COMPARATIVE STUDIES ON THE FUNCTIONAL AND RHEOLOGICAL PROPERTIES OF MYOFIBRILLAR PROTEINS FROM FINFISH AND SHELLFISH DURING STORAGE

Thesis submitted in partial fulfilment

of the requirements

for the degree of

Ph. D. (Post-Harvest Technology)

By

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DEDICATION

To

My parents

SHRI. SHIVDAYAL MEHTA

SMT. GEETA MEHTA

Dated :

CERTIFICATE

Certified that the thesis entitled "**COMPARATIVE STUDIES ON THE FUNCTIONAL AND RHEOLOGICAL PROPERTIES OF MYOFIBRILLAR PROTEINS FROM FINFISH AND SHELLFISH DURING STORAGE**" is a record of independent bonafide research work carried out by **Mr Naresh Kumar Mehta** during the period of study from (October 2011) to (December 2015) under our supervision and guidance for the degree of **Doctor of Philosophy (Post Harvest Technology)** and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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I hereby declare that the thesis entitled "**COMPARATIVE STUDIES ON THE FUNCTIONAL AND RHEOLOGICAL PROPERTIES OF MYOFIBRILLAR PROTEINS FROM FINFISH AND SHELLFISH DURING STORAGE"** is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship fellowship or any other similar title.

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ACKNOWLEDGEMENTS

I wish to express my heartfelt gratitude to Dr. B. B. Nayak, Principal Scientist, Fisheries Resource Harvest and Post-Harvest Management Division, for his able guidance and immense support. He has helped me to put my best to complete this project. He has been a guide and a source of inspiration and his patience has led me through this endeavour.

I am extremely grateful to the advisory committee members Dr. A. K. Balange, Dr. S.K. Gupta and Dr. Gayatri Tripati for their timely suggestions, consistent help and critical comments especially during the course of research work.

I sincerely thank Dr. G. K. Saxena, the Director, In charge and Dr. W.S. Lakra, former Director, Central Institute of Fisheries Education, Mumbai for providing the necessary facilities to carry out this work. I am highly grateful to Dr. S. K. Chakravarty, Principal Scientist and Head, Fisheries Resource Harvest and Post-Harvest Management Division, for providing necessary facilities and advice throughout the programme.

My special thanks to Dr. G. Ventaswaralu, ADG (Education), ICAR, New Delhi, Dr. Sanath Kumar Sir, Dr. M. K. Chauksey, Dr. Martin Xavier Sir, Dr. Nagalaxmi and Manjusha madam, Phande Sir, Avinash Sir and Samba ji for their continuous support and help during my research work. I would like to thank staff of Library, CIFE, Mumbai for the help in literature search.

I express my sincere thanks to my loving seniors Dr. K. Elaversan, Scientist (CIFT, Cochin) Dr. Meena Madam, Ajita Madam and Madonna madam for their consistent suggestions and help during my degree programme. I would never forget the company of my few classmate friends Srijeet, Satish Minimol and my loving friends Abhishek Thakur, Snehal, Shrinu, Arvind, Manish, Brijesh, Tarachand, Irshad, Veerendra and Prem. The love and care received from my junior friends Baru, Namita, Surekha, Utkarsha, Yadi, Gonmei, and Deepitha will remain forever with me.

I cannot forget the encouragement and financial help from my lovely friends Mukesh, Bhanu, Arun, Manoj, Madan, Deepika, Jagdish, Pushkar, Anil and Ghanshayam at every moment of the my life.

The constant encouragement given by my Grandpa Sri. Kallu Prasad ji and Grandma Late Smt. Dhaniya Bai, uncle Shri. Mangal Singh and my sisters Rubi, Meena and Maneesha, whose blessings, love and sacrifices are the driving force for completion of doctoral degree programme. I thank my dear wife Shakshi for all the love and sacrifice she made during this period.

The sacrifice made by my parents Sri. Shivdayal Mehta and Smt. Geeta Mehta to realise my dream cannot be written hence, here just want to thank them for everything.

The senior research fellowship received from Indian Council of Agricultural Research (ICAR) for carry out this work is gratefully acknowledged.

Mumbai .....

December, 2015 (NARESH KUMAR METHA)

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1. INTRODUCTION

This study was conducted to characterise the functional and rheological properties of myofibrillar proteins and to understand muscle architecture of some finfish and shellfish during ice storage. The role of nutritious diet in human health is well established and seafood occupies prominent place in human diet for nutrition. Seafood is recognised as a source of cheap protein and delicacy around the world. Seafood, constitutes different kind of finfish and shellfish species, offers large variety, each one of them is different from others in taste, keeping quality and amenability to industrial processing. These variations are related to the fact that the seafood are drawn from a wide range of phyla of the animal kingdom like mollusc to crustacean to chordates i.e. both invertebrate to vertebrate. Not only their niche differs, their entire constitution differs.

As India is blessed with vast coastal and inland water resources, the production and potential of various finfish (like sciaenid), molluscs (squids, cuttle fish) and crustaceans (prawns, crabs etc.) are also enormous. This is worth mentioning that, in the last decade Indian fish and fishery product export has taken a large leap and achieving new heights in every forthcoming year in India. During the financial year 2014-15 export of fish and fishery products has crossed 10.51 lakh tonnes in terms of quantity and fetched more than Rs. 33,441 crores (MPEDA, 2015). The seafood export has been growing around 6 % per year in India. Shrimp becomes the main commodity in terms of volume and value earner in export. This huge surge in quantity as well as in foreign exchange became possible only because of enormous production (3,53,413 MT) of white leg shrimp in Indian coastal states (MPEDA, 2015). While frozen shrimps are the single most export earner, surimi contributed significantly to the export with 45000 tonnes of production as well as export. For production of value added products (from surimi or surimi based products or specialty products) low value white flesh marine fishes are still preferred. The croakers are one of the major fishery along the coast of Maharashtra and Gujrat, and belong to commercially important family of sciaenids having white flesh. The sciaenids contribute 8.45 % in the total marine fish catch of Maharashtra with an estimated value of Rs. 2215 crores (CMFRI, 2006-2007). It has a wide range of distribution in the Indian Ocean,

usually extending from Pakistan to the Andaman Islands (Froese and Pauly, 2012). The total catch of sciaenids from India was about 1,52,914 t for the period 2001-2011 which is about 21.87 % of the demersal catch and 5.34% of the total fish catch (CMFRI, 2001-2011).

Indian squid (*L. duvauceli*) is a commercially important cephalopod contributing substantially to Indian marine produce basket and distributed on a broad area of Indo-pacific waters. It dwells up to a depth of 120 m or even beyond but most of stock is found in depth of about 50 to 60 m from the shore (Mohamed *et al.,* 1993). In 2010, the total production of cephalopods was 1, 22,777 tonnes. Out of which almost 90 % comes from the west coast. On the west coast, Indian squid contribute 50 % of the total production of cephalopods (DADF, 2011). In addition to this, frozen squid alone contribute 6.62 % to the total seafood export and fetched more than 1275 crore rupees in the year 2014-15 (MPEDA, 2015).

Similarly, in the recent years, the production of white leg shrimp (*L. vannamei*) single handedly transformed the Indian seafood industry. The overall export of shrimp during 2014-15 was 357305 tonnes in which white leg shrimp alone contributed more than 81 % and earned more than 15000 crore rupees (MPEDA, 2015). However, a major part of seafood is still being exported in the form of raw or semi-processed food hence, there is a good scope for value addition of the exportable material. At present, the contribution of the value-added products in India's seafood export basket is only 17 per cent and it will increase in the wake of higher financing to the seafood processing industry (MPEDA, 2015). Further, if we need a diversified and sustainable Indian seafood export scenario with good share of value addition, then we need to create the facilities for all types of produce lands along the 8000 km long coastline of the country. Development and upgradation of value added seafood product industry in India will need huge infrastructure, technology and technical guidance, with regard to the preservation, as well as quality and shelf life maintenance of products.

Though, all different fish species (finfish, crustaceans and cephalopods) dwell in the similar environment but their mode of living, nature of

feeding, chemical composition and the structure, affect eating quality. Eating quality is comprised of textural quality, moisture, extractives, water-holding capacity besides the flavour and taste. These parameters are interdependent. Nevertheless, the most important factors affecting the eating quality or gastronomic quality is quality of proteins. Proteins get affected when they are subjected to any processing method. Processing of fish is known to alter the protein structure, thereby affecting many functional properties. As soon as the fish and shellfish is taken out of the water it is kept in ice for short term preservation and hence, changes occur in the functional constituents of the muscles during initial storage itself. The effect of processing techniques like icing on the fish muscle constituents has been the subject of investigation over few decades and on many varieties of fish species. The degree of the effect of processing is primarily dependent on species, method of harvest, pre-processing condition, and method of icing and temperature of icing storage. The nature of quality deterioration in shrimp, squid and finfish is important from the point of their freshness for consumption.

The use of proteins as natural emulsifiers,binders, gelling agents, etc. in composite food is widespread and is increasing. The most widely used proteins in the food industry are from soybean and milk. Fish proteins have not gained similar position in the market since suitable extraction techniques to preserve the functionality and to remove components affecting proteins quality, have been underdeveloped (Margrét Geirsdóttir, 2005). The functional and rheological properties of myofibrillar proteins derived from fish, are regarded as very important properties of protein as they contributes to texture and sensory characteristics. The myofibrillar proteins, contribute to 55-65% of total proteins are the important protein types responsible for the physicochemical properties of the protein in a food system (Lanier,1986). Myofibrillar proteins are the most functional proteins which comprise more than 15 different fractions. The functional and textural characteristics of meat depend mainly on myofibrillar proteins (Goll *et al.,* 1977). They consist of different fractions like myosin, actin, tropomyosin and troponin C, I, and T (Sikorski, 1994). The solublization of myofibrillar proteins is the major factor affecting the functional properties of fish protein during ice and frozen storage (Regenstein and Regenstein, 1984,

Borderias *et al.,* 1985). Actomyosin is the major protein responsible for gelation, which plays an important role in the texture and processing characteristics of meat products (Ahhmed *et al.,* 2009). Heat-induced gelation of fish myofibrillar proteins occurs in three-steps; dissociation of the proteins in the presence of salt, unfolding of protein molecules due to heating and aggregation of unfolded protein domains via hydrogen and disulphide bonds and hydrophobic interactions (Stone and Stanley, 1992; Lefevre *et al.,* 1999). The gel forming ability of fish proteins has been extensively studied and has got direct application in surimi based products (Mohan *et al.,* 2006) but studies on rheological properties of fish proteins are very few or very limited. Various attempts have been made to study the changes in functional properties of proteins from fish, squid and prawn as a function of ice storage (Dileep *et al.*, 2005; Hossain *et al.,* 2005; Mohan *et al.,* 2006; Rattana Sungsri-in *et al.,* 2011; Begum *et al.,* 2011;Mehta *et al.,* 2014).

Commercially important seafood primarily fall in to three categories, the vertebrates (fish), the molluscs (clam, mussels, cephalopods), the Arthropods (crustacean like shrimps). Though, several studies have been made on different aspects of these groups of animals, the variations in gastronomic properties are not fully understood. Besides, all of them are not amenable to similar processing methods. Where, fishes are used for surimi based products, the other aquatic animals from other phyla are rarely used, even-though protein is the main constituent of all these species. Further, heating changes the very nature of protein-protein, protein-fat, and other similar interactions. All other processing methods also have effect on protein based interaction.The understanding of proteins can come from their chemical nature or from their behaviour. Rheology finds many applications in food acceptability, food processing and food handling (Barbosa-Canovas *et al.,* 1996). Rheological measurements are used to physically characterize the raw materials prior to processing, the intermediate products during manufacturing, and the final food products (Tabilo-Munizaga and Barbosa-Cánovas, 2005). The rheological characteristics of fish muscle are thought to be governed by both the myofibrillar and connective tissue proteins, while the sarcoplasmic proteins contribute very little to texture (Barroso *et al.,* 1998). When animal muscle is ground with salt, it forms a viscous sol. The sol turns to an elastic gel upon heating. Both sol and gel are considered as

viscoelastic bodies. The dynamic rheological measurement is a very useful tool for monitoring the sol-gel transitions and characterizing the viscoelastic behaviour of gelled system in the linear region with small distortion. It can give information regarding elastic modulus (*G*'), viscous modulus (*G*") and tan delta (*δ*), which are very important mechanistic parameters relating to formation of the structure of gel. Attempts have been made to study the rheological properties of squid meat (Gómez-Guillén*et al.,* 2003), ribbon fish meat (Dileep *et al.,* 2005), threadfin bream (Karthikeyan *et al.,* 2006), tuna myofibrillar protein (Liu *et al.,* 2014) actomyosin from cold, temperature and warm water fishes (Esturk and Park, 2014) while there are no published reports on the rheological properties of muscles proteins from prawns/shrimps. With the varieties of proteins present in the fish and shellfish, studying the chemical nature of all of them might need years together. On the other hand, the behaviour depends on the collective chemical nature of proteins and can be quantified using sensory and mechanical means. Though, sensory tests are the ultimate evaluation for any food items, they could be subjective. With technological advancement, some of the sensory attributes can be quantified and controlled. Therefore, measures of functional and rheological properties will help in understanding the gaps as to why certain animal muscle is fit or unfit for particular processing operations, or for that matter have differences in chewbility, succulence or taste. Studies based on the comparison of proteins characteristics from fish, squid and prawn are also rare. In this backdrop, it becomes important to understand the interrelation of physico-chemical, functional and rheological properties of proteins from commercially important species of shrimp, squid and finfish. This will help in formulating effective strategies to minimize the deteriorative changes occurring in proteins during low temperature storage, thereby maintaining their functionality.

Hence, keeping these in view following objectives were set

OBJECTIVES

- To assess the physico-chemical properties of proteins from dhoma Fish (*Johnius dussumieri*), shrimp (*Litopenaeus vannamei*)) and Squid *(Loligo duvauceli*) during chilled storage.

- To assess the functional properties of myofibrillar proteins from dhoma Fish (*Johnius dussumieri*), shrimp (*Litopenaeus vannamei*) and Squid *(Loligo duvauceli*) during chilled storage.

- To assess the rheological properties of myofibrillar proteins from dhoma Fish (*Johnius dussumieri*), shrimp (*Litopenaeus vannamei*)) and Squid *(Loligo duvauceli*) during chilled storage.

1.REVIEW OF LITERATURE

Understanding the functional and rheological properties of proteins from dhoma fish, Indian squid and white leg prawn during ice storage will be the key for their effective utilization for human consumption. Further, understanding these properties would provide insight in to the gastronomic differences among the animals studied and their deterioration over storage.

In this section, the composition of fish, effect of ice storage on functional and rheological properties of proteins (especially myofibrillar proteins) from fin fish and shellfish, have been reviewed.

2.1 Proximate composition of finfish and shellfish

Biochemical composition of flesh is a good indicator for the fish quality (Hernandez *et al.,* 2001), physiological condition of the fish and habitat of fish (Aberoumad and Pourshafi, 2010, Shamsan and Ansari, 2010, Ravichandran *et al.,* 2011). The moisture content of 23 marine fishes reported by Kumar*et al.* (2014)ranged between 67.23 to 80.48 **%**.In another study, themoisture content of 20 marine finfish and shellfish reported between 74-82 % (Nurnadia *et al.,* 2011). Similarly, the contents reported for jumbo squid (*Dosidicus gigas*) mantle (Ramirez-Suarez *et al.,* 2008) and white shrimp (Puga-López *et al.,* 2013) were 83-86.5% and 73% respectively.

The protein content of 23 marine fishes studied and it was revealed that majority (69 %) of fishes had protein content between 15-20 % (Kumar *et al.,*2014). In another study, the protein content of prawn (*Metapenaeus affinis*) contained the highest protein (19.12 %). Similarly, the protein contents reported for jumbo squid (*Dosidicus gigas*) mantle (Ramirez-Suarez *et al.,* 2008) and white shrimp (Puga-lópez *et al.,* 2013) were 10.2-12.2% and 20 % respectively.

The fat content varied widely from 0.24 to 14.72%, with *Leiognathus dussumieri* being the most fatty fish (14.72 %). The average ash contents of most (87%) of fishes were < 2%.The fat content of shellfish ranged between 1-2% (Nurnadia *et al.,* 2011). Tang *et al.*(2009)observed lipid content in fillets of large yellow croaker was moderate (17.95%~18.18%). Kumar *et al.* (2014) also studied the content that varied widely from 0.24 to 14.72%, with *Leiognathus dussumieri* being the most fatty fish (14.72 %). Nurnadia *et al.* (2011) studied the fat content

of various shellfishes that ranged between 1-2 %. The content reported for jumbo squid (*Dosidicus gigas*) mantle was 0.2-0.9 % (Ramirez-Suarez *et al.,* 2008). Similarly the fat content for white shrimp was found to be 2.26 %.

The average ash of 23 marine fishes revealed to be < 2% (Kumar *et al.,* 2014). The content for 20 marine fin fish and shell fish has been studied, which found to be ranged between 0.9-2.1% (Nurnadia *et al.,* 2011).. The ash content reported for jumbo squid (*Dosidicus gigas*) mantle was 0.7-1.2% (Ramirez-Suarez *et al.,* 2008). Similarly, the content for white shrimp was about 1.34% (Puga-lópez *et al.,* 2013).Besides, the white shrimp is a good source of minerals and vitamins such as calcium, iron, zinc, copper, vitamin B_{12} and essential amino acids (Yanar and Celik, 2006).

The use of a dietary supplement containing essential fatty acid in correct ratio to body provides a useful assistance in dealing with a large number of systemic and cutaneous disorders. Furthermore, with regard to cholesterol level prevention. A higher concentration of EPA is required in comparison with DHA, while the opposite relative concentration is required during pregnancy or retina protection (DHA>EPA) (Maes *et al.,* 2000). The relative abundance and low cost of fish , increased awareness advertising and studies demonstrating the beneficial effect of these fatty acids has increased the consumption of food rich in these fatty acids**.** The fatty acid concentrations of *S. longiceps* and *P. lineatus* reported by Suvitha *et al.* (2014).A lot of work has been carried out by some other researchers on different fish sample. Osman *et al.* (2001) studied the fatty acid level on 10 different fish species the fatty acid level was varied from 1.46 to 5.77 %. Eswar *et al.* (2014) observed the fatty acid composition on puffer fish and mentioned PUFA like n-3 at the level of 31.17 %, 31.19 and n-6 7.26 %, 7.29 % at puffer fish *L. lunaris*and *L. inermis* respectively.

Dinçer and Aydin (2014) reported the fatty acid profile of jinga shrimp (*Metapenaeus affinis*) in which SFA, MUFA and PUFA were 60.31%, 15.47%, 24.21 % respectively in male whereas in female these were 53.64 %, 19.90% and 25.48 % respectively. Furthermore, a fatty acid profile study of ocean squid species showed that polyunsaturated fatty acids (PUFA) were the most abundant class of fatty acids in mantle tissue of all species, with sum values

between 51.5 % and 58.1 % of the total fatty acid while palimictic acid was most abundant in saturated fatty acid group (Phillips *et al.,* 2002; John Chembian, 2013).

2.2 Fish and shellfish protein properties

Fish protein based on the solubility has been divided in three categories *viz.* sarcoplasmic protein, myofibrillar protein and stroma protein. The sarcoplasmic proteins are soluble in low ionic strength solutions or water make up to 25-35% of total proteins (Regenstein and Regenstein, 1984; Mackie, 1997). The sarcoplasmic fractions are mainly composed of enzymes, membranous tubular structure and some lipoprotein components. The myofibrillar proteins which constitutes around 65-75 % of total proteins are soluble in relatively high ionic strength (> 0.3M) solution (Gopakumar, 2006).

The stroma proteins, consisting mostly of collagen and connective tissue are insoluble in low and high ionic strength solutions and make up to 3-5 % of whole muscle protein. The intra-muscular connective tissues are mainly composed of collagen, which provides integrity to hold muscle (Binsi *et al.,* 2007). As present work mainly focused on myofibrillar proteins and their properties, hereafter the properties of myofibrillar proteins are being reviewed extensively.

Myofibrillar Proteins

Myofibrillar proteins are the functional proteins which comprise more than 15 different fractions. The functional and textural characteristics of meat depend mainly on myofibrillar proteins (Goll *et al.*, 1977). The myofibrillar protein comprises of myosin, actin, tropomyosin and troponin C, I, and T (Sikorski, 1994). Myosin and actin play important roles in contraction and relaxation of the protein muscle; hence they are also called contractile proteins.

Myosin

It is the largest myofibrillar protein which contributes around 55 % of the total myofibrillar proteins. It contains 4-6 polypeptide chains out of which two are heavy chains (MW 205 each) and four light chains (MW 15.25 kDa each) with a total molecular weight of ≈ 500 kDa (Seki and Arai, 1974). The amino end (N - terminal) of the myosin heavy chains forms globular head having two binding sites, one for the ATPase enzyme and the other for the actin. The carboxylic end (C- terminal) forms the helical rod end of the myosin. Myosin molecules are mainly implicated in the gelation process (Iwata *et al*., 1977; Niwa, 1981). It has been well established that heat gelation of myosin is based on an irreversible aggregation of the myosin head, in which disulphide bonds are involved (Samejima *et al*., 1981).

Paramyosin

Paramyosin is large and rod like protein contains two adjacent α-helical chains with combined molecular weight of 220 kDa. It consists of two subunits, which are 120nm long with a molecular weight ranging from 95 kDa to 125 kDa per subunit. It is composed of proportionately high basic amino acid and amide contents such as glutamine (20 to 23.5 %), aspartic acid (12 %) and lysine (9 %) and low proline content. This abundant protein has been isolated from many vertebrates. This protein is also abundant particularly in those molluscan's muscles capable of catch, which is the ability of these muscles to remain is contracted state for long period of time with little or no expenditure of energy. In white abductor muscle of some clams and oysters, 38-48 % of myofibrils are paramyosin. In the red abductor muscles, paramyosin account for 15-30 % of myofibrils. Apart from bivalves, mantle muscle of squid (cephalopods) and foot muscle of turban shell (gastropod) are reported to contain 9-14% of their myofibrils as paramyosin (Srikantha *et al.,* 1990).

Actin

Actin is the second most important fraction of myofibril with a molecular weight of 42 KD. It constitutes around 25-30 % of the myofibrillar proteins by weight (Murray *et al.,* 1993). In the low ionic-strength solution, it remains as monomer which is having globular structure hence also called as

globular actin (G-actin) (Stryer, 1995). No major differences found in G-actin, which have been purified from different fish species (Seki and Arai, 1974). The complete amino acid sequence of actin has been determined (Watabe *et al.,* 1995). The number of amino acid units in actin is 374. G-actin has three binding sites; a nucleotides (ATP or ADP) binding site, a divalent cation (calcium) binding site and a myosin binding site (Xiong, 1997). Actin along with myosin forms an actomyosin complex during muscle contraction and permanent actomyosin complex during rigor-mortis stage (Xiong, 1997). Actin molecules are known to modify the functional properties of myosin molecules (Mackie, 1984). The actin monomer has a globular shape and is designated as G-actin. In the presence of ATP and Mg, actin polymerises into fibrous form called F-actin. The F-actin is in the form of a double stranded helix in which the G-actin beads are stabilised by tropomyosin fibrils.

Tropomyosin

Itis the most abundant regulatory protein in the myofibril (Bailey, 1946; Ooi *et al*., 1962; Woods, 1967; Xiong, 1997). It is a filamentous protein molecule composed of a coiled unit of two α-helices, each approximately of 40 nm lengths. Under the physiological condition it binds to F-actin at the ratio of 1:7 (tropomyosin: actin) and troponin T (1:1) stoichiometrically. It lays in the grove of F-actin and participates in the calcium regulation along with troponin (Ebashi and Ebashi, 1964; Ohtsuki *et al*., 1986). Although the molecular weight of two subunits of vertebral skeletal muscle tropomyosin are reported to be 33 kDa, of the tropomyosins isolated from 25 species of fish, 22 were composed of α-type subunits only, whereas 3 contained α and β sub units at molar ratio of 1:1.

Troponin

Troponin is a complex of three polypeptide chains, Tn C of 18 kDa, Tn I of 24 kDa and Tn T of 37 kDa. The troponin complex is located in the thin filaments of myofibrils at an interval of 38A°. Each troponin complex through tropomyosin regulates the interaction of about seven G-actin units (Stryer, 1995). In many respect, fish tropomyosin C is very similar to the protein isolated from rabbit muscle (Mc Cubbin *et al.,* 1982) and troponin isolated from skeletal muscle

of carps. Tn C and Tn I from lobster and crayfish muscles were found to exist in several isoforms (Nishita and Ojima, 1990).

2.3 Changes in physico-chemical properties of fin fish and shell fish during ice storage

Physico-chemical properties of the meat are reported to have bearing on the functional and textural properties. Therefore, before reviewing functional properties of the finfish and shell fish physico-chemical properties need to be understood.

pH

pH is considered one of the most influential parameters in muscle protein functionality (Ofstad, *et al.,* 1995). *Post mortem* changes in muscle have great functional and economic implications as they can greatly interfere with its quality. An animal's nutritional stage and stress level before an animal's death modify the glycogen concentration stored in muscle, consequently influencing in the *post mortem* pH (Haard, 1992; Massa *et al.,* 2003). The ice storage studies of farmed and wild turbot (Ozogul *et al.,* 2006) and Senegalese sole (Tejada *et al.,* 2007) deduced that initially pH value was slight acidic which slowly increased during whole period of ice storage. Similarly, in case of wild common sole (*Solea solea*), the initial pH value of 6.65 significantly increased to 7.02 on day 6 and then, the pH value decreased on day 10 . After that, it increased steadily until the end of storage study (Özoğul *et al.,* 2011). In general, pH of the muscles increases during ice storage. This increment of pH value can be attributed to compounds accumulated from endogenous and microbial enzymatic reactions (Seabra *et al*., 2011).

pH value for the fresh squid tubes was 6.2 (Jeyasekaran *et al*., 2010). Paarup *et al.* (2002) reported that the pH ranged from 6.8 to 7.8 in squid mantle (*Todaropsis eblanae*) stored at 4°C. Prafulla *et al.* (2000) observed that the pH of squid and cuttlefish muscle stored in ice did not vary significantly. For squid, skin color and pH also are very crucial to know quality of squid proteins. The pH in the fresh farmed and wild harvested white shrimp (*Litopenaeus*

vannamei) shrimp muscle were above 7.0. Similar findings also reported by López-Caballero *et al*. (2007), Tsironi *et al*. (2009) and Seabra *et al*. (2011) in various shrimp species. However, biochemical study of the freshwater prawn revealed no significant variation in pH during 10 days of ice storage (Akintola and Bakare, 2014).

