Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 106 (2008) 451–457
Food Chemistry
www.elsevier.com/locate/foodchem

Physicochemical characterization of muscle proteins from different regions of mackerel (*Rastrelliger kanagurta*)

Mukund Mohan, Dhanya Ramachandran, T.V. Sankar *, R. Anandan

Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Cochin 682029, Kerala, India

Received 2 December 2006; received in revised form 3 March 2007; accepted 9 May 2007

Abstract

Physicochemical characteristics of proteins extracted from muscles excised from four distinct regions of mackerel were examined. Three regions were identified based on their fin position; namely anterior muscle, median muscle, posterior muscle and red muscles from beneath the lateral line. The biochemical characteristics like reactive sulphydryl groups, surface hydrophobicity, Ca^{2+} ATPase activity, turbidity, proximate composition and the functional characteristics such as viscosity, emulsion activity index (EAI) and emulsion stability (ES) were studied in the samples, from different regions. Sarcoplasmic protein (SPP) solubility was found to be higher in red muscles compared to that of white muscles, whereas myofibrillar protein (MFP) solubility was higher in white muscles particularly in the posterior portion. The MFP from posterior white muscles showed better viscosity, surface hydrophobicity and Ca^{2+} ATPase activity, which in turn contributed to the higher EAI and ES of this muscle portion. Variations in composition, electrophoretic pattern of proteins and selected functional properties (EAI) were noticed between red and white muscles, which may be of significance from the utilization point of the fish.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Viscosity; Surface hydrophobicity; Ca^{2+} ATPase activity; Emulsion activity index; Emulsion stability

1. Introduction

Muscle fibers in different regions of fishes have specific physiological function particularly in locomotion and steering. A number of previous studies have been carried out in this regard (Buss & Drapeau, 2000; Stanfield, 1972). Fish myotomal musculature, is made up of two main types of muscle fibers, which in many species are arranged in separate and distinct anatomical regions. The bulk of the musculature often consists of anaerobic white fibers, while the red fibers are found in a thin strip lying just beneath the lateral line. These muscle fibers vary not only in color but also in innervation, blood supply, myoglobin content, fiber size and abundance of mitochondria (Johnston, Frearson, & Goldspink, 1972). The structural and conformational change in proteins was found to be evident in all the studies. The effect of these peculiar characteristics of the proteins from different muscle regions on functional properties, is an area scarcely researched. Physiochemical characteristics of proteins from different regions are of great importance when it comes to value addition.

The anatomy of fish muscle is different from the anatomy of terrestrial mammals, as the fish lacks the tendinous system connecting muscle bundles to the skeleton of the animal (Huss, 1995). But in the case of fish muscle, cells run in parallel and are connected to sheaths of connective tissue, which are anchored to the skeleton and the skin. Fish fillet is heterogeneous, in that the lengths of the muscle vary from head (anterior) to the tail end (posterior). In the case of cod the longest muscle cells were found to be the twelfth myotome, counting from head, with an average length of 10 mm in a fish that is 60 cm long. The diameter of the cells also varies, being widest in the ventral part of the fillet (Huss, 1995).

* Corresponding author. Tel.: +91 484 2666845; fax: +91 484 26668212.
E-mail address: sankartv@sify.com (T.V. Sankar).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.05.024

Most of the fish muscle is white but depending on the species many fishes will have a certain amount of dark tissue of red or brown color. The proportion of dark to light muscle varies with the activity of the fish. In pelagic fishes i.e. in species such as herring and mackerel (which swim more or less continuously) have up to 48% of the body weight made of dark muscles (Love, 1970), while in demersal fishes (which move only periodically) the amount of dark muscle is very small. As in most animal species, ordinary muscle is anaerobic, whose function is to provide energy quickly and intensively. Ordinary muscle tires easily and primarily uses glycogen as energy source but dark muscle on the other hand is designed for long-term exercise and is used by migrating species that travel great distances using oxidative metabolism of lipids as its principal source of energy.

In this study we have explored the structure–function characteristics of fish MFP extracted from different muscle fibers of Indian mackerel (*Rastrelliger kanagurta*), a pelagic shoaling fish which forms almost 8% of India's total marine fish landings. In the year 2004, more than 200,000 ton of mackerel were caught world over (Devi, 2006).

2. Materials and methods

2.1. Materials

Fresh mackerel was procured from a local fish-landing center at Cochin, India. Fishes of average weight 160 ± 20 g and average length of 22 ± 4 cm were selected for the study. 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.

