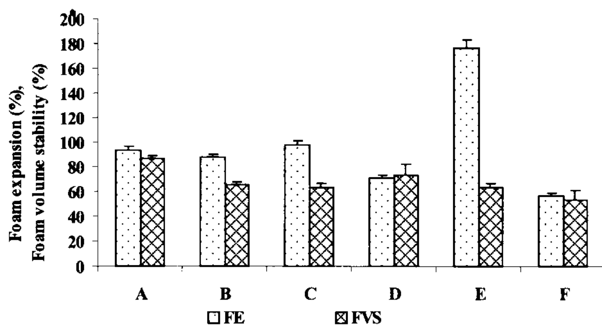

Fig. 3. The foam expansion and foam volume stability of different fishes. (Legends as in Fig 1)

Foam volume stability of the MFP of the fishes was in the range 50 – 90% (Fig 3) and *L. lentjan* had the highest value. The surface hydrophobicity for *L. lentjan* was 36.53 (So). Surface hydrophobicity is a major characteristic, which give proteins better functionality especially in stability of foams and emulsions. A good hydrophobic- hydrophilic balance could have resulted in better foam stability in *L. lentjan*. The surface hydrophobicity and solubility of the MFP of the fishes show that in case of *M. cephalus* and *L. lentjan* a good hydrophobic- hydrophilic balance was noticed. Though *H. molitrix* had better foam expansion (Fig 3), it did not show much foam stability. This could be due to an imbalance of hydrophobic and hydrophilic groups on the surface of proteins, which results in instability of the foam.

The MFP concentrate of *L. lentjan* showed a better gelling property than the other fishes (Table 3). For other fishes the gel strength of the heat induced gels were in the order *M.cephalus* > *O. mossambicus* > *H. molitrix* > *C. carpio* > *L. argentimaculatus*. The type of gel formed by a protein is primarily influenced by its amino acid composition. Other minor form of interactions, namely hydrogen bonding, S-S interchanges, electrostatic interactions and enzyme catalyzed cross-links are also present. There is significant correlation between gel strength and surface hydrophobicity (Table 4). The hydrophobic groups that are exposed during heating are involved in salt soluble protein gel networks, and formation of gel networks obviously results from aggregation of salt soluble protein probably through hydrophobic interaction (Xiong, 1997). A balance of attractive and repulsive forces gives good gel strength. The gel strength could be correlated with the surface hydrophobicity and solubility of the MFP of fishes. *L. lentjan* and *M. cephalus* which have a good hydrophobic-hydrophilic balance shows better gelling properties compared to the other fishes. Hydrophobic interactions between adjacent tail regions (LMM and HMM S-2) during setting forms the basis for an initial structure. The aggregation of myosin head regions (HMM S-1) is accepted as primary mechanism for promoting gel strength. Involvement of sulphydryl and hydrophobic groups is considered predominant (Stone & Stanley, 1992) in gel formation.

The Ca^{2+} ATPase activity, the measure of actomyosin integrity of the myofibrillar proteins, shows comparable values among the fishes from different habitats. Conformational changes such as reactive sulphydryl groups and the surface hydrophobicity increases with decrease in myofibrillar

protein solubility. Viscosity of the MFP increases with increased unfolding of the protein, indicating that hydrodynamic shape and size of the protein has an important role in the functionality of the protein. *H. molitrix* which showed higher viscosity also demonstrated higher EAI and foam expansion. This clearly shows that unfolded flexible proteins are more active at the interface, increasing these functionalities of the protein. Surface hydrophobicity and solubility have a profound influence on the emulsion stability, foam stability and gel strength of the MFP, as these functionalities rely on the hydrophobic-hydrophilic balance of the protein, which enable them to orient themselves at the interface so as to reduce the free energy and stabilize the system. Though similarities in many of the conformational state of proteins were noted with fishes inhabiting same habitat, the functionality of a protein is primarily influenced by the conformational states of the protein.

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