Total volatile base nitrogen (TVBN) and Tri-methyl amine (TMA)

Quantifying the total volatile base nitrogen (TVBN) is one of the routinely carried out analysis for assessing chemical quality of fish. The TVBN content for finfish in the range of 35-40 mgN/100 g of meat is taken as the limit of chemical spoilage (Connell, 1995). The TMA and TVBN value for lizard fish were reported to be increased during 15 day of ice storage (Benjakul *et al.,* 2003). The TVBN and TMA formed in fish during iced storage were probably mediated by psychrotropic bacteria (Sasajima, 1973, 1974). The results suggest that pre-treatment of lizardfish by heading and eviscerating effectively retarded the spoilage during iced storage for up to 15 days. Bennour *et al.* (1991) found a TMA of 5 g/100 g as the rejection value for mackerel. While in case of ray fish (*Raja clavata*), a sharp increment was registered in TVBN content after 10 days of flake and slurry ice storage (Barros-Velazquez *et al.,* 2008). For common sole fish, in the beginning of storage, the TVBN value was 13.44 mg/100 g flesh. This value decreased to 9.73 mg TVBN 100 g flesh on day 6, thereafter significantly increasing to 34.03 mg TVBN /100 g by day 20 as reported by Özoğul *et al*. (2011). The initial storage temperature makes a remarkable difference in the production of total volatile base (Aune *et al.,*2014). The TVBN content of fresh water carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala)* was beyond 30 mg N/100g meat after 17 days of ice storage (Mehta *et al.,* 2014). For hake, the TMA content registered an increase from 13 mg N/100g to 48 mg N/100g in flake ice storage while increment was bit slower (8-19 mg N/100g) in slurry ice in 16 days of storage. For angler fish also the increment of TMA was reported to be higher in flake ice than the slurry ice during 16 days of storage (Barros-Velazquez *et al.,* 2008).

In case of squid increase in TVBN content is relatively slow but reached the limit within 10 days of ice storage (Sungsri-in *et al.,* 2011). The

source of the TVBN mainly arises from degradation of NPN constituents like TMAO, free amino acids, urea and low molecular weight peptides. The mean initial TVBN values of the freshwater prawn (*M. rosenbergii*) samples collected from farms and depots were 5.8 and 6.2 mg/100g respectively, which continuously increased during the progress of storage. In the samples collected from the farm, TVBN value reached upper limit of 25 mg/100g after 6 days of storage while in samples collected from depot, TVBN value reached beyond upper limit after 4 days of storage (Haider *et al.,* 2011).

Non Protein Nitrogen (NPN)

The non-protein nitrogen (NPN) comprises about 10% of total nitrogen in teleost, 25% in crustacean and 30% in elasmobranches (Chinnamma *et al*., 1970; Agarwal, 1984,). The NPN content in the fresh squid muscles was considerably higher (1161 mg/100g)(Paulo Vaz-Pires*et al.,* 2008.) than as reported for freshwater finfishes (282-311 mg N/100g) (Mehta *et al.,* 2014). Ruiz-Capillas *et al.* (2002) also confirmed the high content of NPN in squid. The NPN content of the fresh water prawn decreased from 461 to 170 mg N/100g during 10 day of direct contact of ice storage while value found to be increased in without contact of ice storage. The study suggests that NPN constituents washed off in the melted water of ice in case of direct contact of ice storage (Akintola and Bakare, 2014). The NPN content of fresh ribbonfish accounted for 23% of total nitrogen (Dileep *et al.,* 2005). Karthikeyan *et al.* (2006) showed a considerable reduction (315.97 to 83.33 mg N/100g) in the NPN content of meat after three washings.

Ca^{2+}ATPase enzyme activity

The myofibrillar proteins ATPase activity has been widely used as a measure of actomyosin integrity (Benjakul *et al.,* 1997). ATPase is associated with the *post mortem* disappearance of ATP in fish muscle, leading to rigor mortis (Nambudiri and Gopakumar, 1992). Kamal *et al*. (1991) reported that myofibrillar ATPase activities of ordinary and dark muscles decreased during extended iced storage of 10 days. The effect of ice storage on Ca^{2+} ATPase activity of lizard fish protein studied by Benjakul *et al.* (2003) where results indicated that myosin

underwent some changes in native conformation during iced storage. He also postulated that heading and eviscerating of lizardfish could retard the denaturation of myosin and troponin during iced storage. In ribbon fish stored in ice, the activity reduced to less than 20% of the original at the end of 20 days of ice storage (Dileep *et al.,* 2005). Ramachandran *et al*. (2009) studied Ca^{2+} ATPase activities of various fishes from different habitat and reported that the brackish water fishes have higher ATPase activity compared to fishes from other habitat. Fishes inhabiting colder water have also been reported to have higher ATPase activities compared to their tropical counter parts (Johnston *et al*., 1973). Hence, integrity of myosin head could be related to the temperature of water of inhabitant. The ATPase activity of total proteins from *Labeo rohita* after 18 days of ice storage was below detectable level suggesting the decrement in the solubility of the proteins could be the main factor behind the reduction of ATPase activity (Mehta *et al.,* 2014). The changes in the solubility profile of myofibrillar protein resulting in decreased activity of enzyme was also suggested by Kamal *et al.* (1991). Benjakul *et al.* (2011) reported decrease in the Ca^{2+} ATPase activity of myofibrillar proteins from Kuruma prawn when increase in the temperature due to denaturation of myofibrillar protein.

Surface hydrophobicity

Myofibrillar proteins are important structural proteins implicated in tenderness and water holding capacity of meat. Therefore, information related to their denaturation pattern is of great importance in meat technology. In view of its capacity to monitor subtle changes in chemical and physical state of protein, hydrophobicity can be a suitable parameter to estimate protein denaturation. An increase in surface hydrophobicity was observed in hake stored in ice (Roura *et al.,* 1992). An increase in surface hydrophobicity reflects the loss water holding capacity of muscles, leading to higher exudates in fish; a phenomenon which was observed in cod and haddock muscles kept in ice (Olsson *et al.,* 2003). The surface hydrophobicity in Indian major carps (Sankar and Ramachandran, 2005) and tuna (Liu *et al.,* 2014) was in the range of 60-120 while in Barracuda (Ramachandran *et al.,* 2007) a values of 37.40 was observed indicating higher nonpolarity at the protein surface than Barracuda. Surface hydrophobicity of

actomyosin from post-spawned flounder significantly ($p<0.05$) increased between days 3 and 6 of ice storage and then remained without major changes. However, from pre-spawned flounder linearly increased up to day 6 of storage and thereafter remained unchanged as reported by Paredi and Crupkin (2007). They suggested that unfolding of the α-helical portion of myosin occurs earlier in actomyosin from pre-spawned flounder than in actomyosin from post-spawned ones. Native myosin has hydrophobic residues strongly concentrated in the core of the helix (McLachlan and Karn, 1982) and the surface of the helix is essentially devoid of hydrophobic groups (Borejdo, 1983). Mignino and Paredi (2006) found lower superficial hydrophobicity in myofibrillar proteins from Aulacomya (bivalve molluscs) in compare to squid scallop (*Zygochlamys patagonica*) and squid (*Illex argentinus*) and stated that it could be due to a greater stability of this protein before the chemical environment.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) pattern of proteins

To understand changes at the structural level during ice storage, SDS–PAGE technique was successfully used by many researchers (Gómez-guillén *et al.,* 2003; Benjakul *et al.,* 2003; Dileep *et al.,* 2005; Ramirez-Suarez, 2008; Mignino *et al.,* 2008; Özoğul *et al.,* 2011). SDS–PAGE patterns of proteins extracted from lizard fish revealed that myosin heavy chain was much more susceptible to hydrolysis than actin. Similar findings were reported by Benjakul *et al.* (1997) who reported that myosin heavy chain was more prone to proteolytic degradation than other muscle proteins such as actin, troponin, tropomyosin and pramyosin. From the results, it was found that pre-treatment of lizardfish, by heading and eviscerating, effectively prevented the degradation of myosin heavy chain. Viscera contain a variety of digestive proteinases, which play a role in the softening of abdominal tissues during post mortem storage of fish (Haard, 1994). Therefore, eviscerating resulted in the reduction of digestive enzymes, leading to more retention of myosin heavy chain. Apart from digestive proteinases, viscera contain a number of bacteria, which expedite the spoilage of fish. Furthermore, muscle proteinases, such as cathepsins and calpain, also play an essential role in protein degradation of fish muscle (Haard, 1994).SDS-PAGE pattern of pre

and post spawned flounder showed no significant proteolysis during 10 days of ice storage (Paredi and Crupkin, 2007) and similar PAGE pattern observed for grass carp (*Ctenopharyngodon idella*) fillets stored at -3 °C and 0°C for 21 days in ice storage (Liu *et al.,* 2013). By SDS-PAGE Sotelo *et al.* (2000) established that quantity of the myosin in squid is lesser in comparison to finfish, whereas, percentage of paramyosin reported to be higher (15-17% of myofibrillar protein) than fin fish. An ice storage study on jumbo squid showed that only minor protein changes occurred during the 15 days of ice storage. Further, a slight decrease in the myosin heavy chain (MHC) band intensity occurred over the storage time with a concomitant band appearance at 153 kDa and a slight increase in the paramyosin (PM) band intensity (Ramirez-Suarez, 2008). Ice storage study conducted on myofibrillar protein from freshwater prawn (*Macrobrachium rosenbergii*) showed the gradual disappearance of heavy chain subunits implies the existence of a highly-active enzyme system in the freshwater prawn during 10 days of iced storage (Kye *et al.,* 1988).

Histological changes in muscles

The body musculature of fish is a series of segmental myotomes of complex shape separated from each other by collagenous sheets, called myosepta (Johnston et *al.,* 2000, Johnston *et al.,* 2004). Muscle fibres are orientated at angles up to 40° to the longitudinal axis and, hence, myotomes have complex three-dimensional structure.

Fish musculature is composed of bundles of muscle fibres (long multinucleated cells). Each muscle fibre is composed of bundles of myofibrils containing the contractile materials, myosin and actin filaments. The muscle fibre number and size are one of the characteristics affecting the flesh texture. It is believed that the muscle fibre size and fibre density is correlated to the firmness of the fish flesh (Johnston *et al.,* 2000; Periago *et al.,* 2005). According to various histological studies myofibrils are very stable during ice storage (Taylor *et al.,* 2002, Ofstad *et al.,* 2006). However, myofibre-myofibre detachments might be associated with softening due to loss of hardness (Taylor, 2002). Løje *et al.* (2007) investigated the effect of chilled storage on the structure of smoked salmon fish, and showed that the cells of a smoked salmon sample were more

firmly constrained than those of a control sample. With the length of storage, the extracellular space between the cells of smoked sample gradually became wider.

Sriket *et al.* (2010) studied the histological changes in the pre and post spawned fresh water prawn (*Macrobrachium rosenbergii*) muscle during iced storage and revealed that for both spawning stages, a notable increment in spacing or gaping between the muscle fibres of raw prawn was observed by 7th day of storage. Sharifian *et al.* (2011) studied the effects of cold storage on the microstructure of grouper fillets and results showed that, at day 0, the muscle fibres of control samples displayed fairly homogeneous and relatively normal shapes of the cross-section. After 7 days of cold storage, in comparison to the control, slight shrinkage of extracellular space and fibres were observed, and these changes were further enhanced by 14th day due to the degradation of per-cellular connective tissue.

Roy *et al.* (2012) measured the changes of structure and ultrastructure of cultured Pacific bluefin tuna muscle slices during chilled storage and indicated that the changes of the fish muscle slices were due to loss of myofibers to myofibers adhesion, detachment of the sarcolemma, increase of intermyofibrillar spaces, and adjustment of hexagonal arrangement of thick compared with thin contractile myofilaments. Light microscopy observation of the fillet from grass carp stored at 0 and -3 °C revealed that fillet stored at -3 °C showed gradual loss of integrity as characterized by the formation of detachments between muscle cells and cracks inside the cells before 10th day, while only a few small detachments between muscle cells and cracks inside the cells were occasionally observed for fish fillets stored at 0 °C (Liu *et al.,* 2013).

2.4 Changes in functional properties of myofibrillar proteins from finfish and shellfish during ice storage

Kinsella *et al*. (1976) defined “the functional properties of proteins are those physico-chemical properties of proteins which affect their behaviour in food systems during preparation, processing storage, consumption and contribute to the quality and sensory attribute of food system”. The changes in water holding capacity, Ca^{2+}ATPase enzyme activity, solubility, viscosity and

gelling properties are important physico-chemical and functional properties which are monitored to assess the quality of myofibrillar protein during ice storage (Hossain *et al.,* 2005; Dileep *et al.,* 2005; Rocha-Estrada *et al.,* 2010; Benjakul *et al.,* 2011; Mehta *et al.,* 2014). In addition to these properties, electrophoretic mobility, sulphydryl content are also assessed.

Solubility profile of myofibrillar proteins from finfish and shellfish

Benjakul *et al. (*2005) found that solubility in muscles protein of croaker, lizardfish, thread bream and bigeye snapper decreased continuously during prolonged ice storage period. Solubility profile of myofibrillar proteins from Thai pangas (*Pangasius sutchi*) showed 88.37% solubilisation immediately after death which further decreased to 36.43% at the end of 25 days of ice storage (Hossain *et al.,* 2005).

Myofibrillar proteins constitute about 76% of the total protein content and differ from that of fish and mammals by being more water-soluble. Approximately 85% of the total protein in squid muscle will dissolve in water. The collagen in the squid muscle is somewhat higher than that of fish muscle. The content of collagen in fish muscle ranges from about 1 to 12% of the crude protein. In the mantles of *Loligo* and *Illex*, the amount of collagen is about 3% and 11% respectively. The different amount of collagen in squid mantles varies with the squid species and is presumably responsible for its tough texture (Sikorski and Kolodziejska, 1986). De La Fuente-Betancourt *et al.* (2009) found that pH of more than 10 solubulized more than 80 % jumbo squid proteins. In another study of jumbo squid (Ramirez-Suarez *et al.,* 2008), a significant reduction ($p < 0.05$) in mantle muscle protein solubility from 93.4 ± 4.0 on 0 day to 87.4 ± 4.1 on 4^{th} day was observed.. In general, the muscle protein solubility decreases over the storage period at 0 °C. Solubility of actomyosin of green mussel was found to increase with storage and at the end of storagethe value decreased (Binsi *et al.,* 2006).

A decrease in the solubility of the proteins of fresh water prawn was reported where solubility decreased by 44% in 10 days of ice storage (Begum *et al.,* 2011). Studies conducted with the ice-stored farmed and depot *M.*

rosenbergii showed that the mean initial myofibrillar protein solubilities were 87% and 77% which decreased to 53% and 43% respectively during 10 days of ice storage (Haider *et al.,* 2011).

Viscosity

The measurement of viscosity is considered as more reliable protein quality from fish than protein solubility or emulsion capacity (Jimenez-Colmenero *et al*., 1988). Ramachandran *et al.* (2007) studied the viscosity of MFP from barracuda (*Sphyraena jello*) in smaller and bigger size groups were significantly different ($P<0.05$) at 2.5, 5 and 10 mg/ml concentrations. In smaller size group, 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 bigger group fishes. This indicates that besides concentration, the intrinsic properties of the proteins also contribute to the increased viscosity. Reduced viscosity of myofibrillar protein from ribbon fish registered a sharp increase after 15 days of ice storage (Dileep *et al.,* 2005). The protein–solvent interaction predominates and establishes a linear relationship between viscosity and protein concentration (Chen *et al.,* 1988; Cofrades *et al.,* 1993) and at higher concentration a nonlinear relationship was reported (Cofrades *et al.,* 1993) as reported in this study. A significant decrease in viscosity was observed at days 3 and 9 of storage in actomyosin from post-spawned flounder and at days 6 and 9 in actomyosin from pre-spawned ones due to denaturation of major myofibrillar proteins during storage on ice (Paredi and Crupkin, 2007). The apparent reduced viscosity of the total protein from *Cirrhinus mrigala* (at 3 mg/ml) has shown an increasing trend during ice storage indicating protein-protein interaction as compared to *Catla catla* and *Labeo rohita* (Mehta *et al.,* 2014).

Water holding capacity (WHC)

Lean muscle contains approximately 75% water. The other main components include protein (approximately 20%), lipids or fat (approximately 5%), carbohydrates (approximately 1%) and vitamins and minerals (often analysed as ash, approximately 1%). The majority of water in muscle is held within the structure of the muscle and muscle cells. Specifically, within the

muscle cells, water is found within the myofibrils, between the myofibrils themselves and between the myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscle bundles (groups of muscle cells) (Offer and Cousins, 1992). The WHC has been reported to be influenced by a number of factors, including ultimate pH, protein denaturation, intra- and inter-fascicular spacing and sarcomere lengths (Offer and Knight, 1988). Fish muscle quality is highly affected by water content and its distribution within the flesh. Ofstad *et al.* (1996) have demonstrated a relationship between changes in WHC and microstructural changes and that affected by muscle pH in cod and salmon. The nutritional status at capture influences the post-mortem pH that in turn affects fish quality. Intensive feeding leads to particularly low ultimate pH, which has been shown to result in low WHC of the muscle (Ang and Haard, 1985; Haard, 1992; Love, 1979; Ofstad *et al.,* 1996; Rustad, 1992). Myofibrillar composed of 25% water and 75% of water. Majority of the water is confined within the myofibrils in the space between myosin and actin (Xiong, 1997). Myofibrils are primary sites for intracellular water. Other cellular components may also contribute to water binding in meat. Entrapment and mobility of water present outside of the cell could be affected by many environmental factors (Xiong *et al.,* 2000). There are two major types of forces that contribute to water retention in meat viz. the polarity including surface charges and the capillary effect (Xiong, 1997). Any changes in the surrounding of the myofibrillar proteins that increase charges (any pH away from protein isoelectric point) would lead to the increase the water holding capacity of meat (Xiong, 1997). Ramirez-Suarez *et al.* (2008) reported a slight decrease in water holding capacity of squid gels (82.9 ± 8.6% at day 0 versus 75.3 ± 14.0% at day 15 during 15 days of ice storage period. WHC of halibut muscle registered an increase up to 4th day followed by a continuous decrease up to 15 days of cold storage (Olsson *et al.,* 2003). It has been suggested that the increase of the WHC during ageing of meat is due to reduced water content described as the ‘‘leaking out’’ effect (Moeseke and De Smet, 1999).

Emulsion capacity

The ability of the protein molecule to emulsify a given volume of the oil mainly depends on the hydrophobic groups on the surface of proteins that orient towards the oil phase and the hydrophilic group orienting to the aqueous phase, thus lowering the interfacial free energy (Kato and Nakai, 1980). Borderias *et al.* (1985) reported that the emulsion capacity (EC) of sarcoplasmic and myofibrillar protein of various fish species and found a correlation between the EC and the concentration of solubility of soluble proteins. The emulsion capacity of myofrillar proteins of rohu was 11.09 m^2/g in fresh condition which doubled at the end of 6 days of but again back to 11.04 m^2/g on the 25 days of ice storage (Mohan *et al.*, 2006). A study conducted on common carp showed that the initial EC value of carp meat in the solution containing no phosphate salt was 253.15 ml oil/g protein and increased to 290.06 ml oil/g protein as a function of phosphate salt level incorporated. Furthermore, similar trend was observed for the samples with different levels of salt content (Yapar *et al.,* 2006).

The EC reported for octopus calamari, mussel and cuttle fish was ranged between 7.32-7.63 ml oil/mg protein (Ozalp and Karakaya, 2009) which was higher than reported for shark meat and Indian major carps (Mathew and Shamasundar, 2002; Mehta *et al.,* 2014).

Gel forming ability

Gel forming ability of fish proteins is achieved by heating and gelatinize muscles ground with salt. Apparently various cross links between myosin molecules in the myofibrillar proteins, and between these actin molecules, are increased in the heating process. These cross-linking manifest in gel elasticity and as a result, the skeleton of the gel is formed. The formation of gels involves the partial denaturation of the protein followed by an irreversible aggregation, resulting in a tri-dimensional network (Lanier *et al.,* 2004). The characteristics of the myofibrillar proteins, the proteins mostly responsible for the functional properties, are of great importance for the elaboration of gel products (Xiong, 1997). Some cephalopod species show poor gelling capacity due to high proteolytic activity, affecting one of the major myofibrillar proteins (Hurtado *et al.,* 1999; Gómez-Guillén *et al.,* 2002, 2003), and studies with *Illex argentines* have revealed the presence of proteolytic activity even during frozen storage (Paredi *et*

al., 2006; Mignino *et al.,* 2008, 2013). Studies on cephalopods have reported a decrease in gel strength due to the presence of proteolytic activity that degrades the heavy myosin chain (Nagashima *et al.,* 1992; Gómez-Guillén *et al.,* 2002, 2003). The gelation properties of squid meat have already been investigated by Okada (1953) and Shiba (1990) who found squid meat is very difficult to set and disintegrate. Satelo *et al.* (2000) established that quantity of the myosin in squid is less compared to finfish while the percentage of paramyosin (15-17%) is higher than the fin fish. Therefore, this could be the reason of improper gelation of squid proteins. Gel-forming ability of surimi from three species of mackerel (Indian mackerel, short-bodied mackerel and Frigate mackerel) has been reported in which Kamaboko gel of short-bodied mackerel surimi exhibited the highest breaking force with the lowest expressible drip (Chaijan *et al.,* 2010). The gel forming ability of pink perch surimi was reduced from 211g. cm to 41 g.cm in 6 days of refrigerated storage temperatures (Fazal *et al.,* 2013). In a study, gel strength values of weak fish and squid were determined and revealed lower gel strength value for squid (*Illex argentinus*) in compare to weak fish (*Cynoscion guatucupa*) (Suarez*et al.,* 2014). Washing also affects the gel forming ability. The gel forming ability of freshwater fish *Labeo calbasu* was relatively higher i.e. 680 g.cm which increased considerably after washing to 1110 g.cm (Yathavamoorthi *et al.,* 2010).

Texture profile analysis (TPA)

Texture is considered to be the most important sensory quality since it may change dramatically during extended cooking, while the characteristic flavour develops relatively early during the process and does not change substantially after prolonged heating (Ma *et al.,* 1983). Texture is a multi-point property of foods (Bourne, 1978), and is defined as the properties arising from the structural elements and physiological senses (Szczesniak, 1963; Brady and Hunecke, 1985). In other words, the texture is a multidimensional sensory characteristic evaluated by sensory or instrumental methods (Hyldig and Nielsen, 2001; Sigurgisladottir *et al.,* 1999), and known to vary with several factors, such as pre slaughter stress, storage time and storage temperature *post mortem* (Sigholt *et al.,* 1997; Veland and Torrissen, 1999). The texture is the

function of many characteristics that food possess such as hardness, cohesiveness, stringiness, chewiness, springiness, gumminess and adhesiveness. Each term has been described and its implication in table given below in this section. Toughness, or hardness is among the critical texture attributes in seafood products. Hardness is a property that depends on the connective tissue consisting of mainly collagen (responsible for tensile strength) and the myofibrils, consisting of myosin and actin (Martens *et al.,* 1982).The texture profile analysis (TPA) is an objective method correlated to the sensory analysis of the texture. The TPA determination involves two successive compressions of the material, during which the evolution of the strength is recorded at constant velocity. The value for the grass carp hardness was found to be reduced sharply till 3rd day of ice storage while no significant changes were observed thereafter (Liu *et al.,* 2013). A study conducted on the seabream revealed that hardness, cohesiveness, chewiness and gumminess reduced significantly while springiness remains considerably unchanged during 22 days of ice storage (Ayala *et al.,* 2010). Cardoso *et al.* (2012) studied hardness, cohesiveness gumminess springiness and chewiness of the heat induced gel prepared from sea bream and sea bass 11.7 & 26.1 N, 0.53 & 0.50, 6.2 & 13 N, 0.73 & 0.84 and 4.5 & 10.9 N respectively. A study conducted on jumbo squid revealed that the gel produced from muscles from squid fin had more gel strength compared to exclusively mantle tissue. Gel from fin muscles also registered highest hardness (50.2 N) and cohesiveness compared to gel from the muscles from mantle (De La Fuente-Betancourt *et al.,* 2009).

TPA parameter	Definition	Determination
Hardness	the force required to compress the food between teeth(or between tongue and palate for soft foods) to a given deformation	the peak force during the first(or between tongue and palate for soft foods) to a given compression cycle (‘‘first bite’’)
Cohesiveness	The extent to which food can be deformed before it rupture	The ratio of the positive area during the second compression to that during the first compression
Springiness	The rate at which a deformed material recovers to its undeformed condition after the deforming force is removed	The height that food recovers during the time that elapses between the end of the first bite and the start of the second bite
Gumminess	The amount of the energy required to disintegrate a semi-solid food product to a state ready for swallowing	The product of hardness and cohesiveness
Chewiness	The length of time (or number of chews) required to masticate a solid food	The product of gumminess and springiness
Adhesiveness	The force required to remove food that adheres to the mouth	The negative area for the first mouth bite, representing the work necessary to pull the compressing plunger away from the sample

2.5 Changes in rheological properties myofibrillar proteins from finfish and shellfish during ice storage

The rheological measurements can also be used as a tool for physical characterization of raw material prior to processing (Tabilo-Munizaga and Barbosa-Canovas, 2005). From dynamic rheological tests in the linear viscoelastic range, the storage modulus, G', and the loss modulus G'', and tan δ = (G''/G'), the loss factor, can be obtained. G' value – to measure the deformation energy stored in the sample during the shear process, representing the elastic behaviour of a sample. G'' value – to measure the deformation energy used up in the sample during the shear and lost to the sample afterwards, representing the viscous behaviour of a sample. On the other hand, tan δ (the lost factor or damping factor) reveals the ratio of the viscous to the elastic portion of the deformation behaviour. A phase angle δ = 0° or tan δ = 0 corresponds to an elastic response and δ = 90° or tan δ = 1 is a viscous response. If the phase angle is within the limits of 0 < δ < 90°, the material is called viscoelastic.