2.2. Sample preparation

Fishes were de-skinned and filleted. Three muscle regions were identified for the study based on their fin position; namely anterior muscle (from operculum to first dorsal fin), median muscle (from first dorsal fin to anal fin) and posterior muscle (from anal fin to caudal fin). Red muscles from beneath the lateral line (*M. lateralis superficialis*) constituted the fourth region (Fig. 1). The excised muscles were minced in a kitchen mixer/grinder and boneless meat was used for further experiments. Temperature was maintained at 2–4 °C throughout the experiment.

2.3. Proximate composition

Moisture, crude protein, fat and ash were determined according to the methods of AOAC (1990).

2.4. Extraction of muscle protein fractions

For the extraction of SPP, meat was homogenized with chilled buffer (0.02 M sodium bicarbonate, pH 7.25) maintaining meat to buffer ratio of 1:6 in a Polytron homogenizer (Model PT3000, Kinematica, Switzerland) at 9000 rpm for 1 min (Sankar & Ramachandran, 2001). The homogenate was centrifuged using a refrigerated centrifuge (REMI R24, India) at 10,000 rpm for 15 min maintaining a temperature of 2 °C. Supernatant was collected, and the residue was re-extracted as above and the pooled supernatant was collected as sarcoplasmic protein (SPP). Pellet obtained was extracted twice in chilled Dyer's Buffer (5% NaCl containing 0.02 M $NaHCO_3$) and the supernatant was collected as myofibrillar protein (MFP) (King & Poulter, 1985).

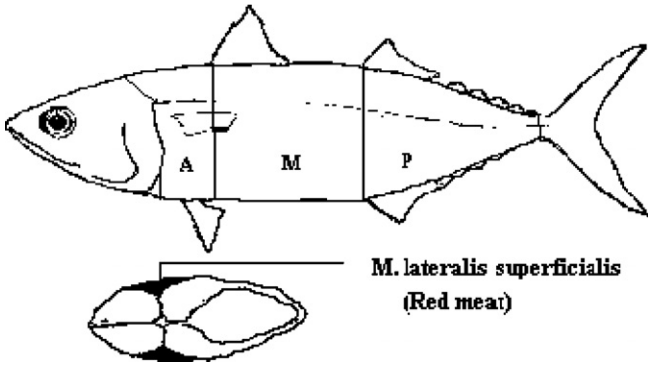

Fig. 1. Diagram showing the anterior, median, posterior and red meat regions from where the meat was excised for the study. A – anterior, M – median and P – posterior.

2.5. Protein estimation

Concentration of proteins in extracted solution was estimated by the Biuret reaction (Gornall, Bradwill, & David, 1949) using alkaline copper sulphate reagent and measuring the color developed at 540 nm using a spectrophotometer (Spectronic 20 Genesis, Rochester, NY, USA). Bovine serum albumin was used as standard.

2.6. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of SPP and MFP of both the groups were performed according to the procedure described by Laemmli (1970) in a 7.5% acrylogel 5.0 premix (BDH, England). Protein (25 μg) were loaded on to each well. Mobility of the protein bands were calibrated with standards of molecular weight markers. After staining and destaining, the gel was scanned using a gel documentation system (Bio-Rad, USA).

2.7. Surface hydrophobicity

The surface hydrophobicity of the extracted myofibrillar proteins from different regions were determined (Kato & Nakai, 1980) using 8-anilino-1-naphthalene sulphonic acid (ANS) as fluorescence probe in a Shimadzu spectrofluorophotometer (Shimadzu Model RF-540, Kyoto, Japan). Emission and excitation spectra were measured at 374 and 485 nm respectively. The initial slope (S_0) of fluores-

cence intensity against protein concentration was taken as the index of protein hydrophobicity.

2.8. Reactive sulphydryl group

Reactive sulphydryl groups were estimated according to Sedlak and Lindsay (1968) in myofibrillar fractions using 5,5′-dithiobis(2-nitrobenzoic acid) [DTNB, Sigma]. Cysteine hydrochloride was used as standard. The color developed was measured at 412 nm using spectrophotometer (Spectronic 20 Genesis, Rochester, NY, USA).

2.9. Ca^{2+} ATPase activity

Ca^{2+} ATPase activity of myofibrillar proteins was assayed according to the method of Jiang, Tsao, and Lee (1987). Inorganic phosphate liberated was estimated using the method of Fiske and Subbarow (1925).

2.10. Viscosity

Viscosity of MFP at a concentration of 5.0 mg/ml was determined with a (Model DV III Ultra, Brookfield, USA) viscometer at shear rate 200 s^{-1} as described earlier (Mohan, Ramachandran, & Sankar, 2006).