The rheological properties of fish gels depend upon factors such as fish species, quality of the meat, salt content, protein concentration and processing methods. The modified Mooney-Rivlin equation developed by Kong *et al.* (2005) concluded that using the modified large deformation equation considering volume changes of samples were more useful to investigate effectively the mechanical behaviour of fish meat gels. The elastic moduli and viscosity were reported to decrease with then moisture contents of the gels (Kong *et al.,* 2005).Prior to gelation, the material shows a typical fluid-like behaviour ($G' < G''$). If the size of protein aggregates becomes large enough, G' increases rapidly, and after some time, a cross-over point ($G' = G''$) is observed. This point and the corresponding time are often referred to as the gel point (gelation point) and the gel time (gelation time) respectively (Clark and Ross-Murphy, 1987; Djabourov, 1988; Clark, 1992). As gelation progresses, G' becomes dominant, showing characteristics of solids ($G' > G''$). In some test systems, a cross-over of G' and G'' may not be observed. As G' is initially close to zero because of the fluid-like nature of the material, the gelation point can be defined as the point where G' begins to increase rapidly. This point may be determined by an extrapolation depending on the sensitivity of the measuring instrument. It should be noted that the gel point determined by this procedure usually depends on the measuring

frequency. Theoretical aspects of determination of the gel point have been considered in the literature (Winter, 1987; Scanlan and Winter, 1991).

The squid (*Loligo vulgaris*) mantle proteins after 4 days of chilled storagehad a significant drop in protein functionality that negatively affected the thermal gelation profile (Gómez-Guillén, 2003). The storage modulus for ribbon fish meat was found to be increased during 25 days of ice storage period and the maximum value obtained was 358.8 kPa at the temperature of 70 °C. They postulated that this increase in the storage modulus may be due to the aggregation/denaturation of protein during ice storage (Dileep *et al.,* 2005). In an another study of the dynamic viscoelastic behaviour of silver carp meat, the gel occurred in two steps 1st step at low temperature of 5 to 35 °C and the 2nd step over 51°C while gel broke up occurred between 35 to 51°C. (Liu *et al.,* 2007). The dynamic visco-elastic behaviour of fresh actomyosin from green mussel revealed the maximum storage modulus (G') of 213.3 kPa AT 63.3 °C during temperature changes (heating) and the sol to gel transition occurred at 56.8°C (Binsi *et al.*, 2006). The slope of G' values as a function of frequency sweep for sol to gel were 0.2803 and 0.0138 for green muscles respectively (Binsi *et al.,* 2006). Dileep *et al.* (2012) studied the dynamic viscoelastic behaviour of fresh bigeye snapper (*Priacanthus hamrur*) mince the maximum G′ value of 378.6 kPa was attained at 63.6°C and two transitions were evident at 43.3 and 63.6 °C. Rheological properties of the actomyosin from cold, temperate and warm water fish species have revealed that stability of myosin from different fish species is related to the environmental temperature at which fish lives and this supported the statement that proteins of the warm water fishes were more thermostable than those of cold water or temperate water species (Esturk and Park, 2014). The tan δ is the ratio of energy lost as a result of viscous flow and energy stored from elastic deformation in the single deformation cycle (Chan *et al.,* 2011).A change in the tan δ indicates the type of the network formed; lower tan δ values represents formation of better 3-dimensional network (Sun and Arntfield, 2010).

3. MATERIAL AND METHODS

3.1 Material

Dhoma fish (*Johnius dussumieri*), Indian squid (*Loligo duvaucelii*) and white leg shrimp (*Litopenaeus vannamei)* were used in the present study. Dhoma fish and Indian squid were purchased from the boat owners, who go for single day fishing, soon after they arrived at Versova landing centre in the north-west part of Mumbai city while shrimps were procured from cultured pond in Raigarh district of Maharashtra. The experimental fish species were transported to the laboratory in iced condition in poly-urethane boxes. The average length and weight of dhoma fish, Indian squid andshrimp used in this study were between 16-28 cm and 132-223 g, 9-14 cm (mantle) and 41-82 g and 10-14 cm and 25-35 g respectively.

Plate 1. Dhoma fish

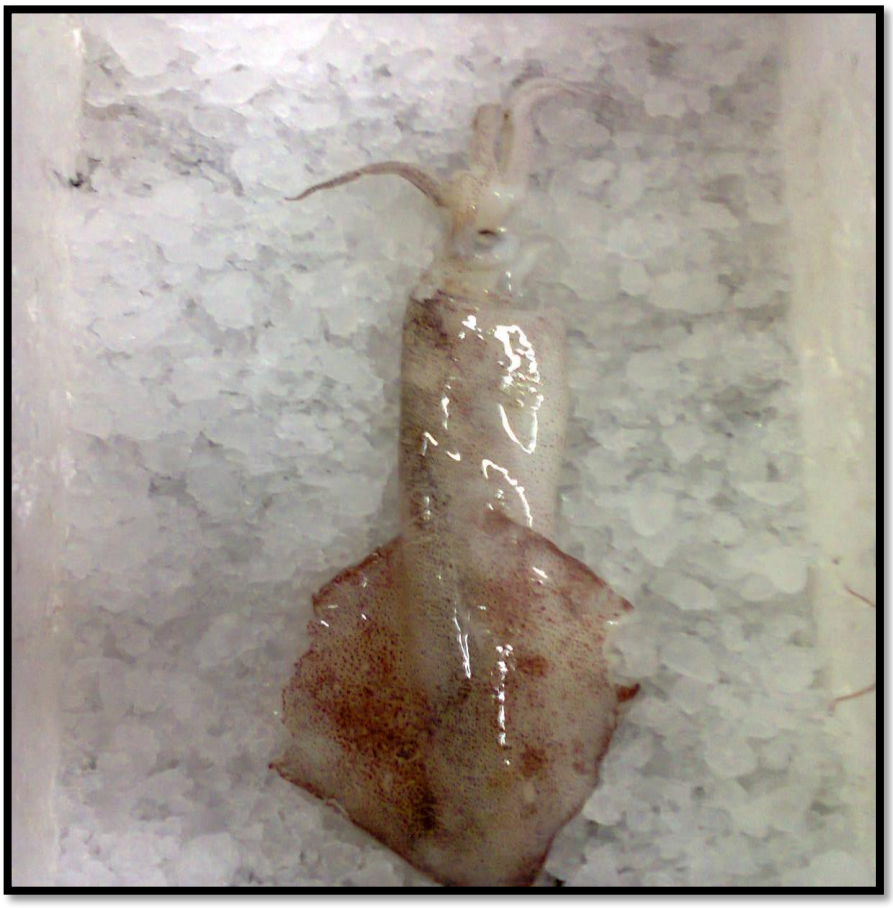


Plate 2. Indian squid

Plate 3. White leg shrimp

3.2 Methods

3.2.1 Icing

Flake ice produced from the laboratory flake ice machine (Icematic, F08c, Germany) used for the ice storage study. Fish from landing centre, after being brought to the laboratory, were washed with chilled water and repacked in alternating layers with ice in the ratio of 1:1 (fish: ice) in thermocole (expanded polystyrene) boxes. The boxes were held at room temperature (28-32 °C) for storage studies. Ratio of fish to ice was maintained at 1:1 for the entire period of study by everyday replacing melt water with fresh ice.

3.2.2 Sample preparation

The samples of dhoma fish were drawn for analysis at 0, 3, 6, 9, 12, 15 and 18^{th} day of ice storage while the squid and the shrimp samples were drawn at 0, 2, 4, 6, 8, 10, 12 and 14^{th} day. The sampling frequency for finfish, squid and shrimp were kept different given the fact that shellfishes spoil faster than the finfish. The muscles samples were analysed for physico-chemical, functional, textural and rheological properties. The term 0 day refers to the sample drawn after 24 h of harvest. Required amount of samples (Dhoma fish, Indian squid and white leg shrimp) were removed from thermocole box and dressed by removing head, viscera, shell and fins. The meat was separated manually from fins, skin and bones and the separated meat was macerated well using pestle and mortar in ice bath and used for further analyses.

3.3 Analysis of physico-chemical properties

3.3.1 Analysis of proximate composition

The proximate composition analysis (moisture, crude protein, fat and ash content) of fresh meat from experimental fish species was carried out using the following standard methods (AOAC, 2010).

3.3.1.1 Moisture

The moisture content was estimated by hot air oven method (AOAC, 2010). About 10 g of meat was taken in triplicates and dried in a hot air oven with mechanical convection at 100 ºC ± 2 ºC for 10-12 h. The samples were cooled to room temperature in a desiccator containing silica gel. Drying and cooing was repeated till constant weight was obtained. The weight loss was expressed as percentage of the moisture content of the meat.

3.3.1.2 Crude Protein

The crude protein content of the meat was determined by estimating total nitrogen content by Kjeldahl method (AOAC, 2010). About 1g sample was weighed accurately and digested with 10 ml of concentrate sulphuric acid and a pinch of digestion mixture {potassium sulphate (10): copper sulphate (1): selenium dioxide(0.25)} in a digestion flask. The digestion flask along with content was heated electrically using a thermostat. The digestion was continued till the solution became clear and heating was stopped. The digested solution was cooled and made up to a known volume with distilled water. Two ml of aliquot was transferred to Kjeldahl distillation unit with 12 ml of 40% sodium hydroxide. The sample was distilled and distillate was collected in 10 ml of 2 % boric acid containing mixed indicators (0.06 % methylene blue and 0.06 % methyl red in 95 % ethyl alcohol). The ratio of methylene blue and methyl red was 2:1 (2 parts of methylene blue and 1 part of methyl red). The distillation was continued till the boric acid turns to green. The boric acid was titrated against N/140 HCl till the pink colour was obtained. The nitrogen equivalent factor of hydrochloric acid was determined using ammonium sulphate as standard. A known quantity of ammonium sulphate was dissolved in distilled water to give a final concentration of nitrogen 1 mg/ml of ammonium sulphate solution. The ammonium sulphate was digested and distillated as explained earlier. The volume of hydrochloric acid consumed for titrating ammonium sulphate solution was used to calculate nitrogen equivalent. The nitrogen factor of 1 ml HCl was found to be 0.1012 mg nitrogen. Crude protein content of meat samples was calculated by multiplying the nitrogen value obtained by a factor of 6.25. The protein content was expressed as percentage of meat.

$$\boldsymbol{Protein}\ (\%) = \frac{\mathbf{14} \times \boldsymbol{N} \times (\boldsymbol{A} - \boldsymbol{B}) \times \mathbf{100} \times \mathbf{100}}{\boldsymbol{W}\ \times \boldsymbol{Sample\ taken\ for\ distillation} \times \mathbf{1000}}$$

N-Normality of HCl

A-Titre value of sample

B- Titre value of blank

3.3.1.3 Crude Fat

The crude fat content of meat was estimated by Soxhlet extraction method (AOAC, 2010). About 1 g of moisture free meat sample was used for fat estimation. The sample was kept inside a Whatman thimble. The thimble was plugged with cotton loosely and placed in a soxhlet unit. Petroleum ether (40º-60ºC grade) was used as solvent. Sufficient solvent was added to soxhlet unit and heating was achieved by thermostatically controlled mantle. The temperature was set to 50º-60ºC and extraction was continued for 14-16 h. The soxhlet flask containing extracted fat and solvent was dried on a water bath so as to remove most of the solvent. Further, the residual solvent was dried by placing the flask in hot air oven set to the temperature 60 ± 5ºC. The flask was cooled in a desiccator and weight was recorded. The drying and cooling was repeated till constant weight was obtained. The fat content in sample was calculated using following formula.

$$\boldsymbol{Fat\ content}\ (\%) = \frac{\mathbf{100} \times\ \boldsymbol{W2} - \boldsymbol{W1}}{\boldsymbol{X}}$$

Where,

X- Weight of the sample

W1- Weight of the empty flask

W2- Weight of the flask after evaporation.

3.3.1.4 Ash

Ash content of meat was determined by method as described in AOAC (2010).About 1 g of moisture free-meat sample was taken in a pre-weighed silica

crucible. Preliminary charring was done by slow heating on a flame without burning. After the charring the sample, was incinerated in a muffle furnace (Phoenix, USA) at 550º ±10 ºC for 6 h. The crucibles were removed and cooled in desiccator and weighed. Ash content was calculated from the weight difference of crucible and expressed as the ash content in percentage on wet weight basis by using following formula.

$$\textbf{Total ash}\ (\%) = \frac{(\mathbf{W2} - \mathbf{W}) \times \mathbf{100}}{(\mathbf{W1} - \mathbf{W})}$$

Where,

W - Weight of crucible

W1 - Weight of crucible + sample

W2- Weight of crucible + ash.

3.3.2 Fatty acid analysis

Total lipids were extracted from muscle according to Folch *et al*. (1957)**.**Fatty acid content and fatty acid composition of muscles were determined simultaneously.Fatty acid analysis was performed in triplicate consisted two consecutive steps, preparation of fatty acid methyl ester (FAME) and chromatographic analysis. The AOAC (2010) method was followed to esterify the lipid extract. Gas chromatography–mass spectrometry (GC-MS) was performed on Shimadzu Qp2010 quadrupole Gas Chromatography Mass Spectrometer (GC-MS) instrument equipped with a carbowax (30 m × 0.25 mm ID; 0.25 mm film thickness) capillary column (Cromlab S.A). Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. Peak area was quantified and expressed as percentage of total fatty acids.

3.3.3 Determination of pH

About 5 g of fish meat sample was macerated with 45 ml of distilled water and the pH of slurry was measured by using pH meter (Systronix µ pH system 361). Before measuring the pH of the slurry, pH meter was calibrated with standard buffer solutions of pH 4.0, 7.0 and 9.2.

3.3.4 Analysis of Total Volatile Base Nitrogen Content

Total Volatile Base Nitrogen (TVBN) content of the fresh and ice stored samples was determined by Conway micro diffusion method (Beatty and Gibbon, 1937). 10 g of the meat sample was macerated with 20 % tri-choloro acetic acid (TCA) solution using pestle and mortar. The slurry was filtered with coarse filter paper and made up to 100 ml with distilled water. 2 ml of boric acid containing mixed indicator (0.066% methyl red and 0.066 % bromo cresol green solution in alcohol in ratio of 1:1) was added into the inner chamber and 1 ml of sample into outer chamber of Conway micro diffusion unit followed by addition of potassium carbonate in the same chamber. Grease was applied on the covering glass of unit to make it air tight. The solution was incubated at 37 ºC for 90 min. After incubation, inner chamber content was titrated against 0.02N sulphuric acid. A blank was run using 2% TCA solution instead of sample. TVBN was calculated using the following formula an expressed in mg %.

$$\mathbf{TVBN}\ (\mathbf{mg}\ \%)\ = \frac{\mathbf{14} \times \boldsymbol{N} \times (\boldsymbol{X} - \boldsymbol{Y}) - \boldsymbol{B} \times \mathbf{100} \times \mathbf{100}}{\boldsymbol{S}}$$

Where, N- Normality of H_2SO_4

X- Volume (ml) of H_2SO_4required for titration of sample

Y- Volume (ml) of sulphuric acid required for blank

S- Weight of sample

3.3.5 Analysis of Tri-methyl amine Content

Tri-methyl amine (TMA) content of fresh and ice stored samples was determined by Conway micro diffusion method (Beatty and Gibbon, 1937).

10 g of the meat sample was macerated with 20 % tri-choloro acetic acid (TCA) solution using pestle and mortar. The slurry was filtered with coarse filter paper and was made up to 100 ml with distilled water. 2 ml of boric acid containing mixed indicator (0.066% methyl red and 0.066 % bromo cresol green solution in alcohol in ratio of 1:1) into the inner chamber and 1 ml of sample into outer chamber of Conway micro-diffusion unit follow by addition of potassium carbonate and formaldehyde in the same chamber. The grease was applied on covering glass of unit to make air tight. Solution was incubated at 37 ºC for 90 min.

After incubation, inner chamber content was titrated against 0.02N sulphuric acid. A blank was run using 2% TCA solution instead of sample. TMA content was calculated using following formula an expressed in mg %.

$$\mathbf{TMA\ (mg\ \%)} = \frac{\mathbf{14} \times \boldsymbol{N} \times (\boldsymbol{X} - \boldsymbol{Y}) \times \mathbf{100} \times \mathbf{100}}{\boldsymbol{S}}$$

Where,

N- Normality of sulphuric acid

X- ml of sulphuric acid required for titration of sample

Y- ml of sulphuric acid required for blank

S- Weight of sample

3.3.6 Analysis of non-protein nitrogen content

Non protein nitrogen (NPN) content was determined by the method as described by Velanker and Govindan (1958). About 3 g of sample was taken and macerated with 10 ml of 15 % TCA for3 min. using pestle and mortar. The slurry was allowed to settle at refrigerated temperature for 30 min. The slurry was filtered and made up to 50 ml with distilled water and 5 ml aliquot was taken for nitrogen estimation by Kjeldahl method. The NPN content was expressed as mg N/ 100 g meat

3.3.7 Determination of Ca^{2+} ATPase enzyme activity

Calcium ATPase enzyme activity was measured by using the method of Naguchi and Matsumoto (1970). About 1 g of fresh and ice stored meat was macerated in 10 ml of 0. 2 M glycine-NaOH buffer, pH 9.2. The slurry was filtered through Whatman no. 1 filter paper and the filtrate was used as enzyme solution. The substrate mixture comprised of 0.06 ml of ATP (0.05M) solution, 0.4 ml $CaCl_2$ (0.1M), and 2 ml glycine-NaOH buffer (0.2 M, pH 9.2). Thereafter, 0.4 ml of enzyme was added to reaction mixture and incubated for 5 min at 27 ºC. The reaction was stopped by adding 2 ml of 15 % TCA. The mixture was filtered through Whatman no. 1 filter paper, and the inorganic phosphorus content was determined by method of Taussky and Shorr (1952).For the analysis of phosphorus, 3 ml of the filtrate was taken to which 2 ml freshly prepared ferrous sulphate–ammonium molybdate solution (10%) was added. The intensity of the color developed was measured at 660 nm (Thermo Spectronic, Great Britain, UK). The liberated inorganic phosphorus was calculated using standard curve obtained by using potassium di-hydrogen phosphate as a standard. The ATPase enzyme activity was expressed as μmoles Pi /min./mg MFP.

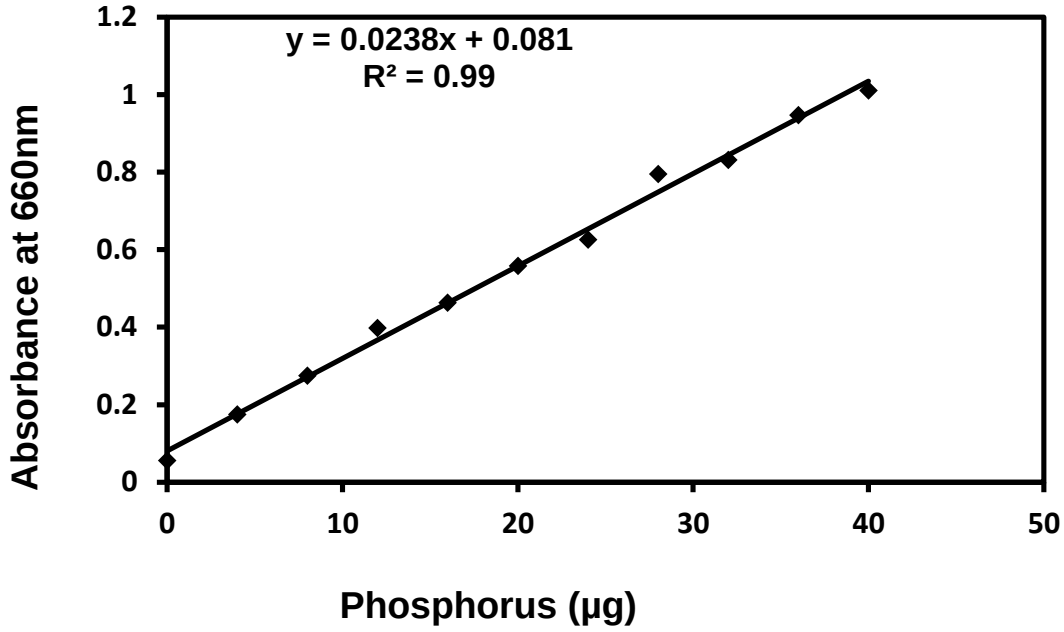


Figure 1. Standard curve of inorganic phosphorus for estimation of Ca^{2+} ATPase enzyme activity. The standard curve was obtained by using Potassium–di-hydrogen phosphate.

3.3.8 Determination of Surface hydrophobicity

In order to determine surface hydrophobicity, myofibrillar proteins (MFP) were extracted from the muscles of dhoma fish, Indian squid and white leg shrimp.

3.3.8.1 Extraction of MFP

Myofibrillar protein fraction was extracted according to the method of Hashimoto *et al.* (1979) with modifications. Meat sample (10g wet weight) was homogenized in 100 ml of ice-cold phosphate buffer, pH 7.5 (15.6 mM Na_2HPO_4, 3.5mM KH_2PO_4) using a homogenizer (Polytron Kinematica, Switzerland). The homogenization process was carried out at 10000 rpm for 1 min with 6 intermissions of 10sec each. The resulting homogenate was centrifuged at 5000 × g at 4°C for 15min using a CR21GIII refrigerated centrifuge (Hitachi, Japan). The supernatant was discarded, while the pellet was collected and re-suspended in 10 volumes of ice cold phosphate buffer, homogenized and centrifuged at 4°C again. After two repeated cycles of homogenization and centrifugation, the resulting pellet was suspended in 10 volumes of ice-cold 50mM phosphate buffer of pH 7.5 containing 1.1 M KCl (pH value was adjusted with stock acid or base when necessary). The mixture was homogenized and centrifuged at 5000 × g for 15 min at 4°C. After the centrifugation clear supernatant was used as myofibrillar protein (MFP). Extracted MFP content was estimated by biuret method (Robinson and Hodgen, 1940) using bovine serum albumin as standard.

3.3.8.2 Estimation of MFP

MFP was estimated by biuret method as described by Robinson and Hodgen (1940). Aliquots (0.5 ml) of MFP were dispensed in 10 ml capacity glass test tubes and 2 ml of biuret reagent was added all the tubes. The mixture was agitated by shaking to apparent homogeneity and incubated at room temperature for 30-45 min. The pre-calibrated spectrophotometer (Thermo Spectronic, Great Britain, UK) was used for measuring the absorbance at 540 nm. A blank was set in parallel with the samples and was prepared by taking distilled water instead of MFP solution and was incubated at room temperature

for 30-45 min under the same conditions described earlier. The blank was used to adjust (zero) the spectrophotometer before readings were recorded. MFP concentration values (mg/ml) were estimated using a standard curve earlier plotted from absorbance values of known concentrations using standard protein i.e. Bovine Serum Albumin (BSA) (Fig.2).

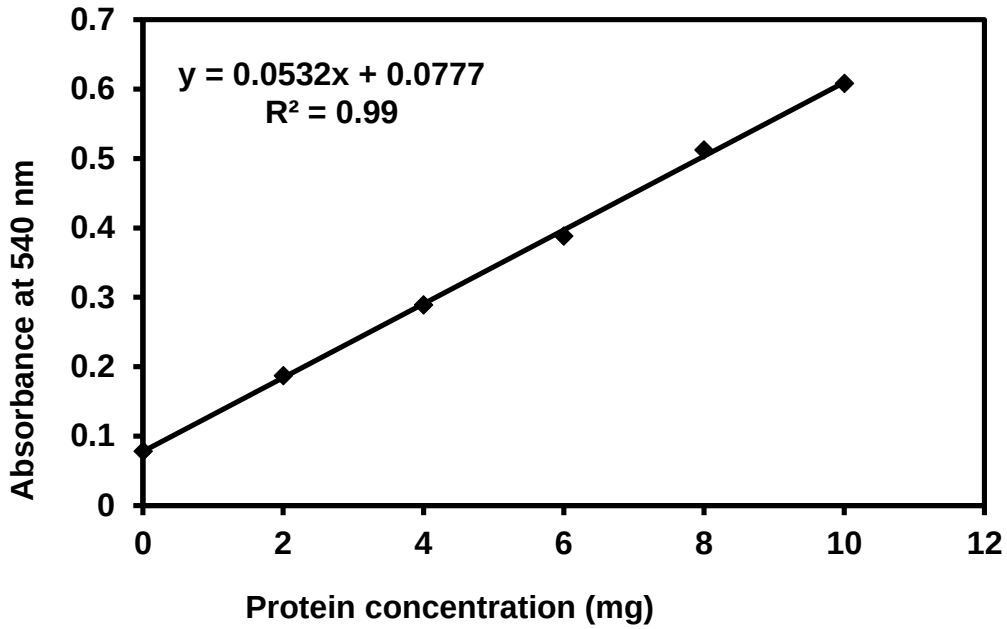


Figure 2. Standard curve for protein estimation using bovine serum albumin as standard by biuret method.