2.11. Turbidity

Turbidity of the MFP solutions at a concentration of 1 mg/ml was estimated as absorbance at 500 nm.

2.12. Emulsion

The ability of the MFP to form emulsions was estimated as emulsion activity index (EAI) according to the methods of Pearce and Kinsella (1978) as per modification of Cameron, Weber, Idziak, Neufeld, and Cooper (1991). Protein at different concentrations was homogenised with oil (sunflower oil) in the ratio 3:1, by high-speed homogenization (13,500 rpm) using an emulsion probe (PT-DA 3012/2EC) attached to a Polytron homogeniser (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 as already described (Mohan et al., 2006).

2.13. Statistics

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

3. Results and discussion

3.1. Proximate composition

Proximate composition of white and red muscle regions in mackerel varied widely (Table 1). Among the white muscle groups, the posterior region had higher moisture content. Red muscles had comparatively lower moisture content (66%) when compared with the white muscles. Nitrogen content varied widely between the red and white muscles. Dark muscle relies on oxidative metabolism of stored lipids as the source of energy and this account for the higher content of crude fat in the muscle (Hultin & Kelleher, 2000). Adipocytes are widely distributed among white muscles in the case of mackerel (Bone, 1978). An inverse relationship between moisture and fat content has been reported (Devi, 2006; Ninan, 2003) and the same was noted in this study. The red muscles were also found to have the higher ash content which indicates the higher content of minerals.

Table 1
Proximate composition (%) of muscle blocks from different regions of mackerel

	Moisture	Total nitrogen	Crude fat	Ash
Anterior (A)	70 ± 0.67	23.5 ± 0.23	6.5 ± 0.23	1.2 ± 0.10
Posterior (P)	72 ± .078	23.3 ± 0.12	3.3 ± 0.35	1.6 ± 0.16
Median (M)	70 ± 1.02	22.6 ± 0.19	6.1 ± 0.12	1.6 ± 0.09
Red (R)	66 ± 0.66	14.9 ± 0.14	13.8 ± 0.25	6.7 ± 0.19

Values are mean ± SD. $n = 3$, $p \leqslant 0.05$, values in the same row bearing unlike letters differ significantly.

3.2. Protein solubility

Higher MFP solubility of 150 mg/g was observed in the posterior muscles when compared to other regions studied (Fig. 2). Red muscles recorded the lowest MFP solubility among the muscle groups studied and among the white muscles the lowest solubility was observed in the case of median muscles. Higher SPP solubility was observed in the case of red muscle proteins and lower in the case of anterior muscles. Variation in solubility was reported by Xiong and Brekke (1991b) in the case of post-rigor fast twitch and slow twitch chicken muscles. Protein extraction

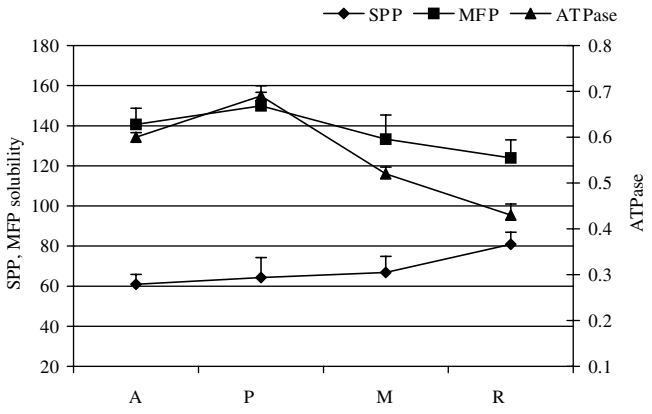

Fig. 2. Sarcoplasmic and myofibrillar protein solubility (mg/g) profile of anterior (A), posterior (P), middle (M) and red (R) muscle regions in mackerel (*R. kanagurta*). Ca^{2+} ATPase activities in MFP from different muscle regions are also plotted. All values are mean ± SD.

was also found to be affected by divalent cations present in muscle. The effect of calcium and magnesium is quite complex and differs for white and red muscle proteins. In the case of white muscles MFP solubility increased in the presence of 2.5–5 mM $CaCl_2$ or $MgCl_2$ but decreases on further increase in ionic strength. However, in red muscles solubility reached a maximum at 5 mM concentration and remained the same with a further increase in ionic strength (Xiong & Brekke, 1991a). Presence of various fast and slow isoforms of calcium and magnesium binding proteins, like myosin light chain, troponin, etc., may be contributing to the fiber type dependent solubility differences. The different rates of MFP extraction from red and white muscle fibers are related to ultra structure of Z bands and the polymorphism of α-actinin fibers and certain subsidiary structural proteins including M proteins. Different myosin isoforms are known to differ in solubility and physicochemical characteristics (Ramachandran, Mohan, & Sankar, 2006). Hence, even if thick filaments are depolymersied the different solubility of white and red myosin are another decisive factor that can influence the total amount of soluble protein in white and red muscles.

3.3. SDS-polyacrylamide gel electrophoresis of proteins

Electrophoretic band patterns of SPP and MFP from white muscles were similar, but when compared with red muscle, the changes were very much evident (Fig. 3). There was wide variation in band intensities between the white and red muscle proteins. In the electrophoretograms of red muscle MFP myosin light chains were not detected. A unique protein fraction of 6.5 kD was detected in red muscle MFP. A distinct 55 kD band in the red muscle MFP showed a higher intensity compared to the corresponding bands separated out from white muscle. Another 40 kD fraction was absent in red muscle MFP that shows higher intensities in white muscles.

Myosin and actin are the major proteins which contribute to most of the functional properties of myofibrillar proteins. The band intensities of myosin and actin separated on SDS-PAGE of MFP from various muscle blocks were compared (Fig. 4). Band intensity of myosin and actin were found to be the highest in the case of posterior white muscle blocks and the lowest in red muscle blocks. The ratio of actin and myosin holds great significance in food chemistry. Myosin, is prone to proteolysis than actin *in vivo* (Crupkin, Montecchia, & Trucco, 1988), and the hydrolysis of myosin is likely to affect the functional properties adversely. Further, modification in the myosin/actin ratio in natural actomyosin, resulted in substantial change in the rigidity of the gel (Samejima, Ishioroshi, & Yasui, 1982; Yasui, Ishioroshi, & Samejima, 1980). There are reports indicating the significance of myosin/actin ratio in influencing the viscosity (Roura, Montecchia, Goldemberg, Trucco, & Crupkin, 1990), Ca^{2+} ATPase activity (Ramachandran et al., 2006; Roura & Crupkin, 1995) and Mg^{2+} ATPase (Beas, Crupkin, & Trucco, 1988; Roura & Crupkin, 1995).

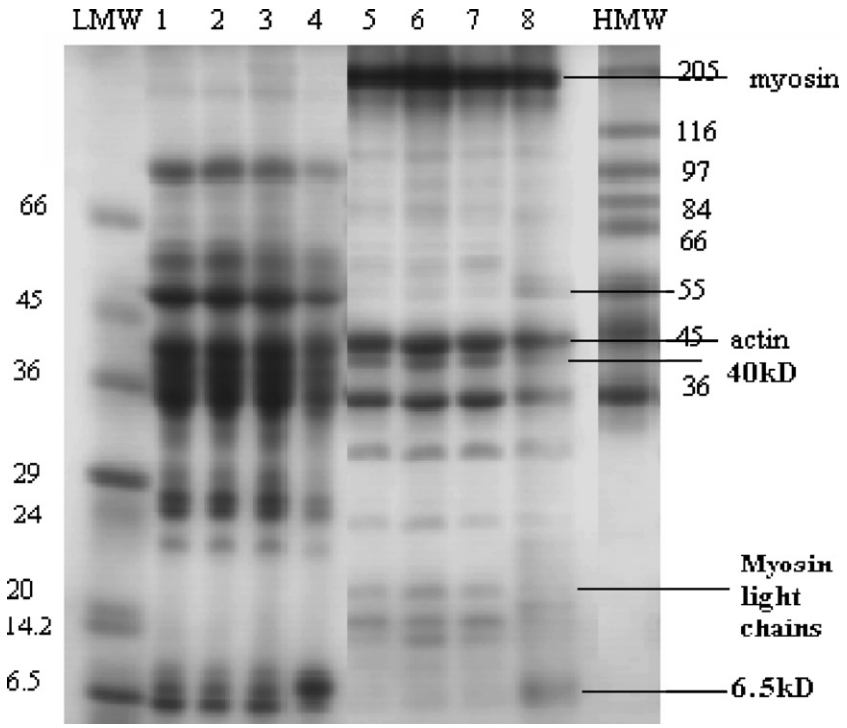

Fig. 3. SDS-PAGE of sarcoplasmic and myofibrillar proteins from anterior (A), posterior (P), middle (M) and red (R) muscle proteins of mackerel (*R. kanagurta*). HMW and LMW are high and low molecular weight markers. Lane 1–4, SPP of A, P, M and R respectively and lane 5–8, MFP of A, P, M and R respectively.