3.3.8.3 Estimation of surface hydrophobicity

Surface hydrophobicity of MFP extracted from muscles of dhoma fish, Indian squid and white leg shrimp was determined by the method described by Chelh *et al.* (2006). Hydrophobicity of MFP was determined using bromophenol blue (BPB) sodium salt of electrophoresis grade. One ml of MFP solution was taken and 200µl of BPB (concentration 1mg/ml) was added and mixed well. A control without MFP consisted of 200 µl of BPB added with 1 ml of 20mM phosphate buffer at pH 6 and mixed. Samples and control were kept under agitation on a shaker (Pelican equipment, ROTEX-LSV, Japan) at room temperature for 10 min and then centrifuged for 15 min at 2000 × g at the 4 °C. The absorbance of the supernatant was taken at 595nm against a blank of phosphate buffer. The amount of BPB bound protein is given by the formula:

BPB bound µg = 200 µg (A control - A sample)/A control

With A = absorbance at 595 nm

3.3.9 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) analysis

SDS-PAGE was carried out according to Laemmli (1970).The electrophoresis was performed using slabs of 16 ×18 cm (length × width). In order to prepare the sample for loading in PAGE instrument (Amersham Bioscience, USA), sample buffer (containing 10% SDS, 2% glycerol, 0.2% mercaptoethanol and 0.02% bromophenol blue) and MFP solutions were mixed in the ratio of 1:1 and boiled for 2 min, cooled and kept in the frozen condition. For each sample, the final MFP concentration in the vials was loaded in the range of 5-6mg/ml. Polyacrylamide concentrations of separating gel and stacking gel were 10% and 4% respectively. Electrophoresis was carried out with a constant current mode. The gels were stained for3h with 0.12% coomassie blue prepared in methanol. Thereafter, the gels were de-stained in a destaining solution (7.5% acetic acid and 5% methanol) until the bands appeared to be clear. A broad range molecular weight un-stained protein marker procured from Takara Bio.Inc.(Japan) was used to estimate molecular weight of the proteins.

3.3.10 Histological analysis

Histological studies of fresh and ice-stored dhoma fish, Indian squid and white leg shrimp were carried out as described by Ando *et al.* (2004) with a slight modification. The muscle samples were drawn from the dorsal side of the fish. Samples were prepared by cutting into cubes of approximately 4mm sides with a sharp razor blade. The samples were preserved in 10% neutral formalin buffer solution (100ml Formalin (37-40% stock solution) 900ml Water 4g/l NaH_2PO_4 monobasic) for 24 h at room temperature. After dehydrating the cubes by passing them for 30 min. each in increasing (10%) concentrations of (70-100 %, v/v) ethanol, fish samples were embedded in paraffin. Thin sections (2mm thick) were prepared by a microtome and stained with Mallory's trichrome staining solution (a mixture of 20g/l of orange G, 5 g/l of methyl blue and 20 g/l of oxalic acid). The micro-structure was visualized using a light microscope (Carl Zeiss, Jena, Germany) under high power objective.

3.4 Evaluation of functional properties

3.4.1 Solubility

The solubility of MFP pellet extracted from dhoma fish, Indian squid and white leg shrimp during ice storage was determined using phosphate buffer (15.6 mM Na_2HPO_4, 3.5 mM KH_2PO_4, containing 1.1 M potassium chloride, pH 7.5). The MFP pellet was homogenized in phosphate buffer at 9000 rpm for 2 min with the help of laboratory homogenizer and then centrifuged at 9000 ×g for 15min at 4ºC. The pellet homogenate before centrifugation and the supernatant obtained after the centrifugation were analysed for the amount of soluble protein (Robinson and Hodgen, 1940).The MFP solubility was calculated as follows

$$\text{Solubility} = \frac{\text{Soluble protein after centrifugation} \times 100}{\text{Soluble protein before centrifugation}}$$

3.4.2 Apparent reduced viscosity

The apparent reduced viscosity of MFP from dhoma fish, Indian squid and white leg shrimp during ice storage was determined by Ostwald viscometer. The apparent reduced viscosity was determined using Ostwald

viscometer with a capillary diameter of 0.5mm. The MFP prepared in chilled condition was equilibrated to 25ºC using water bath and 10-12 mlof MFP was loaded to viscometer.

The time taken for protein solution to travel between two points was recorded with the help of stop watch. The experiment was repeated three times and average flow time (in sec) was taken for calculation. The relative viscosity **($\eta_{rel} = \frac{t_1}{t_0}$)** was determined by noting the time taken for protein solution (t_1) and that for the solvent (t_0) separately. The relative viscosities at different protein concentrations were determined and reduced viscosity (η_{red}) was calculated using equation given by Yang (1961).

$$\eta_{red} = \frac{{}^{t_1}/_{t_0} - 1}{C(mg/ml)}$$

Where,

t_1= time taken for MFP solution in sec

t_0 = time taken for solvent (phosphate buffer) in sec

C= concentration of protein (mg/ml)

A plot of η_{red} viscosity *vs* MFP concentration was obtained and η_{red} viscosity at single MFP concentration (5mg /ml) was derived from the plot.

3.4.3 Water holding capacity (WHC)

The WHC for fresh and ice stored meat samples were measured according to the method as described by Jauregui *et al.* (1981) with modifications. Sample of 5g meat was weighed and put between two layers (4 pieces each) of filter paper (Whatman No. 1). Sample along with the filter paper was placed in 50 ml centrifuge tube and centrifuged at 5000 × g for 10 min at 4°C. Immediately after centrifugation, the sample was removed and reweighed. WHC was calculated as follows.

$$\text{WHC}\ (\%)\ = \frac{\text{W1} - \text{W2}}{\text{W1}} \times 100$$

Where, W1 is the initial weight (g) and W2 is the final weight. Average of three replicates was reported as value of WHC.

3.4.4 Emulsion capacity

Emulsion capacity (EC) of MFP was determined according to the method of Swift *et al.* (1961) with modifications. About 12.5g of MFP solution, 37.5 ml of chilled phosphate buffer and 50 ml of refined sunflower oil were added and homogenized at 9000 rpm for 10 sec using homogenizer (Polytron Kinematica, Switzerland) as dispersing tool. Homogenization was continued at high speed (23000 rpm) with addition of oil at the rate of 0.5-0.6 ml/sec until visual phase inversion was recorded. Emulsion capacity was calculated from total volume of oil added till phase inversion including the initial 50 ml and was expressed as ml of oil per mg of MFP. Average of three replicates was reported as emulsion capacity value.

$$\textbf{Emulsion capacity}\ (\textbf{ml/mg})\ = \frac{\textbf{Total oil consumed}}{\textbf{12.5} \times \boldsymbol{concentration\ of\ MFP}}$$

3.4.5 Assessment of gel forming ability and gel quality

3.4.5.1Preparation of heat-induced gel

Using pestle and mortar, about 300g of separated meat was macerated with 2.5 % NaCl in chilled condition (4-5 ºC) for 10min. To maintain temperature (< 5 °C), the meat was pre-chilled and the maceration was performed by putting mortar in ice bath. After maceration, about 100 g of viscous minced meat was stuffed into krehalon casing of 50 mm× 250 mm (dia × length) using a hand stuffer. One end of casing was sealed prior to stuffing and the other end of casing was sealed after stuffing. The stuffed casings were heat processed at 90 ºC for 45 min in a water bath and cooled in chilled water bath for 15 min. The prepared gels were kept at 4 ºC in refrigerator for 24 h and were used for the gel strength measurement.

3.4.5.2 Measurement of gel strength

The gel strength was measured using texture analyser (TA. XT2i Stable Micro System, Surrey, England). The prepared gels were brought to room temperature and cut into 25mm × 30mm (dia × length) and placed on the platform of texture analyser. A 5 mm spherical probe was used for measurement. A trigger force of 10 g and distance of 20 mm was programmed in the instrument. During the measurement spherical probe pierces the gel to a distance of 20 mm and the peak load exerted by the instrument was recorded. The gel strength was calculated by multiplying the peak load (g) and distance (mm) at which first breakage occurs. Gel strength of the sample was expressed in g.cm. The average of five replicates was reported as gel strength of sample.

3.4.5.3 Gel color measurement

Heat induced gels were prepared from dhoma fish, Indian squid and white leg shrimp as mentioned in the section 3.4.5.1 and color was measured with Lab scan XE-colorimeter (Hunter lab scan XE, USA). Measurements were recorded using the L*, a* and b* colour scale (CIE, 1986). The L* represents the degree of lightness of the sample, a* (redness/greenness) and b* (yellowness/blueness) were measured. Before making the measurements for the samples, the instrument was calibrated with white and black standards.

3.4.5.4 Texture Profile Analysis (TPA)

The TPA was performed for the heat induced gel prepared from dhoma fish using Texture Analyser (TA, Surry, England) mentioned above using a 75 mm compression plate with 50 Kg load cell. The gels were cut into 30 mm size and subjected for analysis. The texture parameters measured include hardness, cohesiveness, springiness, gumminess and chewiness.

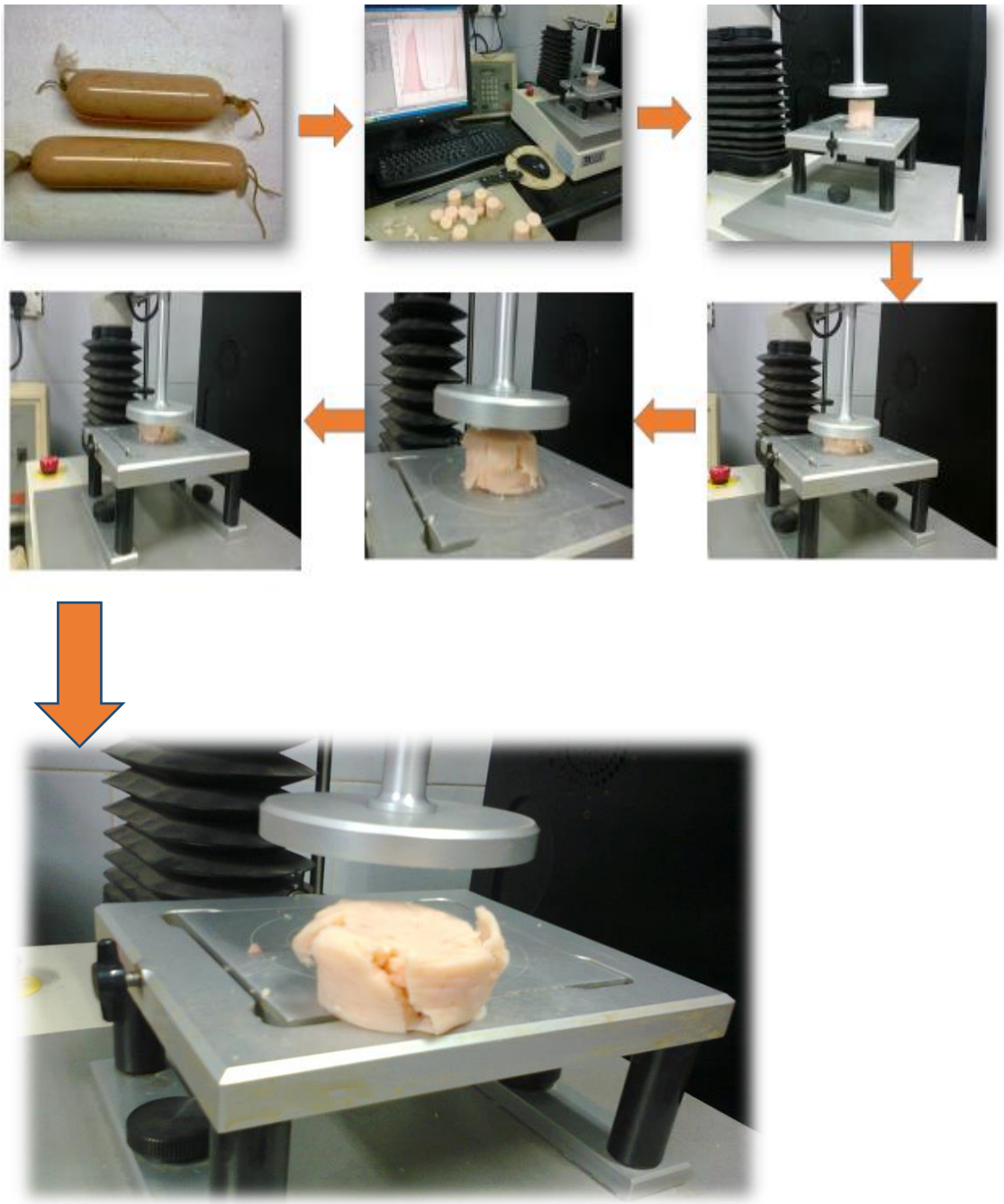


Plate 4. Texture measurement of the gel.

Plate 4. Texture measurement of the gel

3.5 Analysis of rheological properties

3.5.1 Sample preparation

Fresh and ice stored meat obtained from dhoma fish, Indian squid and white leg shrimp was used for rheological measurements. 4g homogeneous meat devoid of skin, scales and bones was taken for analysis. The meat was macerated well using pestle and mortar manually. To maintain temperature (< 5 °C), maceration was performed by putting mortar in ice bath. After making homogeneous paste, 2.5% sodium chloride was added and maceration was continued for another 5min. The total time of maceration was 10 min.

3.5.2 Dynamic rheological properties

Dynamic rheological properties were studied using a Physica MCR 101 rheometer (Anton Paar, Germany) in oscillatory mode. A 20 mm parallel steel probe geometry with a 1 mm gap (between peltier plate and probe) was used and the sample was surrounded by a liquid paraffin to prevent drying. The sample was heated at a rate of 1°C/min from 5 to 90°C. The oscillation stress was 0.6 Pa, and the oscillation frequency was 0.1Hz. The storage modulus (G') and the loss modulus (G") and the damping factor (tan δ) were recorded.

3.6 Statistical analysis

The data obtained were analysed by one way analysis of variance (ANOVA) using SPSS 16.0 software. The ANOVA was carried out for number of days of ice storage with all the parameter. Data were also subjected to determine Karl Pearson correlation coefficient between different parameters.

Plate 5. Rheometer Physica MCR 101 rheometer (Anton Paar, Germany).

4. RESULTS

Experimental results of physico-chemical, functional and rheological properties of meat proteins obtained from dhoma fish, Indian squid and white leg shrimp during ice storage are presented in this section.

4.1 Proximate composition of dhoma fish, Indian squid and white leg shrimp in fresh condition

The proximate compositions of all the three experimental species are given in Table 1. Moisture content of Indian squid found to be 84.58 % and that of dhoma fish and white leg shrimp were 81.42 % and 73.45 % respectively. The crude protein contents of white leg shrimp, dhoma fish and Indian squid were 19.90 %, 15.38% and 14.17% respectively. The fat contents of the three species were found to be less than 2% while ash content was varied from 0.87 -1.56 %.

Table 1. Proximate composition of fresh minced meat of dhoma fish, Indian squid and white leg shrimp.

Proximate composition	Dhoma fish	Indian squid	White leg shrimp
Moisture	81.42 ±0.70	84.58 ±0.74	73.45 ±0.29
Protein	15.38 ±0.21	14.17 ±0.63	19.90 ±0.57
Fat	1.28 ±0.05	0.72 ±0.40	1.82 ±0.28
Ash	1.01 ±0.11	0.87 ±0.10	1.56 ±0.21

Values are means ± SD, n = 3

4.2 Yield of meat from dhoma fish, Indian squid and white leg shrimp

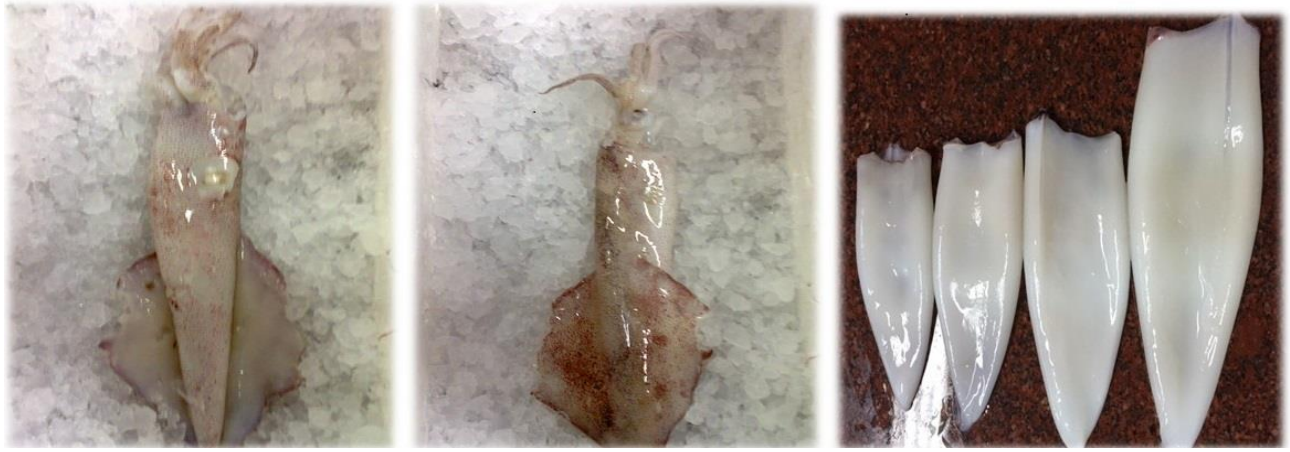
The yields of mince, edible portions without head and with head from the three test species examined are summarised in the Table 2. The yield mince from dhoma fish was 37.41%, that of only edible portion of without head and with head were 57.99 % and 75.49% respectively. The yields from Indian squid were 30.46 % (mantle without fin and tentacles) and 39.59% (finned mantle without tentacle). The weight percentage of the whole edible parts was 64.35 % (Tentacles+fin+mantle) of the total weight of Indian squid. The yields of edible parts from white leg shrimp when peeled and deveined was 52.93% and when headless shell on condition was 63.64 %.

Table 2.Yield of edible portion from dhoma fish, Indian squid andwhite leg shrimp.

Yield of dhoma fish		
Mincemeat (%)	Edible portion without head (%)	Edible portion with head (%)
37.41±2.41	57.99±3.04	75.49±2.45
Yield of Indian squid		
Mantle without fin (%)	Mantle+ fin (%)	Tentacles +fin+mantle (%)
30.46±2.94	39.59±3.73	64.35±2.76
Yield of white leg shrimp		
Peeled and deveined (P&D) (%)		Head less and shell on (%)
52.93±1.26		63.64±5.95

Values are mean ± SD, n=10

Dhoma fish



Indian squid

White leg shrimp

Plate 6. Experimental species at different stages of yield determination.

4.3 Fatty acid profile of dhoma fish, Indian squid and white leg shrimp

The fatty acid profiles of all the three experimental species are depicted in Table 3 and presented in Fig. 3. The principal saturated fatty acid (SFA) was palmitic acid (C16) in the fats extracted from three experimental species in fresh condition. The proportional contents of palmitic acid in fats extracted from fresh dhoma fish, Indian squid and white leg shrimp were 49.09 %, 51.98 % and 50.15 % respectively. The myristic acid (C14) was also found to be present in the considerable proportion in Indian squid (6.41 %) while in the case of white leg shrimp and dhoma fish the content was lesser (< 3 %).The proportions of monounsaturated fatty acid (MUFA) were 24.78 %, 16.5 % and 10.23 % in white leg shrimp, Indian squid and dhoma fish respectively. The percentage of the oleic acid (C18:1, n-9) in the fat extracted from white leg shrimp, Indian squid and dhoma fish were 23.80 %, 13.98 % and 10.23 % respectively.

The contributions of polyunsaturated fatty acid (PUFA) found to be in the range of 14.55 % -19.71 % in all the three experimental species. The total percentage contribution of n-3 fatty acids was slightly higher in dhoma fish (14.91 %) as compared to Indian squid (13.59 %) and white leg shrimp (13.02 %). The total n-6 fatty acids were near 5 % in dhoma fish and Indian squid while found less 2 % in white leg shrimp. The n-3/n-6 ratio was considerably higher for white leg shrimp (8.51) as compared to two other experimental species.

Table 3. Fatty acid profiles of dhoma fish, Indian squid and white leg shrimp.

Fatty acid	Dhoma fish	Indian squid	White leg shrimp
C13	6.16	-	-
C14	2.30	6.41	2.77
C15	0.34	1.18	1.79
C16	49.09	51.98	50.15
C17	6.95	1.78	-
C18	4.17	2.76	5.97
C19	-	0.27	-
C22	1.06	0.61	-
SFA	**70.07**	**64.99**	**60.68**
C18:1(n-9)	10.23	13.98	23.80
C18:2(n-9)	-	-	0.98
C20:1(n-9)	-	2.27	-
C22:1(n-9)	-	0.25	-
MUFA	**10.23**	**16.5**	**24.78**
C20:4(n-6)AA	4.80	4.92	1.53
C20:5(n-3)EPA	3.55	6.80	9.96
C22:6(n-3)DHA	11.36	6.79	3.06
PUFA	**19.71**	**18.51**	**14.55**
Σn-3	**14.91**	**13.59**	**13.02**
Σn-6	**4.80**	**4.92**	**1.53**
n-3/ n-6	**3.11**	**2.76**	**8.51**

SFA**:**saturated fatty acid, MUFA**:**monounsaturated fatty acid, PUFA**:**polyunsaturated fatty acid, AA = arachidonic acid, EPA**:**eicosapentaenoic acid, DHA**:**docasahexaenoic acid

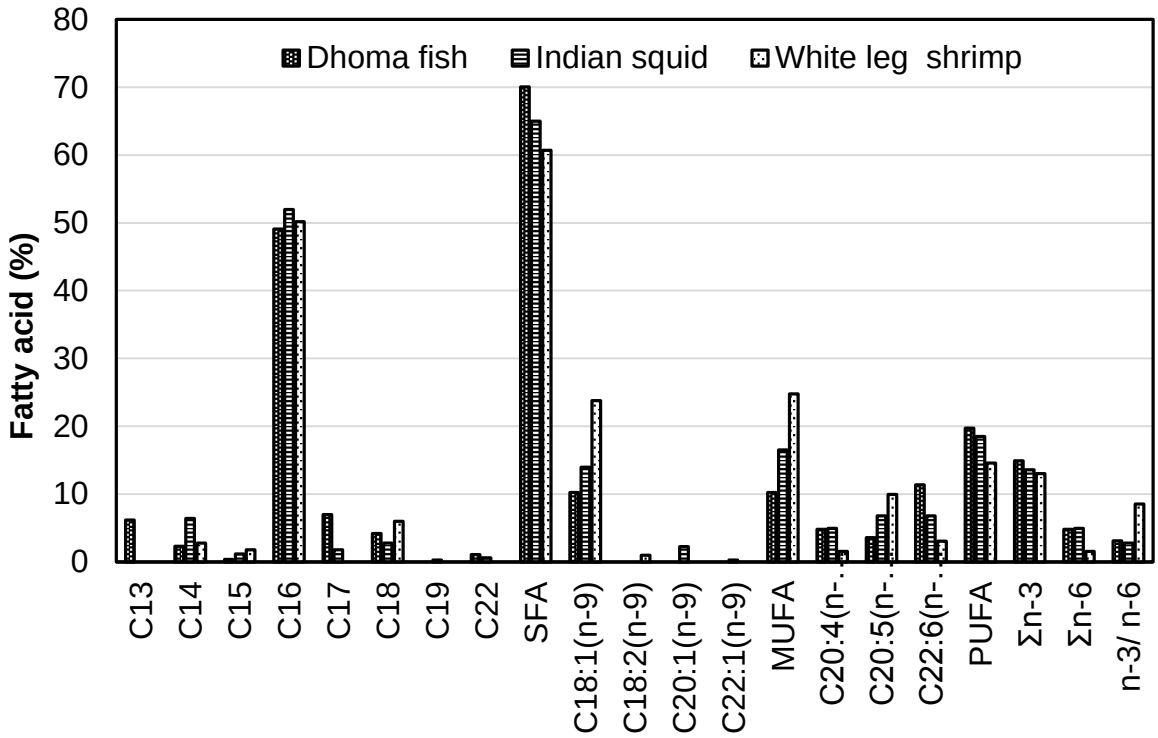


Figure 3. Fatty acid profiles of the fresh meat of dhoma fish, Indian squid and white leg shrimp.

4.4 Effect of ice storage on physico-chemical properties

In this section, the results of analyses of the effect of ice storage on the physico-chemical properties *viz.* pH, TVBN, TMA, NPN, Ca^{2+} ATPase activity, surface hydrophobicity of the meats of the three experimental species are given. The SDS-PAGE profile of the MFP and histological observations of muscles of the species during ice storage are also given.

pH

Changes in pH of meat from dhoma fish, Indian squid and white leg shrimp as a function of ice storage period is given in Table 4 and Fig. 4 A, B and C. pH values of the meat obtained from dhoma fish, Indian squid and white leg were 7.29, 6.73 and 6.62 respectively. In the case of dhoma fish, pH value changed significantly and reached to 7.58 at the end of 18 days of ice storage period. The pH of meat obtained from white leg shrimp and Indian squid crossed pH 7 on 4th and 8th day of ice storage and further the values increased to 7.62 and 7.37 respectively at the end of 14 days of storage period.

Table 4. Changes in pH of meat from dhoma fish, Indian squid and white leg shrimp during ice storage.

Ice storage (Days)	pH		
	Dhoma fish	Indian squid	White leg shrimp
0	7.29 ±0.05^{b}	6.73 ±0.03^{a}	6.62 ±0.03^{a}
2	-	6.82 ±0.02^{b}	6.79 ±0.05^{b}
3	7.24 ±0.03^{a}	-	
4	-	6.95 ±0.01^{c}	7.09 ±0.12^{c}
6	7.39 ±0.02^{c}	6.96 ±0.06^{c}	7.31 ±0.04^{d}
8	-	7.06 ±0.03^{d}	7.38 ±0.05^{d}
9	7.48 ±0.03^{d}	-	
10	-	7.30 ±0.01^{f}	7.50 ±0.06^{e}
12	7.51 ±0.02^{d,e}	7.19 ±0.05^{e}	7.53 ±0.06^{e,f}
14	-	7.37 ±0.02^{g}	7.62 ±0.03^{f}
15	7.55 ±0.02^{e,f}	-	-
18	7.58 ±0.02^{f}	-	-

Values are mean± SD, n=3, p< 0.05.
Value in the same column bearing unlike letters differ significantly.