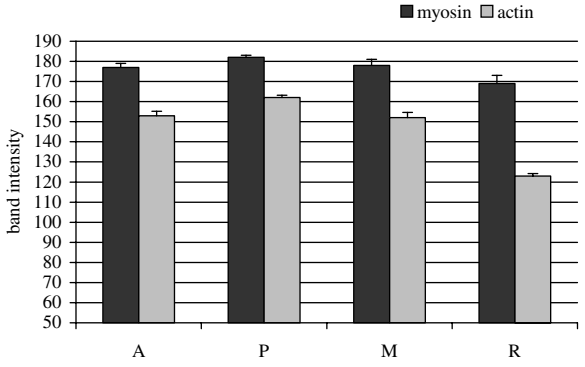

Fig. 4. Band intensities of myosin and actin separated on SDS-PAGE of MFP from different muscle blocks, anterior (A), posterior (P), middle (M) and red (R) muscle proteins. All values are mean ± SD.

3.4. Surface hydrophobicity

Most proteins have their own characteristic surface hydrophobicity which plays a role in influencing the functional properties of the protein. In the posterior and median muscle proteins, the aromatic fluorescent probe 8-anilino-1-naphthalene sulphonic acid (ANS) imparted hydrophobicity (S_0) was found to be 29.6 and 29.2 respectively (Fig. 5). In the anterior muscle proteins, surface hydrophobicity was found to be less when compared to other regions. Even though higher hydrophobicity was recorded in the case of both posterior and median muscle proteins, its effect on protein solubility was found only in the case of latter. Surface hydrophobicity contributes to its functional properties and an ideal balance of polar and non-polar groups in a protein helps it to function as a better emulsifying agent. The structure of myofibrils is a factor that contributes to the solubility of muscle proteins; in this study any peculiarity in the structure of myofibrils in the posterior muscles of mackerel could have contributed to this higher solubility (Xiong, 1997) and higher surface hydrophobicity.

3.5. Reactive sulphydryls

Among the white muscles, higher concentration of reactive sulphydryl groups were observed in proteins extracted from anterior and posterior regions (Fig. 5). Red muscle proteins were found to have the lowest concentration of reactive sulphydryl groups i.e. 24 μmol sulphydryl per gram protein. Reactive sulphydryl concentration did not show any correlation with surface hydrophobicity. This clearly proves that protein extracted from each muscle group included in the study have their own characteristic structural properties. The variation in the concentration of sulphydryl groups, surface hydrophobicity, etc. is not due to protein unfolding but it is the characteristic of the native protein studied. Accessibility for the relatively large reagent (DTNB), restricts the method to detect only the sulphydryl groups that are located on the surface or in accessible cavities (Visschers & Harmen de Jongha, 2005).

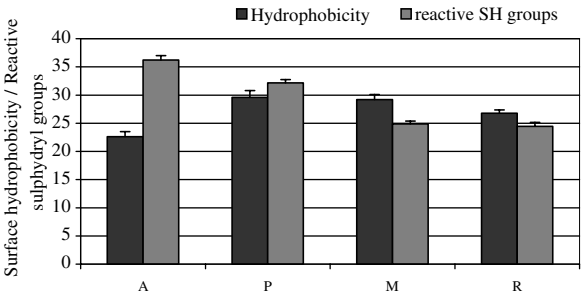

Fig. 5. Surface hydrophobicity (S_0) and reactive sulphydryl group concentration among myofibrillar proteins from different muscle regions. All values are mean ± SD.

3.6. Ca^{2+} ATPase activity

The fish myosin similar to many mammalian myosins, possesses ATPase activity, regulated by the presence of calcium and magnesium ions (Venugopal, 2006). The posterior muscle MFP were found to have Ca^{2+} ATPase activity of 0.69 μmol Pi/mg protein/min which was the highest and red muscles recorded an activity of 0.43 μmol Pi/mg protein/min, which was the lowest (Fig. 2) in this study. Lower ATPase activities in the case of red muscle proteins, were reported by earlier investigators in the case of Coal fish (*Gadus virens*), North sea cod (*Gadus morhua*) and Plaice (*Pleuronectes platessa*). Ca^{2+} ATPase activity correlated well with solubility profile of the various muscle groups. The lower Ca^{2+} ATPase activity in some cases can be attributed to the apparent instability of fish myosin (Johnston et al., 1972). The high Ca^{2+} ATPase activity of posterior muscle MFP also indicate higher muscular activity associated with it.