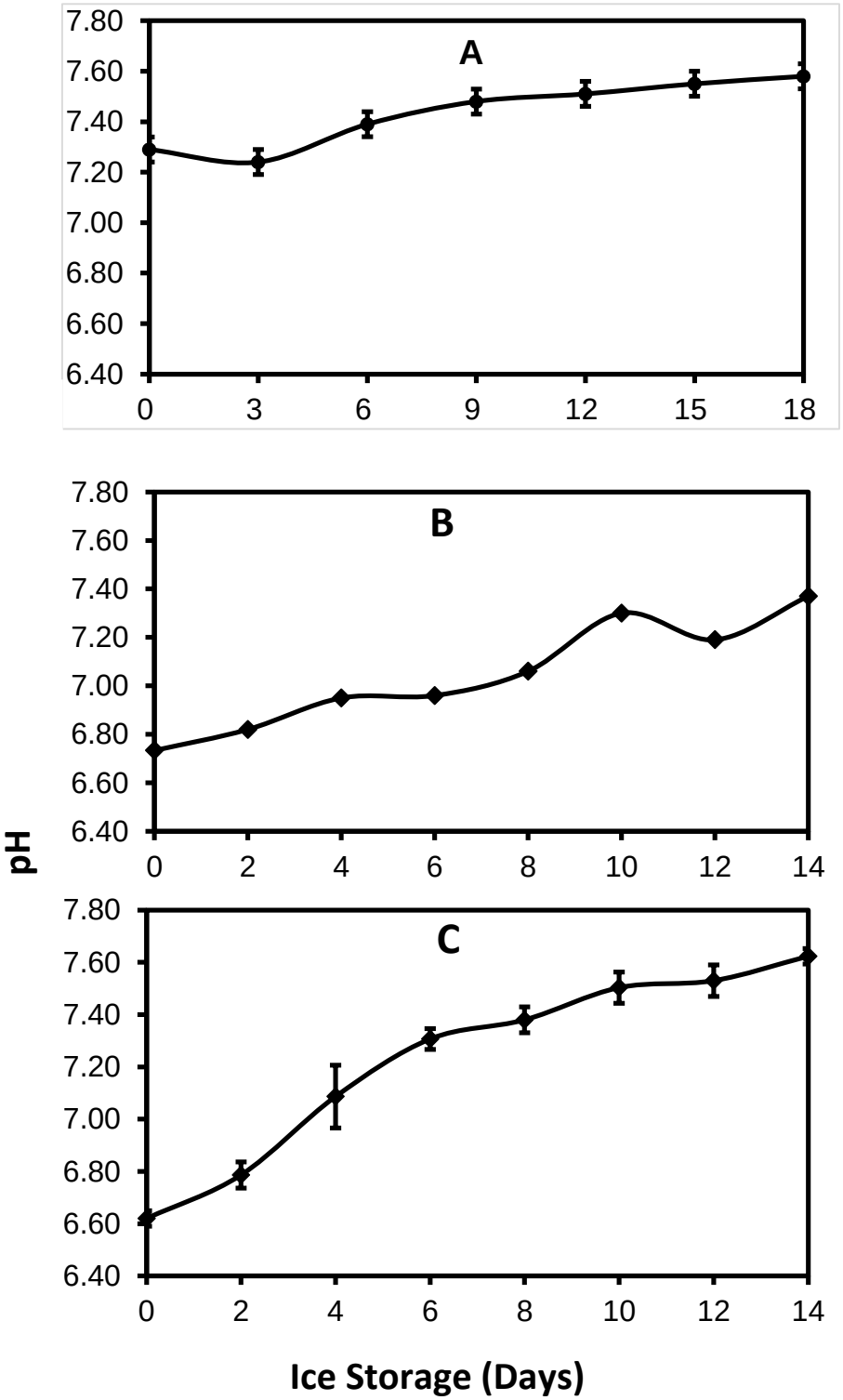


Figure 4. Changes in pH of meat obtained from A: dhoma fish B: Indian squid and C: white leg shrimp during ice storage.

TVBN

The TVBN values of the ice stored samples from experimental species at different periods are depicted in Table 5 and Fig. 5 A, B and C. Initially, TVBN values were 3.22, 4.20 and 4.67 mg N/100g meat for Indian squid, white leg shrimp and dhoma fish respectively. Dhoma fish samples showed an increase in TVBN value at the end of 9 days storage and after a small decline, the values again increased till the end of storage. At the end of ice storage the TVBN values for dhoma fish, Indian squid and white leg shrimp were 65.33, 74.60 and 56.00 mg N/100 g meat.

Table 5. Changes in total volatile base nitrogen (TVBN) content of meat from dhoma fish, Indian squid and white leg shrimp during ice storage.

Ice storage (Days)	TVBN (mg N /100 g meat)		
	Dhoma fish	Indian squid	White leg shrimp
0	4.67 ±0.82^{a}	3.22 ±1.61^{a}	4.20 ±0.00^{a}
2	-	4.83 ±0.00^{a}	4.67 ±0.81^{a}
3	9.92 ±0.82^{a}	-	-
4	-	6.98 ±0.93^{a}	11.67 ±1.62^{b}
6	26.83 ±1.65^{b}	14.49 ±1.61^{b}	12.13 ±1.62^{b}
8	-	25.76 ±3.22^{c}	22.40 ± 2.42^{c}
9	42.00 ±5.72^{c,d}	-	-
10	-	40.79 ±2.46^{d}	43.47 ± 1.27^{d}
12	37.33 ±3.30^{c}	66.55 ±5.65^{e}	46.67 ± 4.28^{d}
14	-	74.60 ±3.35^{f}	56.00 ±2.80^{e}
15	51.33 ±8.73^{d}	-	-
18	65.33 ±3.30^{e}	-	-

Values are mean ± SD, n=3, $p< 0.05$.
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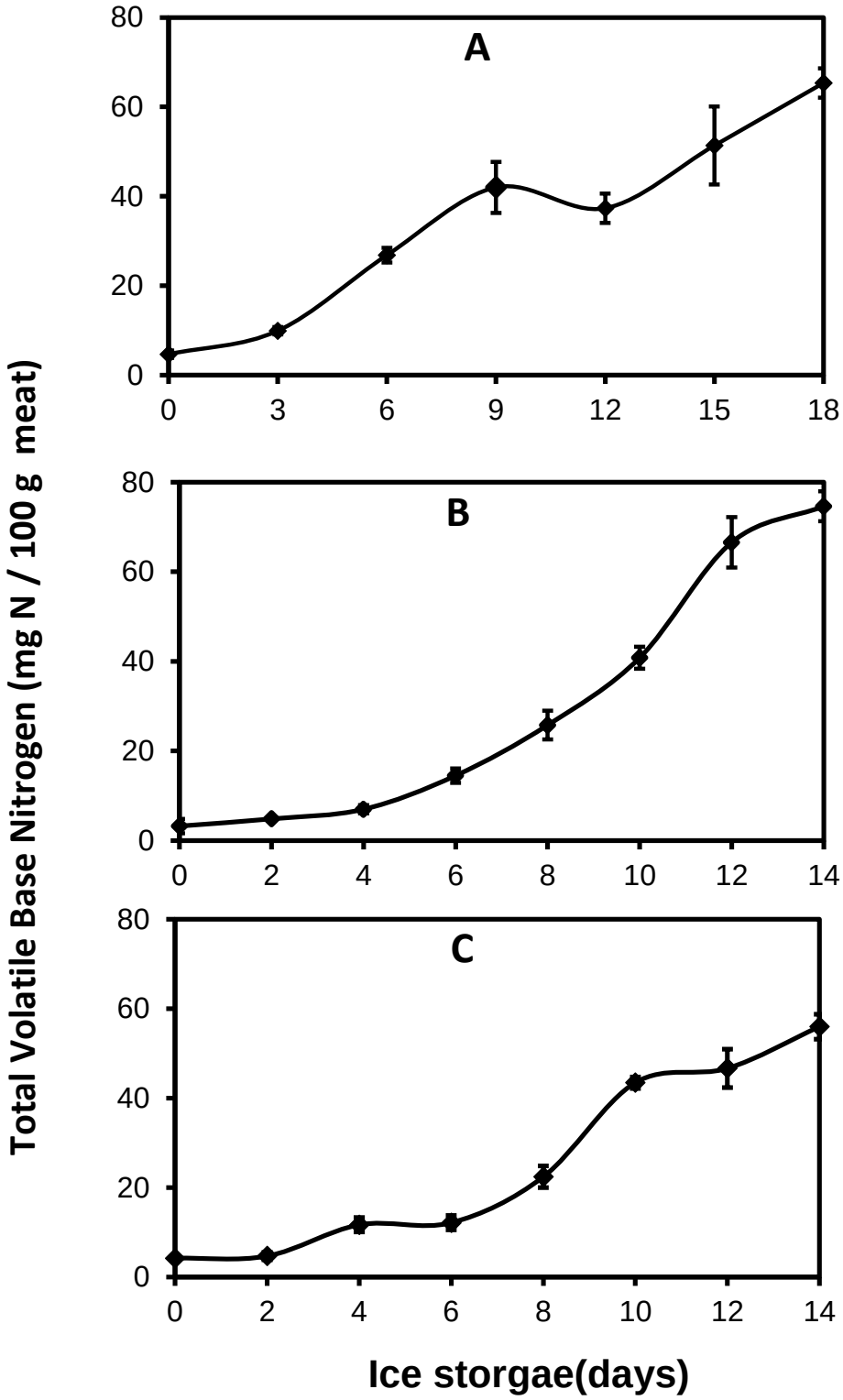

Figure 5. Changes in total volatile base nitrogen (TVBN) content of meat of A: dhoma fish B: Indian squid and C: white leg shrimp during ice storage.

Trimethylamine(TMA)

TheTMA values of samples from experimental aquatic species at different periods of ice storage are presented in Table 6 and Fig. 6 A, B and C. TMA values for the fresh samples of Indian squid, white leg shrimp and dhoma fish were 1.61, 2.80 and 3.50 mg N/ 100 g meat respectively. Dhoma fish samples showed an increase in TMA value till 9th day followed by a decline and further increase after 12 days in ice storage the value progressed. The values recorded to be increased in all the case during ice storage period.

Table 6.Changes in tri-methyl amine (TMA) content in meat of dhoma fish, Indian squid and white leg shrimp during ice storage.

Ice storage period (Days)	TMA (mg N /100 g meat)		
	Dhoma fish	Indian squid	White leg shrimp
0	3.50 ±0.00^{a}	1.61 ±0.00^{a}	2.80±0.00^{a}
2	-	2.68 ±0.93^{a}	3.27±0.81^{a}
3	8.17 ±1.65^{a,b}	-	-
4	-	5.37 ±0.93^{a,b}	7.00±1.40^{b}
6	14.00 ±4.95^{b,c}	9.12 ±0.93^{b}	9.33±1.62^{c}
8	-	13.95±1.86^{c}	10.73±0.81^{c}
9	32.67 ±8.73^{d}	-	-
10	-	22.54±3.22^{d}	28.93±1.62^{d}
12	23.33 ±3.30^{c,d}	40.79±3.35^{e}	29.87±1.62^{d}
14	-	41.86±3.22^{e}	40.13±1.62^{e}
15	44.33 ±3.30^{d,e}	-	-
18	51.33 ±3.30^{e}	-	-

Values are means ± SD, n=3, $p< 0.05$.
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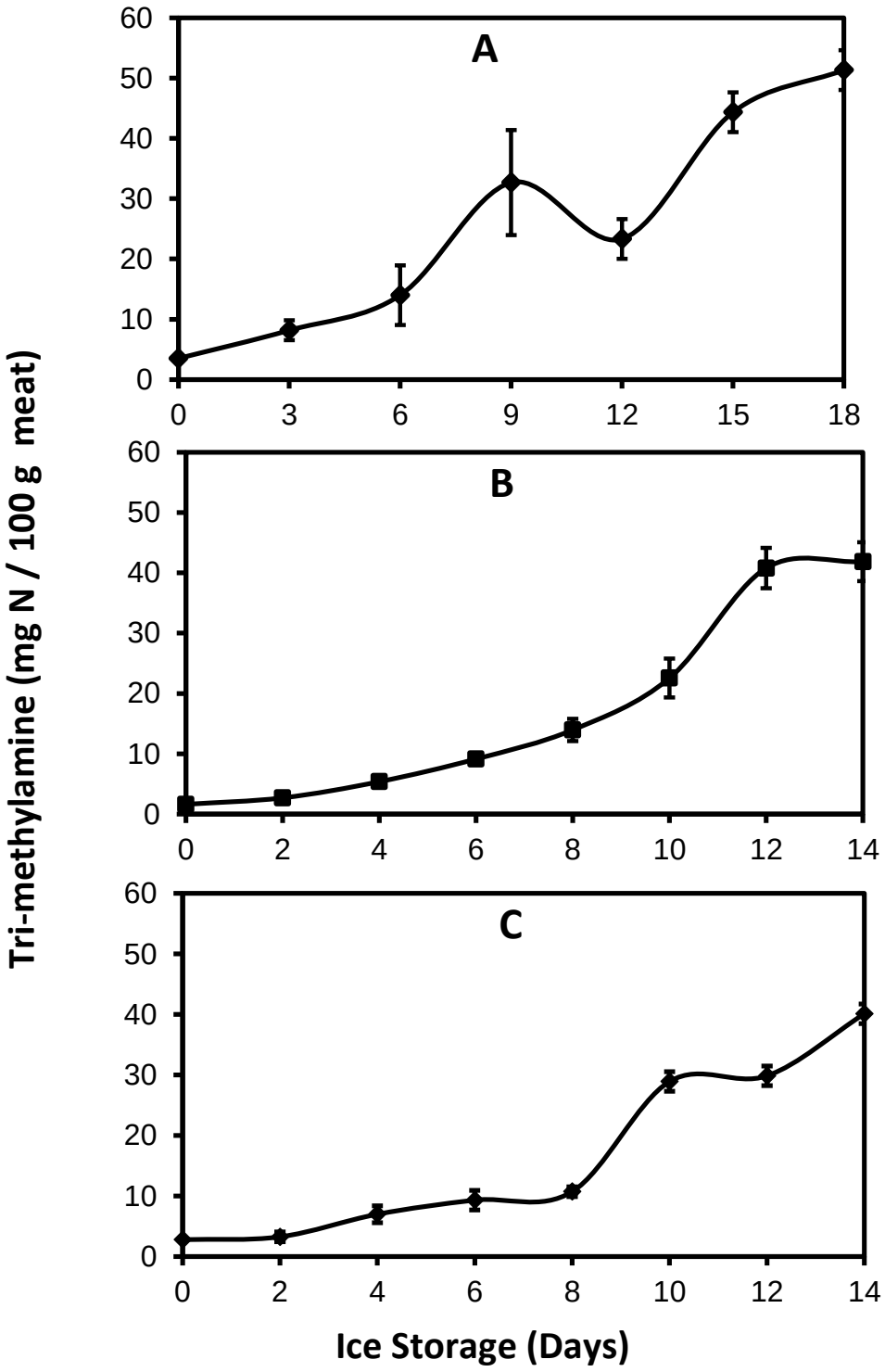

Figure 6. Changes in trimethylamine (TMA) content of meat of A: dhoma fishB: Indian squid and C: white leg shrimp during ice storage.

NPN

The effect of ice storage on NPN content of meat obtained from dhoma fish, Indian squid and white leg shrimp is given in Table 7 and Fig. 7 A, B and C. The NPN values of dhoma fish, Indian squid and white leg shrimp were 641, 309.66 and 297.67 mg N/100 g meat respectively in the fresh condition. In case of dhoma fish and Indian squid the values decreased almost linearly up to end of the ice storage study while in case of white leg shrimp the value showed two peaks on on 4th and 8th day of storage and further decreased to 186.67 mg N/100 g on 14th day of ice storage.

Table 7. Changes in non-protein nitrogen (NPN) content in meat of dhoma fish, Indian squid and white leg shrimp during ice storage.

Ice storage (Days)	NPN (mg N /100 g meat)		
	Dhoma fish	Indian squid	White leg shrimp
0	641.00 ±58.00^{a}	309.66 ±10.97^{a}	297.67 ±17.50^{c}
2	-	237.33 ±4.51^{b}	227.67 ±30.60^{a,b}
3	439.67 ±35.67^{b}	-	
4	-	170.33 ±2.31^{c}	440.42 ±5.06^{a}
6	401.00 ±48.88^{b,c}	151.00 ±10.54^{d}	221.67 ±20.21^{d,e}
8	-	101.67 ±2.89^{e}	367.50 ±17.50^{b}
9	349.33±58.50^{b,c,d}	-	
10	-	87.67 ±8.62^{f}	271.25 ±8.75^{c,d}
12	310.33 ±33.49^{c,d}	52.00 ±6.93^{g}	239.17 ±20.21^{c,d,e}
14	-	38.67 ±2.31^{h}	186.67 ±20.21^{e}
15	252.33 ±33.49^{d,e}	-	-
18	194.33 ±33.49^{e}	-	-

Values are means ± SD, n=3, p< 0.05.
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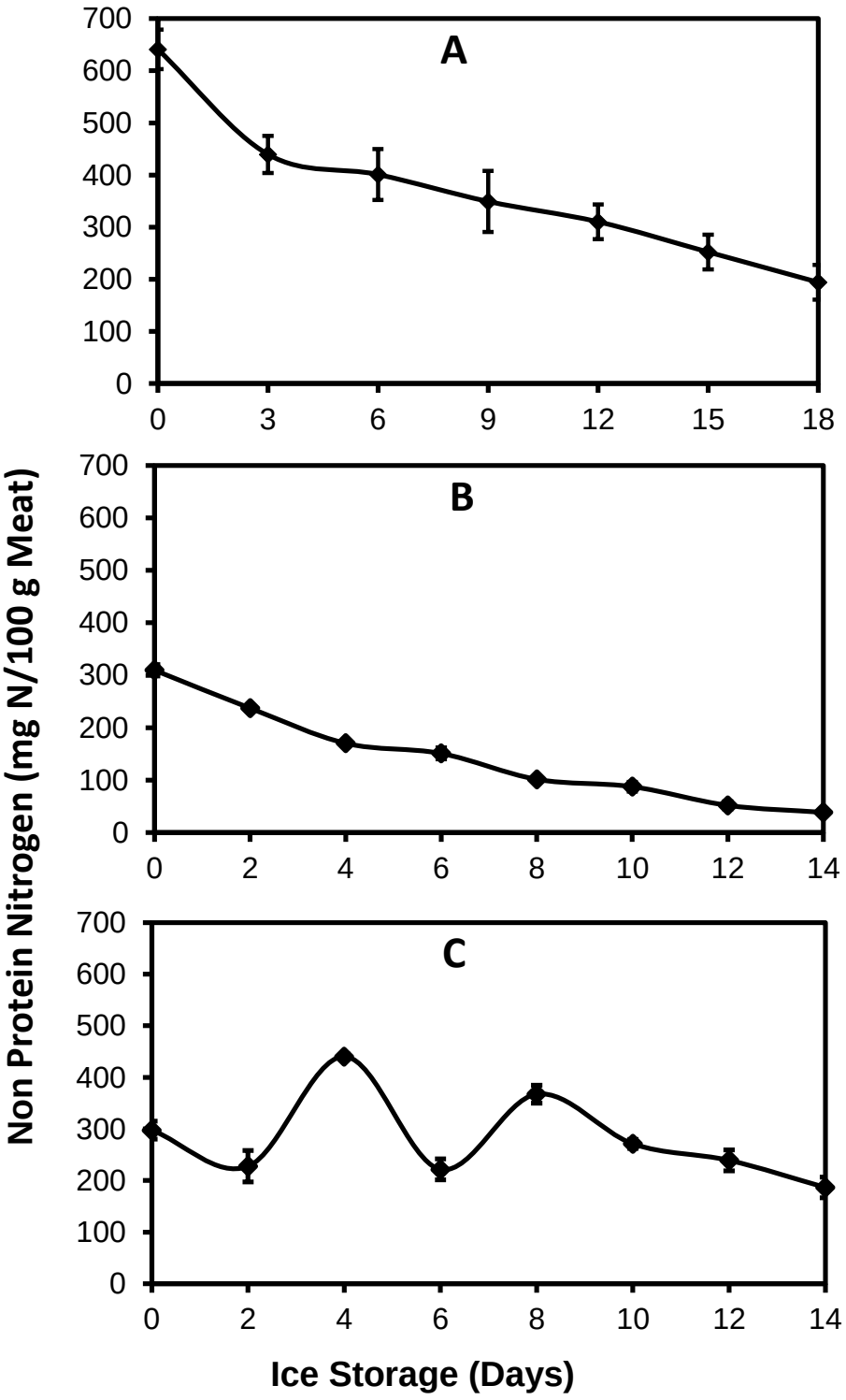


Figure 7.Changes in non-protein nitrogen of meat obtained from A: dhoma fish, B: Indian squid and C: White leg shrimp during ice storage.

Ca^{2+} ATPase enzyme activity

TheCa^{2+} ATPase enzyme activity values of the samples from the experimental species at different periods of ice storage are presented in Table 8 and Fig. 8 A, B and C. In the fresh condition, the activities observed were 0.138, 0.118 and 0.114 µmoles Pi/min./mg protein for Indian squid, white leg shrimp and dhoma fish respectively. In case of dhoma fish and white leg shrimp, activity sharply reduced till 6th day, while a steep decrement was also observed for Indian squid between 4th to 6th day of ice storage. However, in case of white leg shrimp activity could not be detected on 14th day of ice storage.

Table 8. Changes in Ca^{2+} ATPase enzyme activity of MFP obtained from dhoma fish, Indian squid and white leg shrimp during ice storage.

Ice storage (Days)	Ca^{2+} ATPase enzyme activity (µmoles Pi/min./mg MFP)		
	Dhoma fish	Indian squid	White leg shrimp
0	0.114 ±0.01^{a}	0.138 ±0.02^{a}	0.118 ±0.02^{a}
2	-	0.131 ±0.01^{b}	0.088 ±0.01^{b}
3	0.075 ± 0.01^{b}	-	
4	-	0.129 ±0.01^{b}	0.045 ±0.01^{c}
6	0.035 ± 0.01^{c}	0.058 ±0.01^{c}	0.025 ±0.01^{c,d}
8	-	0.046 ±0.02^{d}	0.019 ±0.01^{d,e}
9	0.023 ± 0.00^{d}	-	
10	-	0.044 ±0.00^{e}	0.013 ±0.00^{e}
12	0.020 ± 0.00^{e}	0.041 ±0.02^{e}	0.011 ±0.00^{e}
14	-	0.034 ±0.00^{f}	ND
15	0.010 ± 0.00^{f}	-	
18	0.011 ± 0.00^{f}	-	

Values are means ± SD, n=3, p< 0.05.
Value in the same column bearing unlike letters differ significantly.
ND-Not Detected

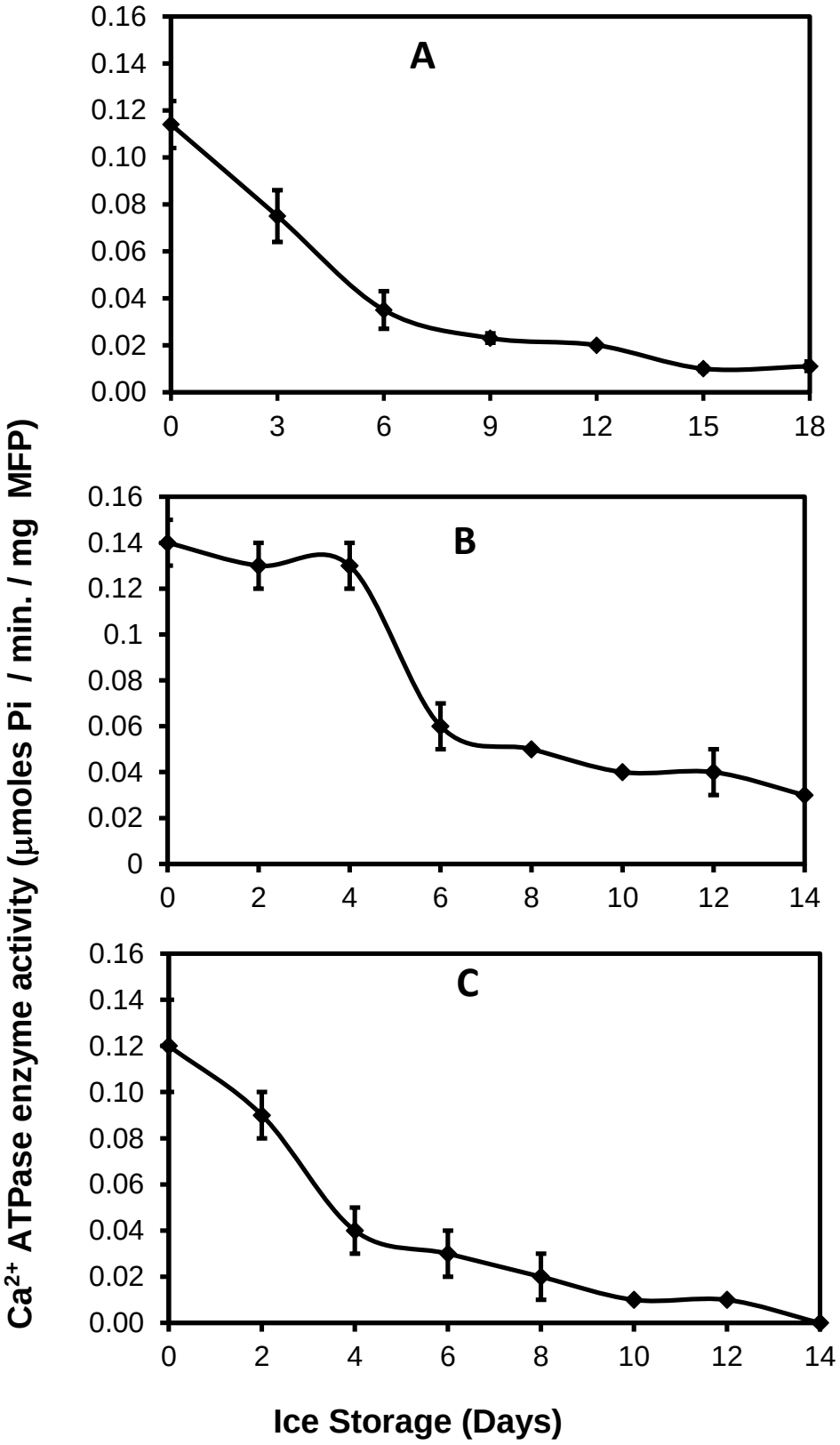


Figure 8. Changes in Ca^{2+} ATPase enzyme activity of MFP obtained from A:dhoma fish B: Indian squid and C: white leg shrimp during ice storage.

Surface hydrophobicity

Changes in surface hydrophobicity of myofibrillar proteins extracted (as described in section 3.3.8.1) from meat obtained from dhoma fish, Indian squid and white leg shrimp as a function of ice storage period is given in Table 9 and Fig. 9 A, B and C. The surface hydrophobicity of myofibrillar protein extracted from Indian squid and white leg shrimp in fresh condition were 14.93µg and 12.65µg respectively, while the value recorded for dhoma fish was 18.98 µg on 0^{th} day of the storage. Surface hydrophobicity did not show significant changes up to 6^{th} day in case of dhoma fish and white leg shrimp while value shot up significantly on 2^{nd} day in case of Indian squid, remained constant for two sampling days and steadily increased till last day when the value recorded was 75.81 µg. Overall an increment was recorded in the values of surface hydrophobicity as ice storage progressed.

Table 9.Changes in surface hydrophobicity of MFP obtained from dhoma fish, Indian squid and white leg shrimp during ice storage.

Ice storage (Days)	Surface hydrophobicity (µg)		
	Dhoma fish	Indian squid	White leg shrimp
0	18.98 ±1.96^{a}	14.93 ±1.26^{a}	12.65±2.93^{a}
2	-	22.46 ±3.53^{b}	13.28±2.76^{a,b}
3	22.53 ±0.87^{a}	-	-
4	-	23.12 ±2.33^{b}	16.70±1.48^{a,b}
6	21.98 ±7.37^{a}	24.32 ±1.07^{b}	18.74±4.87^{b}
8	-	34.68 ±2.57^{c}	18.76±2.26^{b}
9	32.44 ±8.59^{a,b}	-	
10	-	38.91 ±2.04^{d}	26.18±2.15^{c}
12	55.45 ±6.23^{b,c}	57.44 ±3.36^{e}	35.41±4.29^{d}
14	-	75.81 ±0.96^{f}	45.39±3.34^{e}
15	64.44 ±10.43^{c}	-	-
18	76.03 ±13.05^{c}	-	-

Value are means ± SD, n=3, $p< 0.05$.
Value in the same column bearing unlike letters differ significantly.

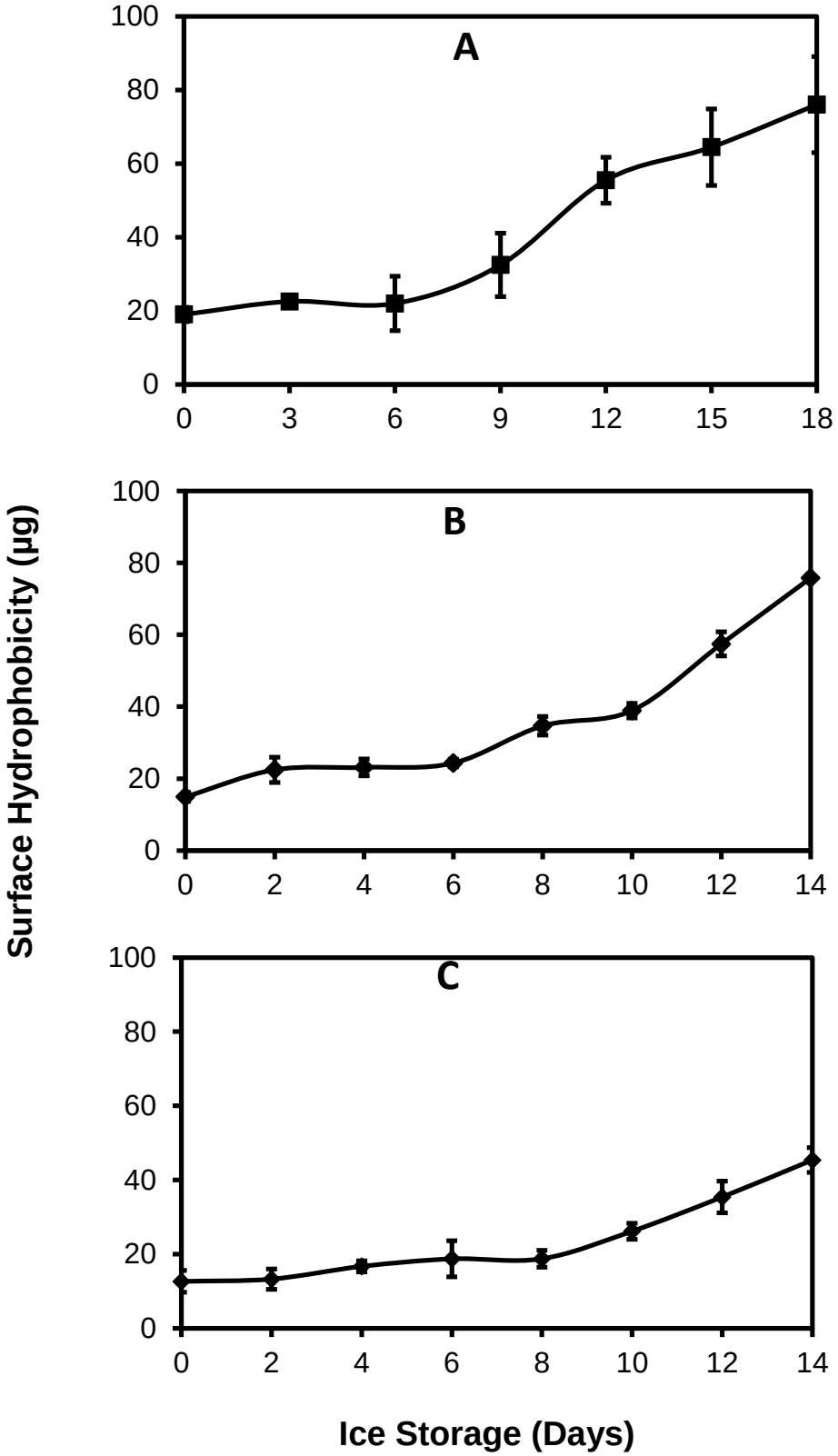


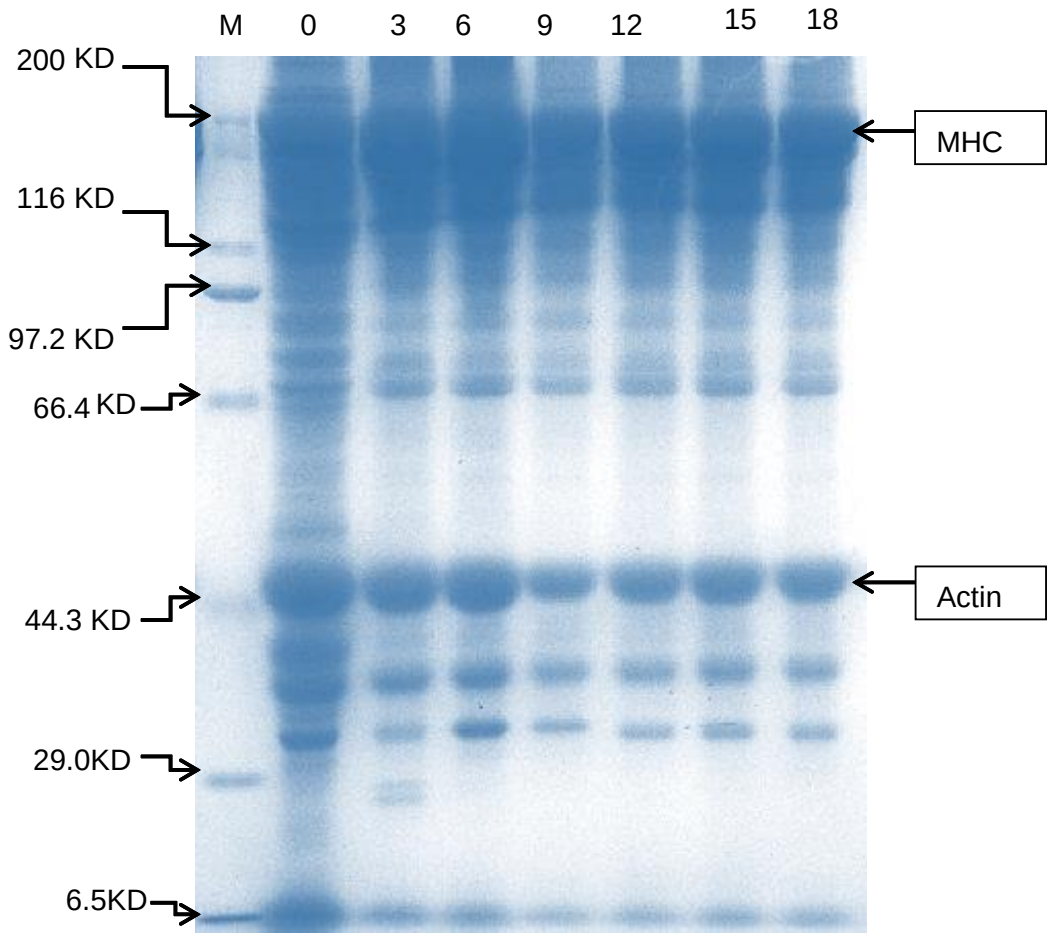
Figure 9. Changes in surface hydrophobicity of MFP obtained from A: dhoma fishB: Indian squid and C: white leg shrimpduring ice storage.

SDS-PAGE

Changes in SDS-PAGE pattern of myofibrillar proteins extracted (as described in section 3.3.7.1) from meat of dhoma fish, Indian squid and white leg shrimp during ice storage period are given in Fig. 10, 11 and 12 respectively . In case of dhoma fish and white leg shrimp, major sub fragments of myofibrillar proteins were myosin heavy chain (MHC) and actin, while in case of Indian squid in addition to aforesaid fragments paramyosin was also observed. In case of dhoma fish MFP SDS-PAGE pattern, a small variation in the intensity of the myosin and actin can be observed on 2^{nd} day of storage while on 9^{th} day, the band thickness decreased notably which further recovered with progression of ice storage period.

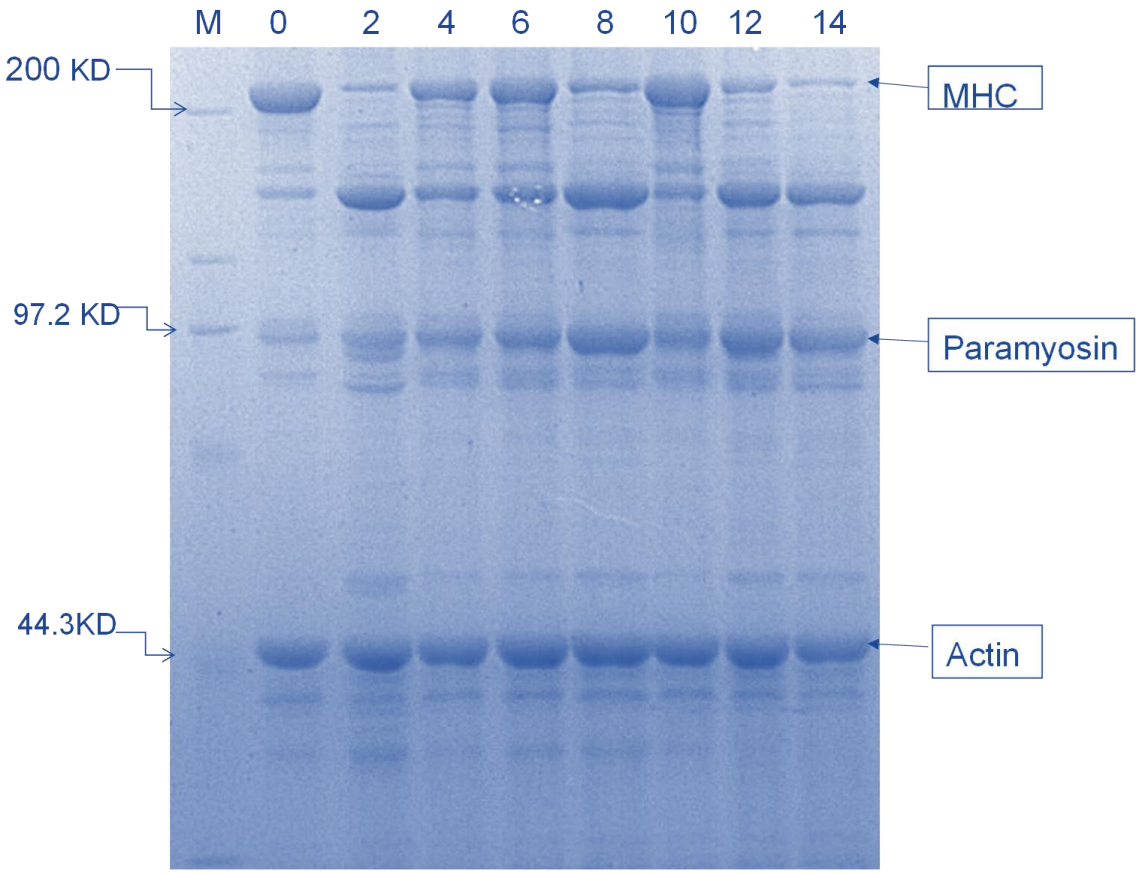
The SDS-PAGE pattern of MFP obtained from Indian squid showed an interesting phenomenon during different period of ice storage as evident in the Fig. 11. The MHC band intensity and thickness both reduced remarkably on 2^{nd} day. Though the intensity recovered in the subsequent sampling, at the end of 14^{th} day the intensity reduced considerably. The band of actin did not show any variation in intensity during 14 days of ice storage.

The SDS-PAGE pattern of MFP extracted from white leg shrimp showed a small and relatively insignificant reduction in intensity of the myosin band on 2^{nd} and 4^{th} day. Actin band recorded no change over the ice storage period (Fig. 12).



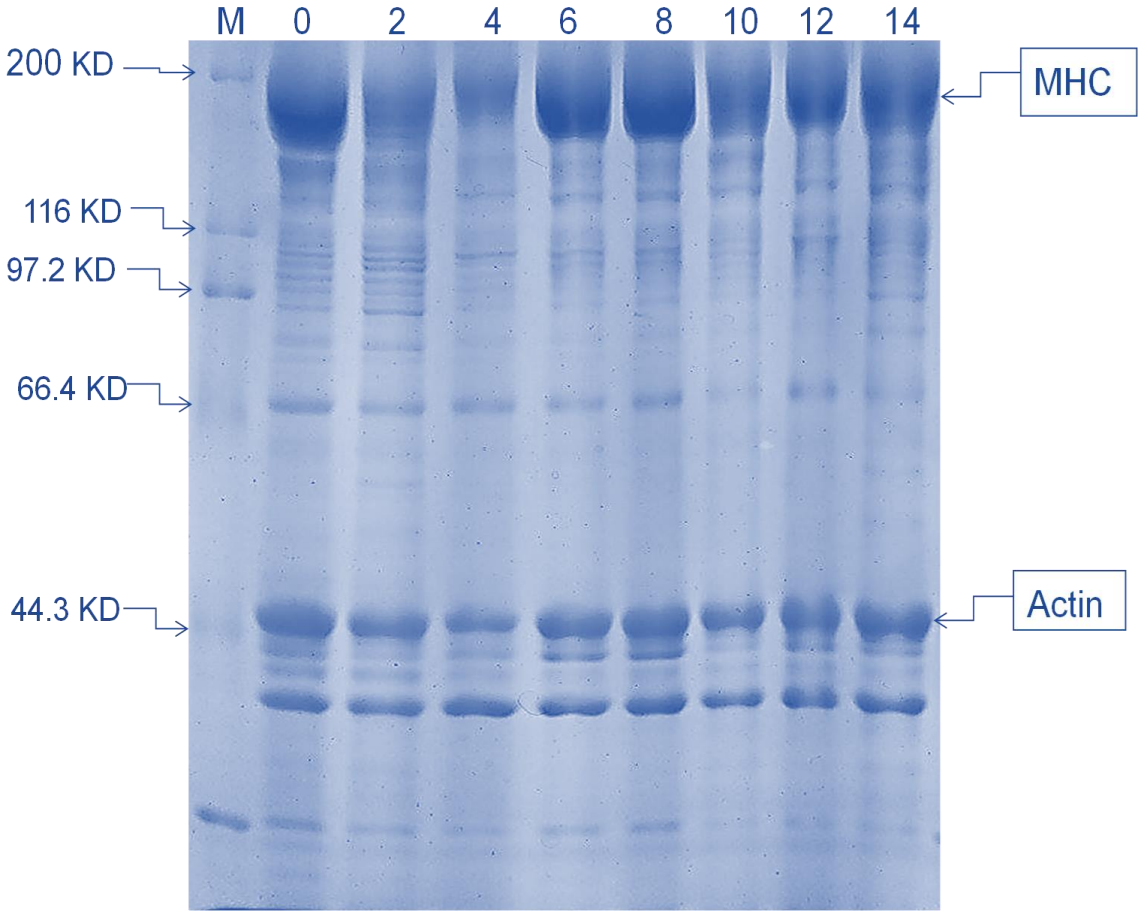
L1(M): Broad range protein marker ; L2-L8,marked as 0, 3, 6, 9, 12, 15 and 18 refers to ice storage days

Figure 10. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS- PAGE) pattern of MFP from dhoma fish on different sampling days.



L1(M): Broad range protein marker; L2-L8,marked as 0, 2, 4, 6, 8, 10, 12 and 14 refers to ice storage days

Figure 11. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS- PAGE) pattern of MFP from Indian squid on different sampling days.



L1 (M): Broad range protein marker; L2-L8 marked as 0, 2, 4, 6, 8, 10, 12 and 14 refers to ice storage days

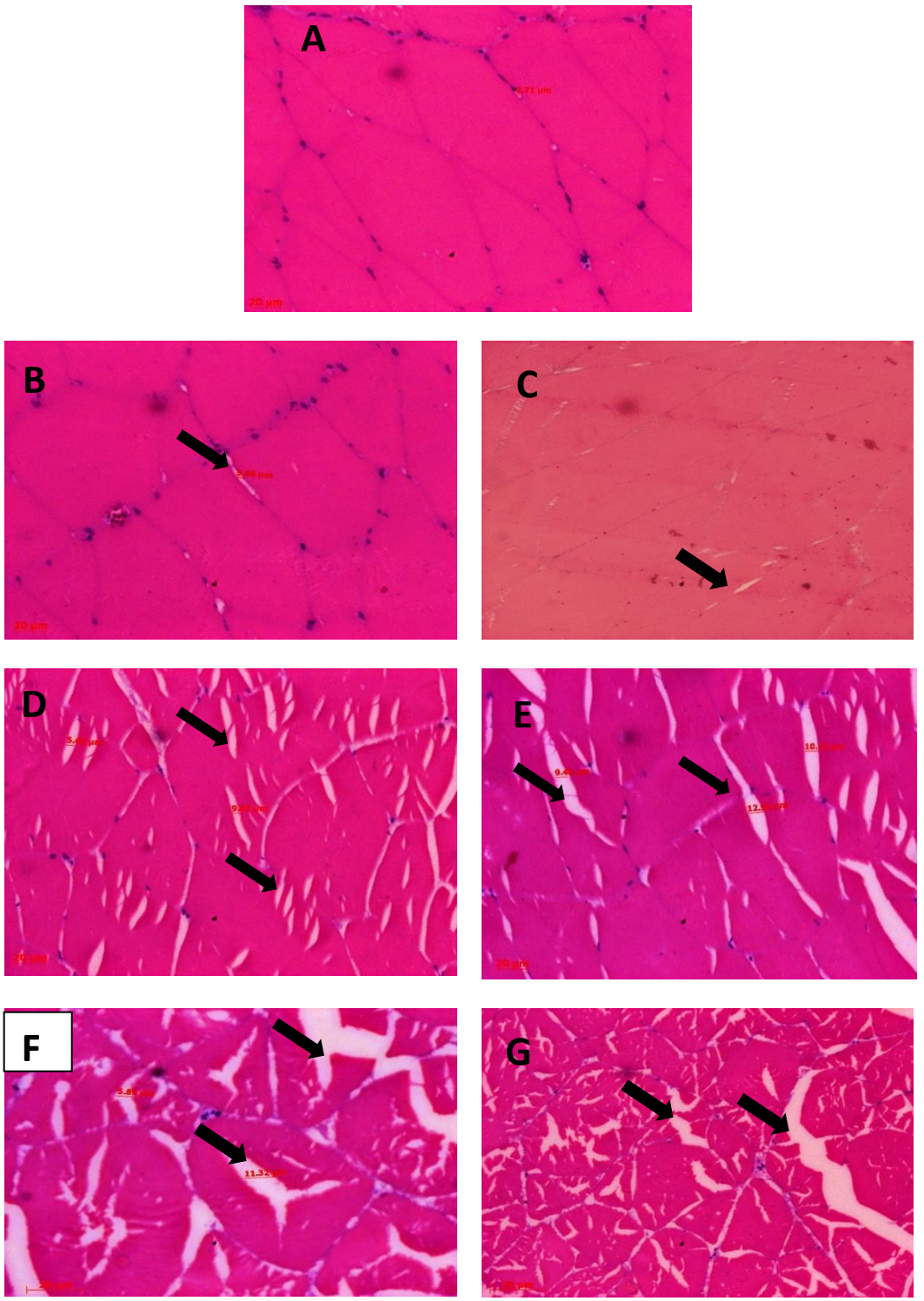
Figure 12. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS- PAGE) pattern of MFP from white leg shrimp on different sampling days.

Histological observation

Histological changes in musculature of dhoma fish, Indian squid and white leg shrimp are depicted in Fig. 13, 14 and 15 respectively. Histological architecture of dhoma fish muscle was found to be intact up to 6th day of ice storage (Fig. 13 A, B and C). However, gaping among the myofibrils was evident from 9th day onwards (Fig. 13 D to G).

The histological observation of fresh muscles (0 day) of Indian squid (Fig. 14 A) showed strong muscle architecture with fine and long myofibrils supported with special linings distributed at equal distance. Up to 8th day of ice storage, samples showed no considerable disruption (Fig. 14 A to D). However, gaping between the fibrils registered an increasing trend with the increase in the ice storage period (Fig. 14 E to H).

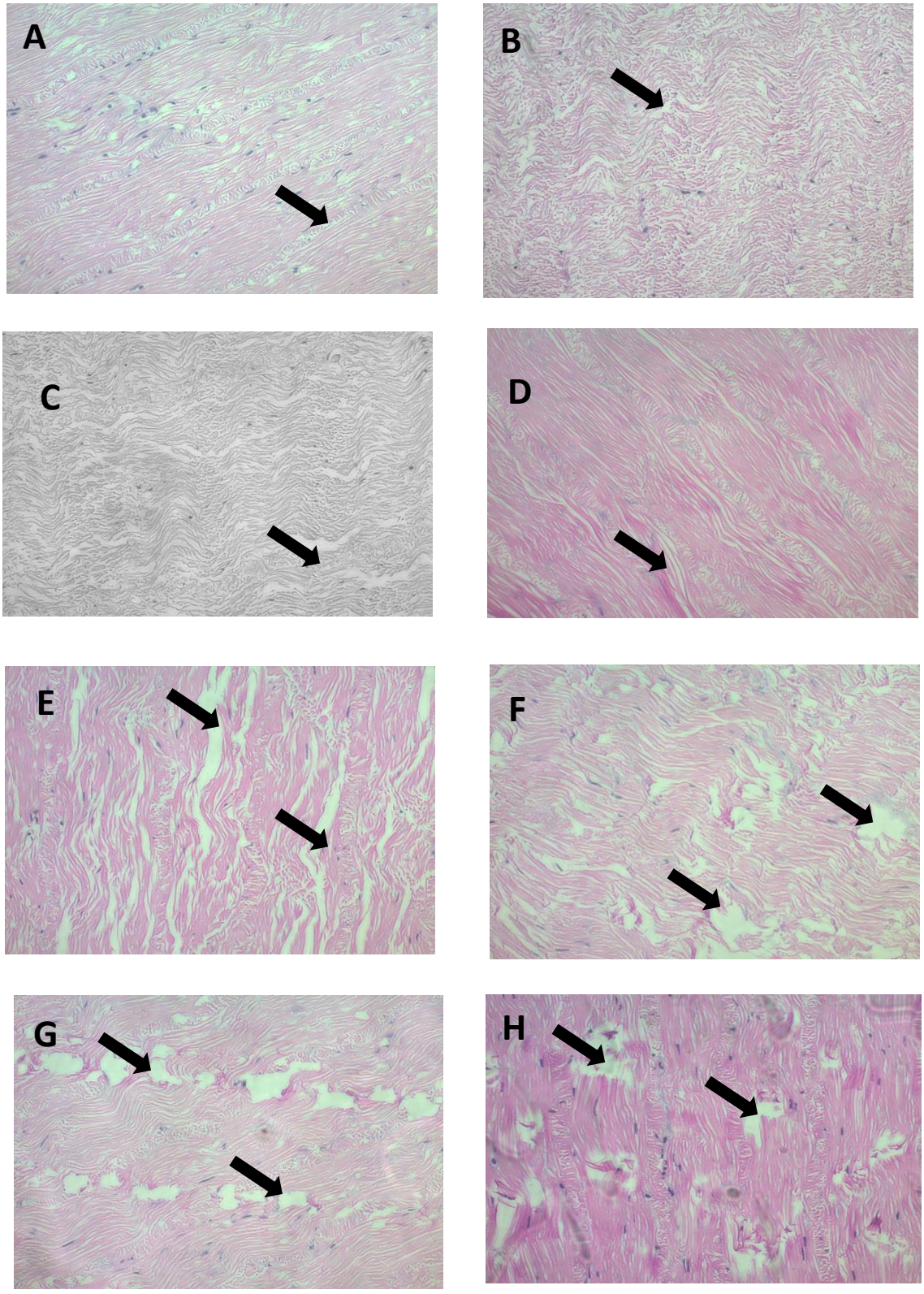
Histological observation of the muscles of white leg shrimp showed no notable changes up to 4th day (Fig. 15 A to C) of ice storage thereafter, gaping became evident and wider with progression of storage period (Fig. 15 D to H).