3.7. Viscosity

Viscosity, is a functional property which is greatly exploited when proteins are added to liquid foods as thickeners, and it affects several other functional properties. Myosin present in muscle proteins, is the major contributor to the viscosity of aqueous muscle extracts. The unique structure of myosin with its large length to diameter ratio of the rod portions makes myosin highly viscous in salt solutions. When the viscosities of MFP from white muscle regions were compared, posterior muscle blocks were found to have a higher value (Fig. 6). The MFP from anterior and middle regions recorded almost the same viscosity. Viscosity of red muscle MFP was very low when compared with that of MFP from white muscle regions studied. Viscosity showed a direct correlation to solubility and myosin content.

Salt soluble proteins extracted from porcine *longissimus dorsi* muscle, which constitutes 75% white muscle fibers, generally has a greater viscosity than that extracted from *serratum ventralis* (43% red fiber) (Robe & Xiong, 1993). These results further substantiate that certain intrinsic

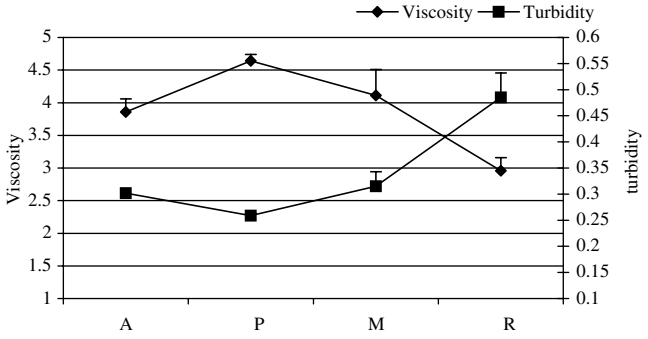

Fig. 6. Viscosity (cP) and turbidity (Abs) of myofibrillar proteins from different regions of mackerel. All values are mean ± SD.

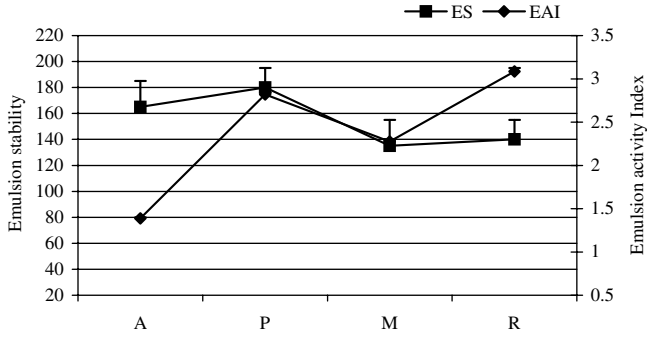

Fig. 7. Emulsion stability (minutes) and emulsion activity index (m^2/g) compared among myofibrillar proteins extracted from different muscle fibers from mackerel. All values are mean ± SD.

factors exist in white and red muscle fibers, which produce rheological variations in MFP. Myosin polymorphism and the ability of white muscle myosin to form longer filaments than red myosin may explain at least in part, the viscosity differences between white and red myofibrillar proteins. Exact flow behavior of muscle proteins is quite complex and depends on both intrinsic (molecular size, surface charge, shape, and ease of deformation) and extrinsic factors (pH, temperature, ionic strength, ion type and shear rate). In this study the extrinsic factors were kept constant, and therefore clearly demonstrate that the change in viscosity of MFP extracted from different muscle groups are due to the intrinsic factors.

3.8. Turbidity

Turbidity indicates the degree of aggregation of protein (Acton, Hanna, & Satterelee, 1981; Sankar & Ramachandran, 2005). In this study turbidity was observed to be the highest in case of red muscle MFP (Fig. 6). Turbidity shows a negative correlation with viscosity, solubility and Ca^{2+} ATPase activity. This clearly shows the variation in stability of MFP extracted from distinct muscle blocks, especially myosin.

3.9. Emulsion

The ability of proteins to bind fat in comminuted meat is of great importance. Proteins being amphoteric molecules are surface-active agents and thus concentrate on fat-water interface. This ability varies from protein to protein; as it is affected by the amino acid profile and the conformational characteristics of the protein. In this study, MFP from red muscles recorded the highest EAI (Fig. 7). The lowest was observed in anterior MFP (1.3 m^2/g). Among the white muscle proteins studied MFP from posterior region was found to have the highest EAI (2.87 m^2/g). The MFP from posterior muscles also showed high emulsion stability (ES) in the range of 180 min, which is the highest among the groups studied (Fig. 7). This higher EAI and ES in posterior muscle proteins points out the scope for utilizing particular muscle groups for value addition.

EAI is reported to have a clear correlation with surface hydrophobicity, solubility and viscosity of the proteins. Increased concentration of myosin in the extracted MFP from posterior white muscles, could have contributed to its enhanced EAI. Higher surface hydrophobicity and viscosity was observed in the case of MFP extracted from posterior and middle white muscle fibers and this substantiates the higher EAI in these group of proteins. The myosin:actin ratio was not found to have an influence on EAI and ES. Surface hydrophobicity, solubility, viscosity and reactive sulphydryl groups were considerably low in the case of red muscle MFP. Besides higher fat content, the particular myosin isoforms present in red muscle could have contributed to their enhanced EAI. The EAI of red muscle MFP being higher still, shows the lowest emulsion stability. This could be correlated with turbidity of the protein solution. The proteins being in a highly unfolded state enhanced the EAI. But the tendency of the proteins to aggregate resulted in decreased emulsion stability.

4. Conclusion

From the results obtained, it can be concluded that muscle proteins extracted from different regions of mackerel varies in its structural and physicochemical characteristics. The protein from different regions, particularly from the posterior region, could be used as material for value addition as they differ uniquely in characteristics and functionality. Myosin polymorphisms and/or the quantity and quality of myosin in the extracted proteins could be the reason behind the variation in the functional behavior of MFP extracted from different fish muscle blocks. The possibility of utilizing specific muscle blocks of fish for particular value addition process may be of significance from a commercial point of view.

Acknowledgements

The authors express their sincere thanks to Dr. K. Devadasan, Director, Central Institute of Fisheries Technology,

Cochin, India for granting permission to publish this paper and Dr. P.G. Viswanathan Nair, Head, Biochemistry and Nutrition Division for his keen interest shown in this study. The financial assistance from Indian Council of Agricultural Research, New Delhi in carrying out this study is gratefully acknowledged.

References

Acton, J. C., Hanna, M. A., & Satterelee, L. D. (1981). Heat induced gelation and protein–protein interaction of actomyosin. *Journal of Food Biochemistry, 5*, 101–103.

AOAC (1990). *Official methods of analysis of the association of the official analytical chemistry* (15th ed.). Arlington, Virginia: Association of Official Analytical Chemists.

Beas, V. E., Crupkin, M., & Trucco, R. E. (1988). Gelling properties of actomyosin from pre and post spawning hake (*Merluccius hubbsi*). *Journal of Food Science, 53*, 1323.

Bone, Q. (1978). Myotomal muscle fibre types in *Scomber* and *Katsuwonus*. In G. D. Sharp & A. E. Dizon (Eds.), *The Physiological ecology of tunas* (pp. 183–205). New York: Academic Press.

Buss, R. R., & Drapeau, P. (2000). Physiological properties of zebrafish embryonic red and white muscle fibers during early development. *Journal of Neurophysiology, 84*, 1545–1557.

Cameron, D. R., Weber, M. E., Idziak, E. S., Neufeld, R. J., & Cooper, D. G. (1991). Determination of interfacial areas in emulsions using turbidimetric and droplet size data: correction of the formula for emulsifying activity index. *Journal of Agricultural and Food Chemistry, 39*, 655–659.

Crupkin, M., Montecchia, C. L., & Trucco, R. E. (1988). Seasonal variation in gonadosomatic index and actin/myosin ratio in actomyosin of mature hake (*Merluccius hubbsi*). *Comparative Biochemistry and Physiology A, 89*, 7.

Devi, V. R. (2006). *Influence of ionic strength on conformation and functional properties of fish muscle proteins*. M.FSc. Dissertation, Central Institute of Fisheries Education, India.

Fiske, C. H., & Subbarow, Y. (1925). The calorimetric determination of phosphorus. *Journal of Biological Chemistry, 66*(2), 375–400.

Gornall, A. C., Bradwill, C. J., & David, M. M. (1949). Determination of serum proteins by means of biuret reaction. *Journal of Biological Chemistry, 177*, 755–766.

Hultin, H. O., & Kelleher, S. D. (2000). Surimi processing from dark muscle fish. In J. W. Park (Ed.), *Surimi and surimi seafood* (pp. 59–77). New York: Marcel Dekker.

Huss, H. H. (1995). Postmortem changes in fish. In *Quality and quality changes in fresh fish*. FAO Fisheries Technical Paper-348, Food and Agriculture Organisation of United Nations, Rome.