Arrow indicating detachment of myofibrils. Scale Bar-20µm

A-0 day, B-3 days, C-6 days, D-9 days, E-12 days,F-15 days, G-18 days

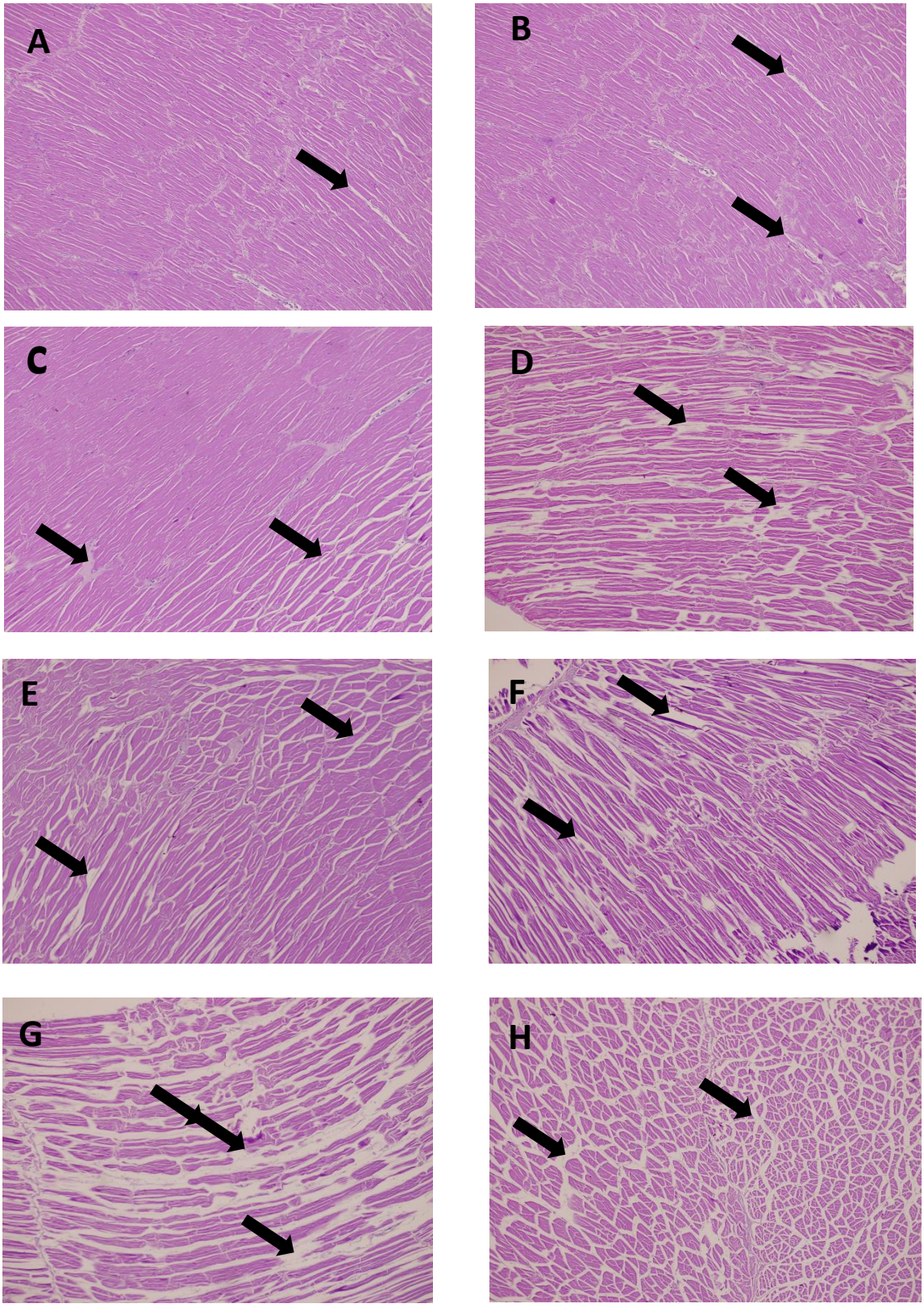
Figure 13. Histological changes in dhoma fish muscles during ice storage.



Arrow indicating detachment of myofibrils. Scale Bar-20µm

A-0 day, B-2 days, C-4 days, D-6 days, E-8 days, F-10 days, G-12 days, H-14 days

Figure. 14 Histological changes in Indian squid muscles during ice storage.



Arrow indicating detachment of myofibrils. Scale Bar-20µm

A-0 day, B-2 days, C-4 days, D-6 days, E-8 days, F-10 days, G-12 days, H-14 days

Figure 15. Histological changes in white leg shrimpmuscles during ice storage.

4.5 Effect of ice storage on functional properties of myofibrillar proteins

In this section, effect of ice storage on functional properties (solubility, apparent reduced viscosity, water holding capacity, emulsion capacity and gel forming ability) of myofibrillar proteins extracted from dhoma fish, Indian squid and white leg shrimp are given.

Solubility

Changes in solubility of myofibrillar proteins of dhoma fish, Indian squid and white leg shrimp as a function of ice storage period is given in Table 10 and Fig. 16 A, B and C. Solubility values of MFP extracted from fresh meat (0^{th} day) of dhoma fish, Indian squid and white leg shrimp were 55.32 %, 89.58 % and 86.76 % respectively. Solubility values for dhoma fish increased to 82.26 % on 6^{th} day which thereafter decreased as the ice storage proceeded (Fig. 16 A). In case of Indian squid, the solubility decreased significantly on 2^{nd} day. Barring 2^{nd} day, solubility profile showed an ordered decrement with increment in the ice storage period. The solubility profile of myofibrillar proteins extracted from white leg shrimp did not change significantly up to 8^{th} day of ice storage, followed by gradual decrement till the 14^{th} day of ice storage.

Table 10. Changes in solubility of MFP obtained from dhoma fish, Indian squid and white leg shrimp during ice storage.

Ice storage (Days)	Solubility (%)		
	Dhoma fish	Indian squid	White leg shrimp
0	55.32 ±3.22^{b}	89.58 ±1.96^{a}	86.76 ±2.96^{a}
2	-	81.14 ±1.65^{c}	87.19 ±1.65^{a}
3	68.32 ±4.02^{c}	-	-
4	-	86.46 ±3.20^{b}	87.22 ±2.00^{a}
6	82.26 ±3.00^{d}	82.11 ±1.54^{b,c}	87.87 ±1.54^{a}
8	-	77.64 ±1.65^{d}	86.42 ±1.65^{a}
9	76.60 ±5.52^{d}	-	-
10	-	76.48 ±0.99^{d}	81.82 ±1.99^{b}
12	48.13 ±6.43^{a}	75.92 ±2.44^{d,e}	79.19 ±2.44^{b,c}
14	-	72.64 ±1.65^{e}	77.06 ±1.85^{c}
15	59.03 ±2.43^{b}	-	-
18	65.72 ±1.12^{c}	-	-

Values are means ± SD, n=3, $p< 0.05$.
Values in the same column bearing unlike letters differ significantly.

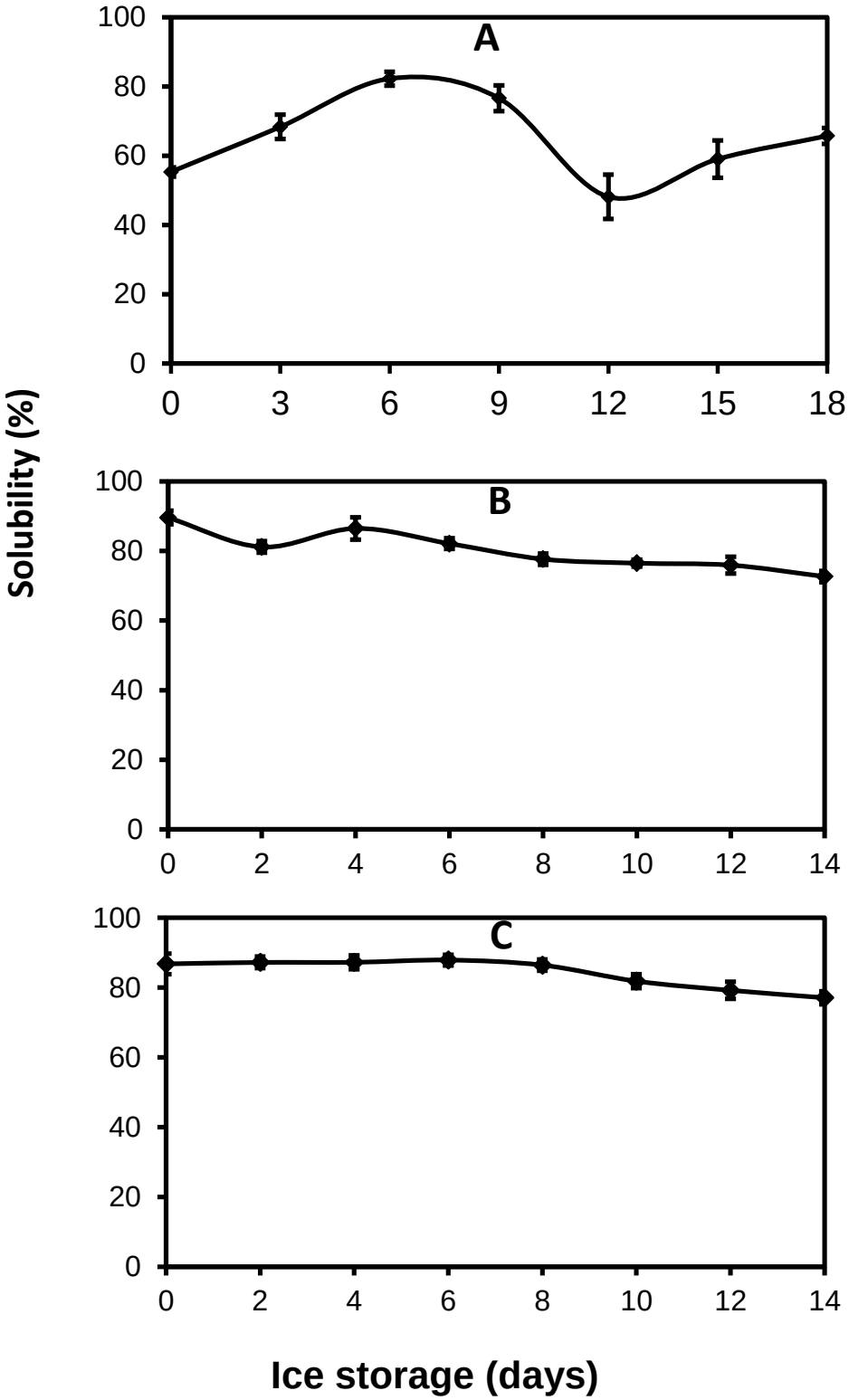


Figure 16. Changes in solubility of MFP obtained from A: dhoma fish B: Indian squid and C: white leg shrimp during ice storage.

Apparent reduced viscosity

The apparent reduced viscosity of myofibrillar proteins extracted from ice stored samples of experimental species are presented in Table 11 and Fig. 17 A, B and C. The apparent reduced viscosity of fresh myofibrillar protein from dhoma fish was 0.36 dl/mg, while the values for white leg shrimp and Indian squid ranged from 0.60 to 0.63 dl/mg respectively. On 15th day, a sharp increase was observed in the viscosity values for dhoma fish protein. In the cases of Indian squid and white leg shrimp, viscosity values were found to reduce significantly up to the end of ice storage study.

Table 11.Changes in apparent reduced viscosity of MFP obtained from dhoma fish, Indian squid and white leg shrimp during ice storage.

Ice storage (Days)	Apparent reduced viscosity (dl/mg)		
	Dhoma fish	Indian squid	White leg shrimp
0	0.36±0.00^{a}	0.63± 0.18^{a}	0.60±0.01^{a}
2	-	0.55±0.10^{a,b}	0.56±0.05^{a,b}
3	0.25±0.01^{b}	-	-
4	-	0.47±0.07^{b,c}	0.53±0.03^{b,c}
6	0.29±0.02^{a,b}	0.46±0.06^{b,c}	0.50±0.03^{c,d}
8		0.43±0.06^{b,c}	0.48±0.01^{c,d}
9	0.26±0.01^{b}	-	-
10	-	0.40±0.04^{b,c}	0.46±0.04^{d,e}
12	0.17±0.02^{c}	0.39±0.04^{b,c}	0.41±0.04^{e,f}
14	-	0.35±0.06^{c}	0.36±0.03^{f}
15	0.60±0.08^{d}	-	-
18	0.24±0.01^{b,c}	-	-

Values presented in the table were measured at 5 mg/ml MFP concentration.
Value are means ± SD, n=3, $p< 0.05$.
Values in the same column bearing unlike letters differ significantly.

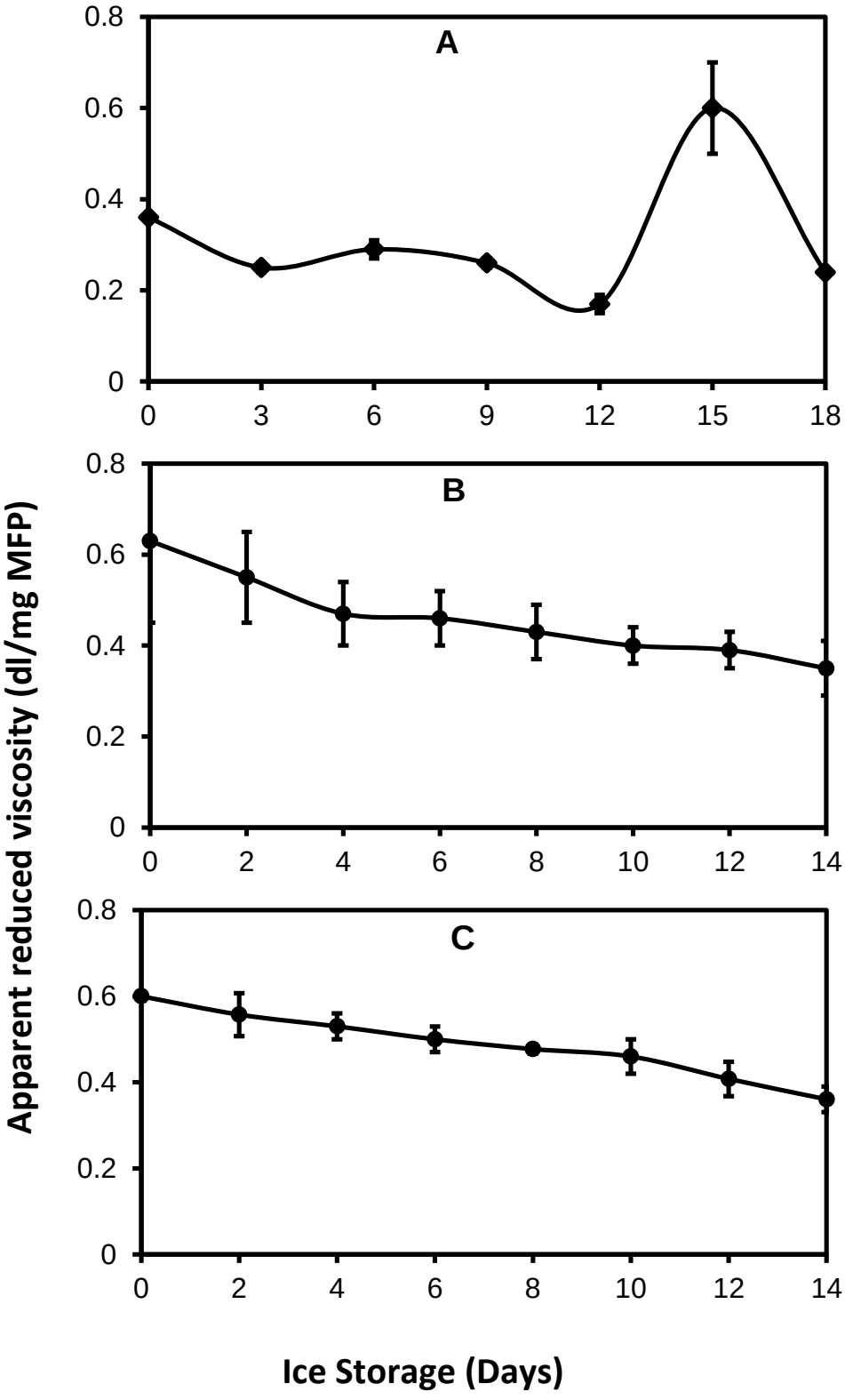


Figure 17. Changes in apparent reduced viscosity of MFP obtained from A: dhoma fish B: Indian squid and C: white leg shrimp during ice storage.

Water holding capacity

The water holding capacity (WHC) of ice stored meat samples obtained from dhoma fish, Indian squid and white leg shrimp are presented in Table 12 and Fig. 18 A, B and C. In the fresh condition, the WHC values for white leg shrimp, dhoma fish and Indian squid were 22.63 %, 37.79 % and 57.83 % respectively. In case of dhoma fish, values were found to decrease initially and subsequently returned to near original value during storage. WHC values for Indian squid showed decrement from 57.83 % to 35.75 % during 14 days of ice storage period. However, the value for white leg shrimp surged to 38.26 % on 6th day and further decreased to 31.22 % at the end of 14th day of ice storage.

Table 12. Changes in water holding capacity of meat proteins obtained from dhoma fish, Indian squid and white leg shrimp during ice storage.

Ice storage (Days)	Water holding capacity (%)		
	Dhoma fish	Indian squid	White leg shrimp
0	37.79±2.82^{b,c}	57.83±1.84^{d}	22.63±1.31^{a}
2	-	49.51±8.06^{c}	30.60±1.05^{b}
3	33.98±3.86^{b}	-	-
4	-	53.74±1.53^{c}	31.15±6.73^{c}
6	24.93±0.74^{a}	46.20±6.72^{b}	38.26±0.39^{b}
8	-	41.51±2.92^{b}	36.44±2.92^{b,c}
9	40.19±1.57^{c}	-	-
10	-	40.43±1.45^{b}	34.37±3.24^{b}
12	34.07±0.74^{b}	37.30±3.30^{a}	31.97±2.83^{b}
14	-	35.75±1.79^{a}	31.22±2.16^{b,c}
15	35.00±0.99^{b}	-	-
18	35.28±2.63^{b,c}	-	-

Value are means ± SD, n=3, $p< 0.05$.
Values in the same column bearing unlike letters differ significantly.

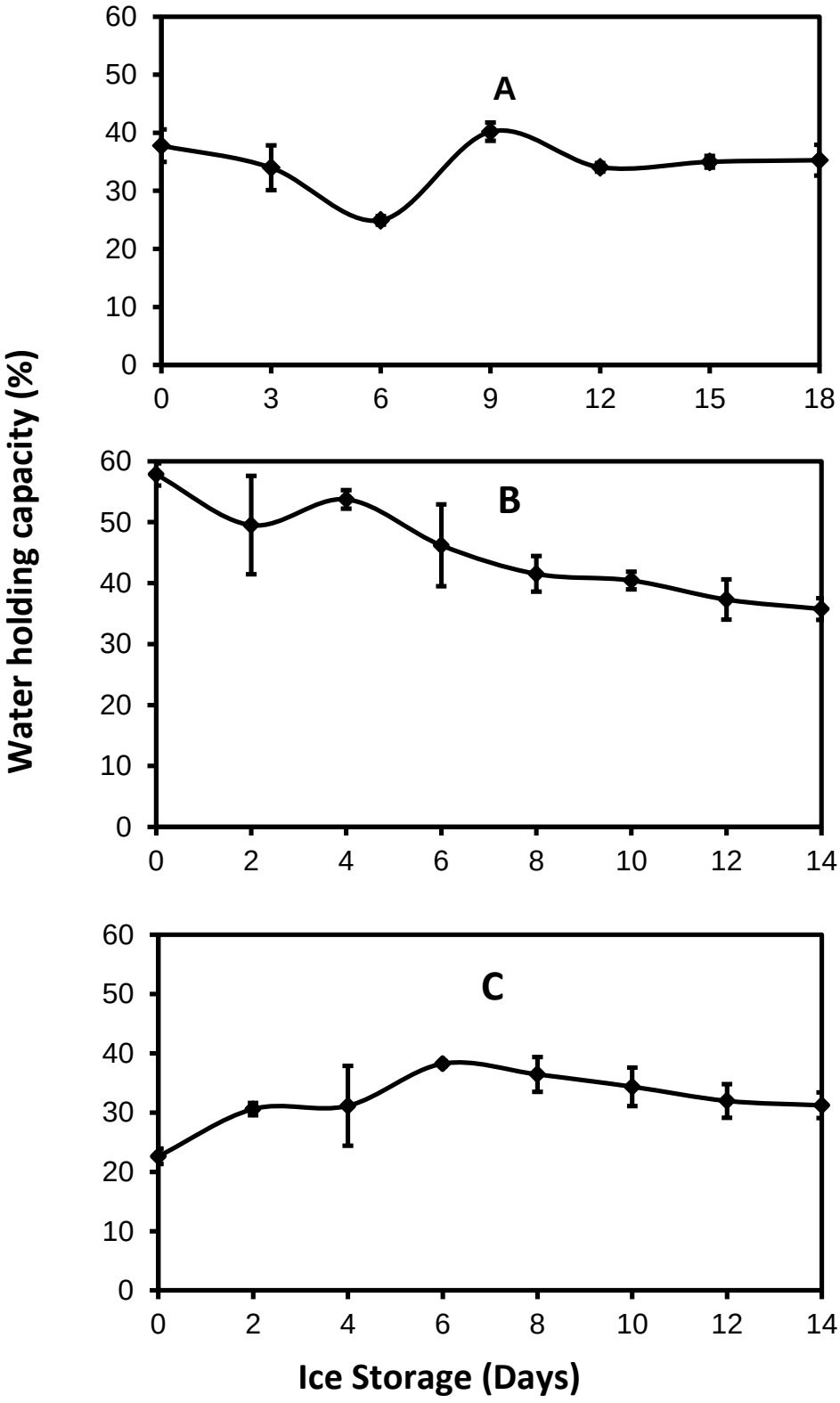


Figure 18. Changes in water holding capacity of meat proteins obtained from A: dhoma fish, B: Indian squid and C: white leg shrimp during ice storage.

Emulsion capacity

The emulsion capacity of myofibrillar proteins extracted from dhoma fish, Indian squid and white leg shrimp are presented in Table 13 and in Fig. 19 A, B and C. In the fresh condition, emulsion capacity of Indian squid, dhoma fish and white leg shrimp were 2.23, 1.09 and 0.89 ml/mg MFP respectively. Emulsion capacity of myofibrillar protein of dhoma fish showed an increment up to 15th day barring 3rd day of storage. In the case of Indian squid, the values of emulsion capacity were found to decrease significantly ($p<0.05$) up to 10th day of ice storage, thereafter, the values fluctuated. The emulsion capacity of myofibrillar protein of white leg shrimp showed increment from 0th day to 6th (0.89 to 0.93 ml/mg MFP) day of ice storage, thereafter a fluctuating trend was recorded.

Table 13. Changes in emulsion capacity of MFP obtained from dhoma fish, Indian squid and white leg shrimp during ice storage.

Ice storage (Days)	Emulsion capacity (ml/mg MFP)		
	Dhoma fish	Indian squid	White leg shrimp
0	1.09 ±0.03^{b}	2.23 ±0.06^{e}	0.89 ±0.04^{a,b}
2	-	2.29 ±0.10^{e}	0.90 ±0.05^{a,b}
3	0.94 ±0.06^{a}	-	-
4	-	1.80 ±0.04^{a,b}	0.91 ±0.05^{a,b}
6	1.14 ±0.09^{b}	1.94 ±0.01^{c,d}	0.93 ±0.06^{b}
8	-	1.84 ±0.05^{b,c}	0.85 ±0.04^{a,b}
9	1.27 ±0.01^{c}	-	-
10	-	1.82 ±0.07^{b}	0.83 ±0.03^{a}
12	1.43 ±0.04^{d}	2.00 ±0.04^{d}	0.89 ±0.02^{a,b}
14	-	1.71 ±0.08^{a}	1.09 ±0.06^{c}
15	1.86 ±0.05^{e}	-	-
18	1.47 ±0.05^{d}	-	-

Value are means ± SD, n=3, $p< 0.05$.
Values in the same column bearing unlike letters differ significantly.