Jiang, S. T., Tsao, C. Y., & Lee, T. C. (1987). Effect of free amino acids on the denaturation of mackerel myofibrillar proteins *in vitro* during frozen storage at −20 °C. *Journal of Agricultural and Food Chemistry, 35*, 28–33.

Johnston, I. A., Frearson, N., & Goldspink, S. (1972). Myofibrillar ATPase activities of red and white myotomal muscles of marine fish. *Seperatum Experientia, 28*, 713.

Kato, A., & Nakai, S. (1980). Hydrophobicity determined by a fluorescent probe method and its correlation with surface properties of proteins. *Biochimica et Biophysica Acta, 624*, 13.

King, D. R., & Poulter, R. G. (1985). Frozen storage of Indian mackerel (*Rastrelliger kanagurta*) and Big eye (*Priacanthus hamrur*). *Tropical Science, 25*, 79–90.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

Love, M. (1970). *The chemical biology of fishes*. London: Academic Press, pp. 395.

Mohan, M., Ramachandran, D., & Sankar, T. V. (2006). Functional properties of Rohu (*Labeo rohita*) proteins during iced storage. *Food Research International, 39*, 847–854.

Ninan, G. (2003). Chemical composition of fish and shellfish. In J. Joseph, P. T. Mathew, A. C. Joseph, & V. Muraleedharan (Eds.), *Product development and seafood safety* (pp. 15–32). India: Central Institute of Fishery Technology.

Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of protein evaluation by a turbidimetric technique. *Journal of Agricultural and Food Chemistry, 26*, 716–723.

Ramachandran, D., Mohan, M., & Sankar, T. V. (2006). Physicochemical characteristics of muscle proteins from barracuda (*Sphyraena jello*) of different weight groups. *LWT Food Science and Technology*. doi:10.1016/j.lwt.2006.09.010.

Robe, G. H., & Xiong, Y. L. (1993). Dynamic rheological studies of salt soluble proteins from three porcine muscles. *Food Hydrocolloids, 7*(2), 137–146.

Roura, S. I., & Crupkin, M. (1995). Biochemical and functional properties of myofibrils from pre and post-spawned hake (*Merluccius hubbsi Marini*) stored on ice. *Journal of Food Science, 60*, 269.

Roura, S. I., Montecchia, C. L., Goldemberg, A. L., Trucco, R. E., & Crupkin, M. (1990). Biochemical and physicochemical properties of actomyosin from pre and post –spawned hake (*Merluccius hubbsi*) stored on ice. *Journal of Food Science, 55*, 688.

Samejima, K., Ishioroshi, M., & Yasui, T. (1982). Heat induced gelling properties of actomyosin: Effect of tropomyosin and troponin. *Agricultural and Biological Chemistry, 46*, 535.

Sankar, T. V., & Ramachandran, A. (2001). Changes in biochemical composition in Indian major carps in relation to size. *Fish Technology, 38*(1), 22–27.

Sankar, T. V., & Ramachandran, A. (2005). Thermal stability of myofibrillar protein fom Indian major carps. *Journal of the Science of Food and Agriculture, 85*, 563–568.

Sedlak, J., & Lindsay, R. H. (1968). Estimation of total, protein bound, and non-protein sulphydryl groups in tissues with Elman's method. *Analytical Biochemistry, 25*, 192–205.

Stanfield, P. R. (1972). Electrical properties of white and red muscle fibers of the elasmobranch fish *Scyliorhinus canicula*. *Journal of Physiology (London), 222*, 161–186.

Venugopal, V. (2006). *Seafood processing: adding value through quick-freezing, retortable packaging and quick chilling*. New York: Taylor & Francis group (pp. 378–445).

Visschers, R. W., & Harmen de Jongha, H. J. (2005). Disulphide bond formation in food protein aggregation and gelation. *Biotechnology Advances, 23*, 75–80.

Xiong, Y. L., & Brekke, C. J. (1991a). Gelation properties of chicken myofibrils treated with calcium and magnesium chlorides. *Journal of Muscle Foods, 2*, 21.

Xiong, Y. L., & Brekke, C. J. (1991b). Protein extractability and thermally induced gelation properties of myofibrils isolated from pre and post rigor chicken muscles. *Journal of Food Science, 56*, 210.

Xiong, Y. L. (1997). Structure–function relationships of muscle proteins. In S. Damodaran & A. Paraf (Eds.), *Food proteins and their applications* (pp. 341–392). New York: Marcel Dekker Inc.

Yasui, T., Ishioroshi, M., & Samejima, K. (1980). Heat induced gelation of myosin in the presence of actin. *Journal of Food Biochemistry, 44*, 61.