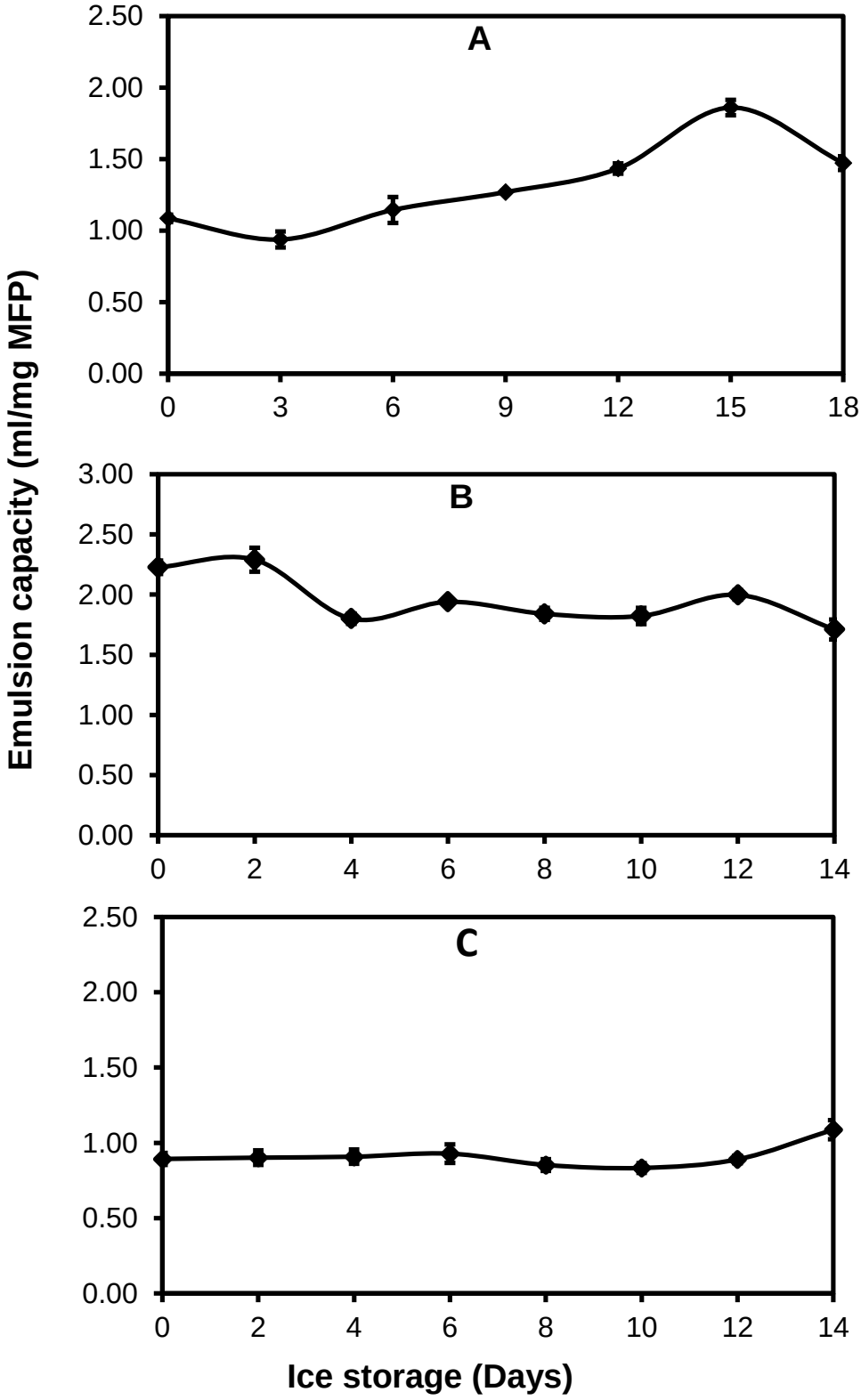


Figure 19. Changes in emulsion capacity of MFP obtained from A: dhoma fish B: Indian squid and C:white leg shrimp during ice storage.

Gel forming ability

The results of gel forming abilities of heat-induced gels prepared from fresh and ice stored meat of dhoma fish, Indian squid and white leg shrimp are presented in Table 14 to 16 and Fig. 20 A, B and C. Breaking force (BF) values of heat induced gel prepared from the fresh meat obtained from dhoma fish, Indian squid and white leg shrimp were 183.43 g, 189.55 g and 114.77 g respectively. Deformation values of heat induced gel prepared from the fresh meat obtained from dhoma fish, Indian squid and white leg shrimp were 17.08 mm, 13.58 mm and 6.99 mm respectively.

Breaking force of dhoma fish gel reduced to 144.06 g on 6^{th} day and consequently, a fluctuating trend was registered on progression of ice storage. In the same species, the deformation values were found to be continuously decreasing from 0^{th} to 9^{th} day (17.08 mm to 13. 62 mm) and, by the end of study, the value slipped down to 7.93 mm. The gel strength of heat-induced gel decreased significantly on 6^{th} day of ice storage and further continuously decreased to 88.76 g.cm on 18^{th} day of ice storage.

Breaking force values of gel produced from Indian squid and white leg shrimp were found to be decreasing significantly by 4^{th} day of ice storage. The changes in deformation values (6.99 mm to 6.41 mm) of gel produced from white leg shrimp were insignificant.

Gel strength values of heat induced gel prepared from the fresh meat obtained from dhoma fish, Indian squid and white leg shrimp were 313.45 g.cm, 257.49 and 80.62 g.cm respectively. In case of dhoma fish, gel strength value of 200 g.cm was maintained up to 9^{th} day and thereafter the values reduced to 88.76 g.cm on 18^{th} day of ice storage. A considerable reduction in gel strength values of heat induced gel prepared from Indian squid and white leg shrimp was observed during 14 days ice storage.

Table 14. Changes in gel forming ability of heat-induced gel prepared from dhoma fish during ice storage.

Gel forming ability			
Ice Storage (day)	**Breaking force (g)**	**Deformation (mm)**	**Gel strength (g.cm)**
0	183.43±2.74^{a}	17.08±0.46^{a}	313.45 ±11.87^{a}
3	180.78±1.17^{a}	16.11±0.77^{a,b}	291.48±15.60^{a}
6	144.06±1.16^{b}	14.91±0.85^{b,c}	214.62±10.86^{b}
9	146.48±1.22^{b}	13.62±0.72^{c}	199.33±8.80^{b}
12	115.34±0.87^{c}	16.73±0.28^{a}	192.97±4.50^{b}
15	150.03±1.30^{b}	7.11±0.07^{d}	106.73±1.68^{c}
18	113.14±16.38^{c}	7.93±0.32^{d}	88.76 ±9.51^{c}

Value are means ± SD, n=5, $p<0.05$.
Values in the same column bearing unlike letters differ significantly.

Table 15. Changes in gel forming ability of heat-induced gel prepared from Indian squid during ice storage.

Gel forming ability			
Ice Storage (day)	**Breaking force (g)**	**Deformation (mm)**	**Gel strength (g.cm)**
0	189.55 ±8.34[a]	13.58 ±0.60[a]	257.49 ±24.64[a]
2	186.78 ±2.72[a]	13.51 ±0.87[a]	252.34 ±19.96[a]
4	171.11 ±3.54[b]	11.24 ±0.66[b]	192.33 ±11.64[b]
6	159.78 ±8.56[c]	9.62 ±0.65[c]	153.91 ±15.85[c]
8	150.48 ±6.19[c,d]	8.30 ±0.91[c]	124.85 ±11.98[c,d]
10	147.14 ±1.53[d,e]	7.96 ±0.38[c]	117.18 ±6.09[d]
12	139.01 ±6.68[e,f]	8.12 ±1.63[c]	113.41 ±27.68[d]
14	134.03 ±6.22[f]	5.58 ±0.50[d]	74.58 ±3.54[e]

Value are means ± SD, n=5, $p<0.05$.
Values in the same column bearing unlike letters differ significantly.

Table 16. Changes in gel forming ability of heat-induced gel prepared from white leg shrimp during ice storage.

Gel forming ability			
Ice Storage (day)	**Breaking force (g)**	**Deformation (mm)**	**Gel strength (g.cm)**
0	114.77±12.99[a]	6.99±0.52[a]	80.62± 14.31[a]
2	114.47±3.78[a]	6.92±0.47[a]	79.37±7.85[a]
4	107.80±8.25[a,b]	6.82±0.38[a]	73.72±9.07[a,b]
6	96.13±10.72[b]	6.86±0.24[a]	66.04±9.06[a,b]
8	92.80±6.67[b]	6.79±0.18[a]	63.09±6.08[b]
10	70.13±5.82[c]	6.59±0.04[a]	46.23±4.04[c]
12	56.80±8.97[c,d]	6.56±0.03[a]	37.23±5.79[c]
14	49.27±6.01[d]	6.41±0.16	31.63±4.60[c]

Values are means ± SD, n=5, $p<0.05$.
Values in the same column bearing unlike letters differ significantly.

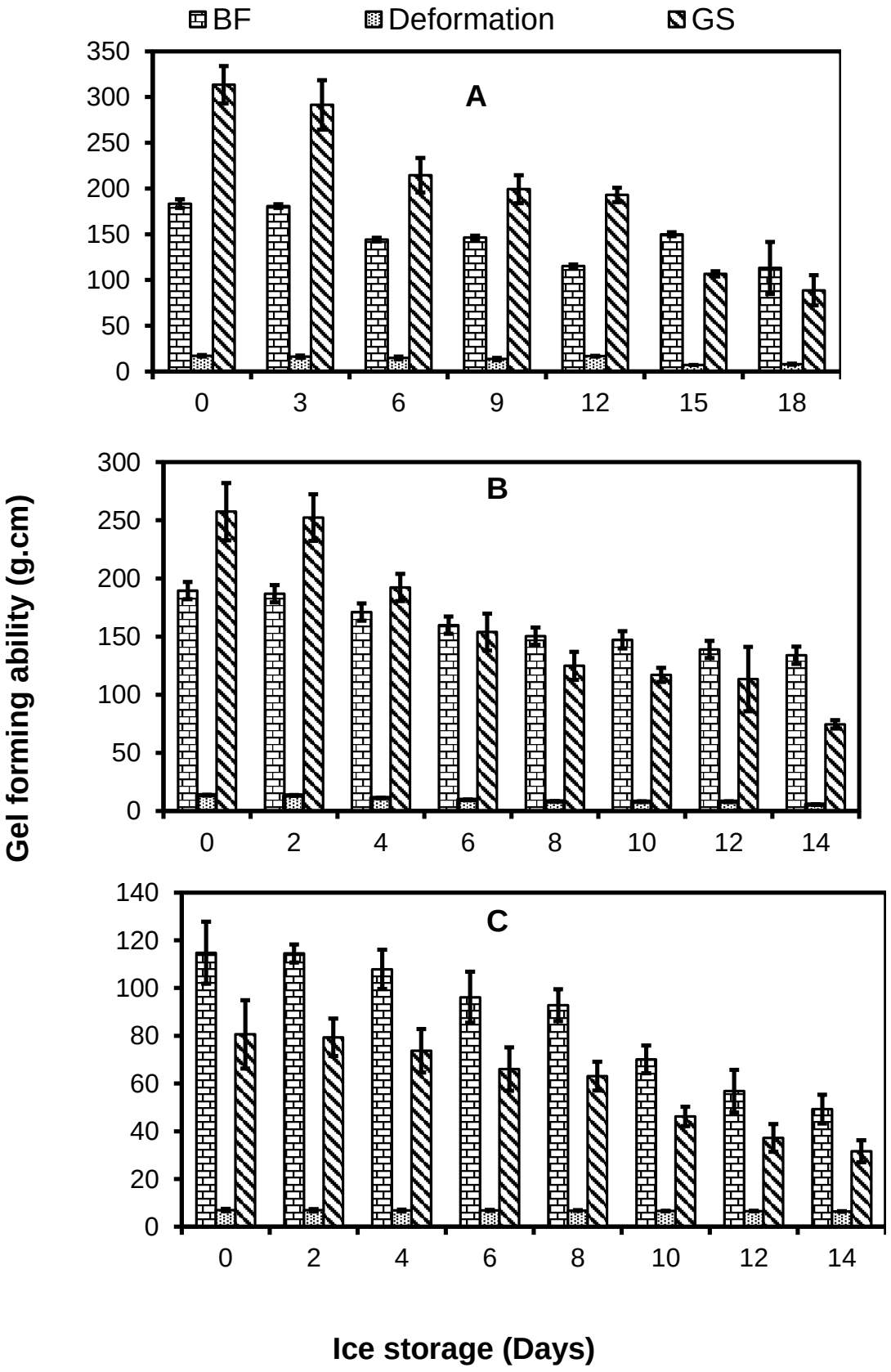


Figure 20. Changes in gel forming ability of heat-induced gel produced from obtained from A: dhoma fish B: Indian squid and C: white leg shrimp meat during ice storage.BF-Breaking force, GS-Gel Strength.

Gel colour measurement

The data on gel colours of heat-induced gel prepared from fresh and ice stored meat of dhoma fish, Indian squid and white leg shrimp is presented in Table 17 to 19 and Fig. 21 A, B and C. The lightness (L*) values for gel produced from fresh dhoma fish, Indian squid and white leg shrimp were 70.05, 74.68 and 80.69 respectively. Redness (a*) and yellowness (b*) values for gel produced from dhoma fish, Indian squid and white leg shrimp in fresh condition were -1.88 & 11.04, -1.64 & 1.37 and 13.40 & 15.95 respectively.

In case of dhoma fish, the lightness values recorded a continuous decrease from 0th day to 18th day of storage while the values of yellowness were found to be increasing up to 9th day, and thereafter the values decreased. Redness value on 0 day was -1.88 which reduced to -1.40 on 14th day of storage.

In case of Indian squid, the lightness and redness values continuously decreased to 76.01 on 8th day and after a slight increase, the value further dipped to 73.75 at the end of 14th day of ice storage period. The values of redness were found to have increased from -1.64 to -0.48 10th day followed by a brief decreased till 14th day of ice storage. Yellowness values recorded significant and continuous increments to 5.51 on 8th day of storage followed by fluctuations over the storage period.

The lightness of gel produced from white leg shrimp did not vary significantly up to 4th day. On subsequent storage, an initial decrease followed by increase was recoded. Redness values did not vary up to 6th day of ice storage but subsequently the values decreased to 11.86 at the end of 14th of storage. If one compares the yellowness values of fresh gel and 14th day produced gel, yellowness was found to have decreased.

Table 17. Changes in color parametersof heat-induced gel prepared from dhoma fish fish during ice storage.

Ice Storage (day)	Color parameters of gel produced from dhoma fish		
	L*	a*	b*
0	70.05 ±0.56^{a}	-1.88±0.31^{a}	11.04±0.31^{a}
3	69.69±0.81^{a}	-1.89±0.31^{a}	11.12±0.79^{a}
6	69.32 ±0.85^{a}	-1.63±0.14^{c}	11.49±0.59^{a}
9	68.44±0.66^{b}	-1.66±0.16^{b,c}	11.62±0.52^{a}
12	68.06±0.80^{b,c}	-1.85±0.05^{a,b}	10.22±0.48^{b}
15	68.01±0.83^{b,c}	-1.74±0.06^{a,b,c}	9.07±0.69^{c}
18	67.42±0.63^{c}	-1.40±0.17^{d}	10.01±1.13^{b}

Values are means ± SD, n=10, p<0.05.
Values in the same column bearing unlike letters differ significantly.

Table 18. Changes in colour parameters of heat induced gel prepared from Indian squid during ice storage.

Ice Storage (day)	Colour parameter of gel produced from Indian Squid		
	L*	a*	b*
0	80.69±1.17[a]	-1.64±0.07[a]	1.37±0.22[a]
2	80.49±1.12[a]	-1.63±0.37[a]	1.45±0.83[a]
4	78.99±0.77[b]	-1.35±0.14[b]	3.12±0.92[b]
6	77.45±1.28[c]	-1.16±0.13[c]	3.54±0.73[b,c]
8	76.01±0.79[d]	-0.69±0.13[d]	5.51±0.41[e]
10	77.08±0.93[c]	-0.48±0.11[e]	4.05±0.49[c,d]
12	74.50±0.70[e]	-0.53±0.21[f]	6.33±0.88[f]
14	73.75±1.34[e]	-0.80±0.08[d]	4.58±0.30[d]

Values are means ± SD, n=10, $p<0.05$.
Values in the same column bearing unlike letters differ significantly.

Table 19. Changes in colour parameters of heat-induced gel prepared from white leg shrimp during ice storage.

Ice Storage (day)	Color parameters of gel produced from white leg shrimp		
	L*	a*	b*
0	74.68±1.17^{a,b}	13.40±0.73^{a}	15.95±0.87^{a}
2	75.34±0.44^{a}	13.93±0.88^{a}	14.94±0.36^{b}
4	75.22±0.34^{a}	13.40±0.16^{a}	15.02±0.29^{b}
6	73.54±0.74^{c}	13.78±0.70^{a}	13.86±0.57^{c}
8	74.29±0.72^{b}	12.02±0.46^{b}	13.37±0.28^{d}
10	73.13±0.78^{c,d}	11.82±0.52^{b}	12.77±0.34^{e}
12	72.50±0.76^{d}	11.85±0.48^{b}	13.46±0.44^{c,d}
14	73.42±0.84^{c}	11.86±0.83^{b}	13.09±0.53^{d,e}

Values are means ± SD, n=10, $p<0.05$.
Values in the same column bearing unlike letters differ significantly.

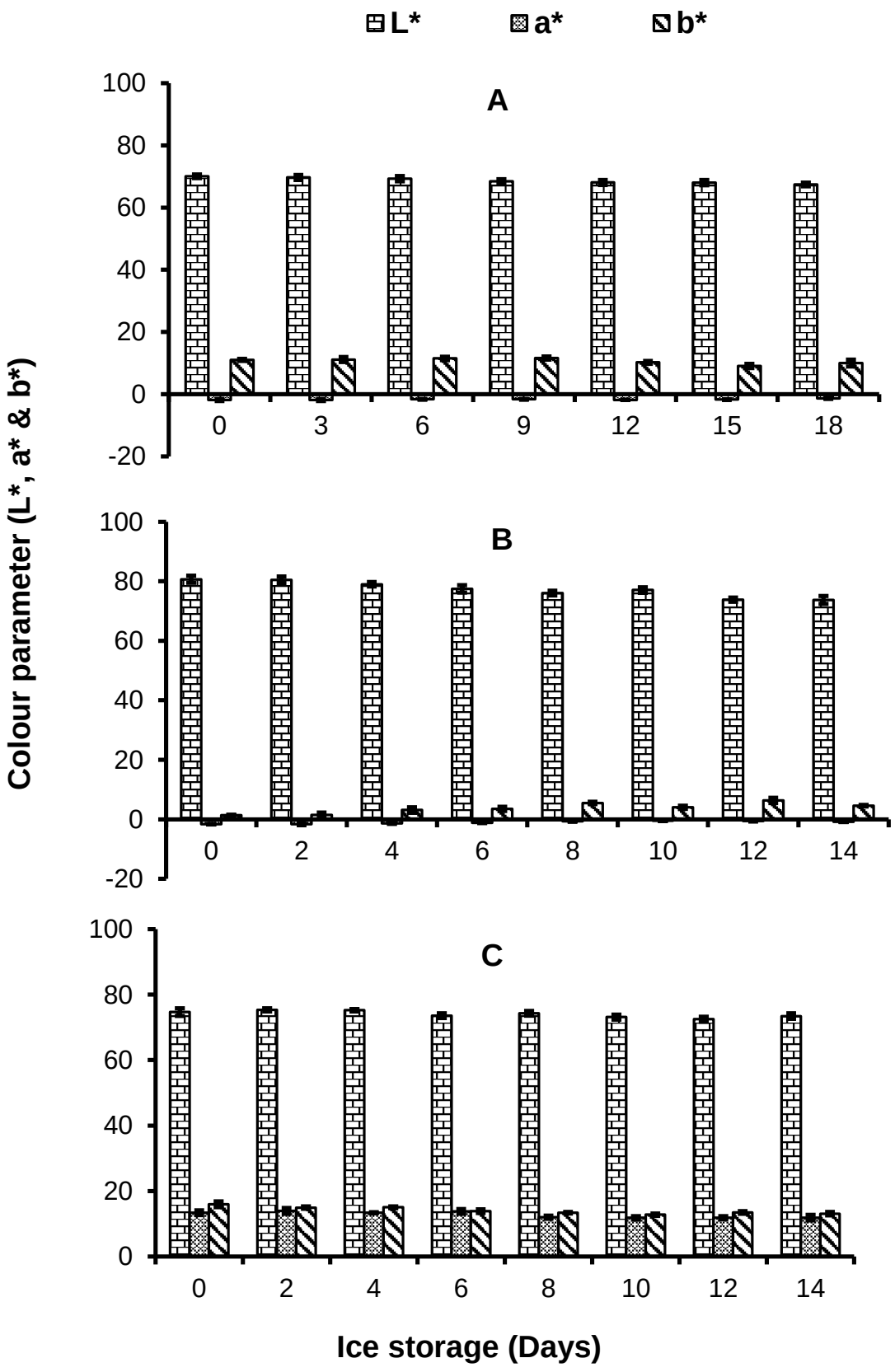


Figure 21. Changes in color parameters (L*, a* and b*) of heat-induced gel produced from obtained from A: dhoma fish B: Indian squid and C:white leg shrimp meat during ice storage.

Texture profile analysis

The results of texture profile analysis (TPA) of heat-induced gel prepared from fresh and ice stored meat of dhoma fish, Indian squid and white leg shrimp is presented in Table 20 to 22 and Fig. 22 A, B and C. Hardness of the gels produced from fresh mince of dhoma fish, Indian squid and white leg shrimp was 17.99 N, 28.09 N and 8.09 N respectively. Similarly, cohesiveness and springiness of the gels produced from fresh mince of dhoma fish, Indian squid and white leg shrimp were 0.30 & 0.67, 0.33 &0.72 and 0.21 & 0.34 respectively. The gumminess and chewiness of the gels produced from fresh mince of dhoma fish, Indian squid and white leg shrimp were 5.39 N & 3.63 N, 8.89 N &14.69 N and 3.99 N & 5.35 N respectively.

In case of dhoma fish, the value of hardness did not vary significantly up to 9th day followed by a steep reduction to 11.87 N on 12th day and further to that, the values increased slightly. Cohesiveness did not change much during ice storage period. Springiness value reached to 0.71 on 18th day of storage. The maximum value for gumminess (6.26 N) was observed on 9th day while at the end of the study the values dipped to 4.67 N. Similarly, maximum value for chewiness (4.91 N) was observed on 3rd day, while at the end of the study the values dipped to 3.29 N.

In case of Indian squid, the hardness almost remained unchanged for 2 days of ice storage, then reduced to 20 N on 4th day and the value further decreased up to 10th day insignificantly. Over all, value decreased considerably during 14 days of ice storage. Cohesiveness of the gel produced on 0th day was 0.33 which increased significantly up to 10th day and recorded a value of 0.48. Over all, value increased considerably during 14 days of ice storage. A maximum value of springiness (0.84) was registered on 4th day of ice storage period. Gumminess and chewiness of gels produced from fresh meat were 8.89 N and 14.69 N which reduced to 4.23 N and 4.35 N during 14 day of ice storage period.

The hardness of gels produced from white leg shrimp found to be decreased from 8.09 N to 3.73 N during 14 days of ice storage period. The cohesiveness more or less remained unchanged during storage study. A significant decreased in the springiness was observed on 9th day while value

further dipped to 0.22 by the end of the storage study. Gumminess value did not vary statistically up to 6th day consequently value decreased to 3.12 on 14th day of storage. A marginal reduction was recorded in the value of chewiness throughout ice storage period.

Table 20. Texture profile analysis of heat-induced gel prepared from dhoma fish during ice storage.

Ice Storage (days)	Hardness (N)	Cohesiveness	Springiness	Gumminess (N)	Chewiness (N)
0	17.99±1.08^{a,b}	0.30 ± 0.00^{b,c}	0.67 ± 0.02^{b}	5.39±0.27^{b,c}	3.63±0.17^{b}
3	20.58±2.45^{a}	0.33 ± 0.01^{a,b}	0.73 ±0.01^{a}	6.75±0.93^{a}	4.91±0.75^{a}
6	19.34±2.19^{a}	0.30±0.03^{b,c}	0.70 ±0.01^{a,b}	5.90±1.19^{a,b,c}	4.14±0.88^{a,b}
9	17.88±1.43^{a,b}	0.35 ± 0.03^{a}	0.68 ± 0.03^{b}	6.26±0.99^{a,b}	4.26±0.84^{a,b}
12	11.87±0.67^{d}	0.29 ± 0.01^{c}	0.59 ± 0.01^{d}	3.48±0.32^{d}	2.06±0.17^{d}
15	13.02±0.58^{c,d}	0.30 ± 0.00^{b,c}	0.63 ±0.02^{c}	3.96±0.23^{d}	2.50±0.07^{c,d}
18	15.53±0.01^{b,c}	0.30 ± 0.01^{b,c}	0.71 ± 0.01^{a}	4.67 ± 0.09^{c,d}	3.29±0.02^{b,c}

Values are means ± SD, n=3, $p< 0.05$.
Values in the same column bearing unlike letters differ significantly.

Table 21. Texture profile analysis of heat-induced gel prepared from Indian squid during ice storage

Ice Storage (days)	Hardness (N)	Cohesiveness	Springiness	Gumminess (N)	Chewiness (N)
0	28.09± 0.93^{a}	0.33± 0.08^{a}	0.72±0.10^{a,b}	8.89±0.66^{a}	14.69±2.65^{a}
2	27.43±1.20^{a}	0.32±0.07^{a}	0.71±0.08^{a}	8.56±1.17^{a}	13.35±3.69^{a}
4	20.96±0.67^{b}	0.45±0.05^{b}	0.84±0.02^{c}	8.22±0.54^{a}	9.58±0.98^{b}
6	20.12±0.79^{b}	0.47±0.03^{b}	0.81±0.00^{b,c}	6.46±0.52^{b}	7.31±1.17^{b,c}
8	19.61±0.54^{b}	0.43±0.07^{b}	0.80±0.02^{a,b,c}	6.12±0.61^{b}	6.35±1.38^{b,c}
10	18.31±2.74^{b}	0.48±0.02^{b}	0.83±0.01^{c}	5.32±0.18^{b,c}	5.27±1.14^{c}
12	16.77±0.02^{c}	0.44±0.02^{b}	0.79±0.03^{a,b,c}	4.28±1.17^{c}	5.23±0.27^{c}
14	14.45±2.74^{c}	0.47±0.02^{b}	0.80±0.01^{a,b,c}	4.23±0.18^{c}	4.35±1.14^{c}

Values are means ± SD, n=3, $p< 0.05$.
Values in the same column bearing unlike letters differ significantly.

Table 22. Texture profile analysis of heat-induced gel prepared from white leg shrimp during ice storage.

Ice Storage (days)	Hardness (N)	Cohesiveness	Springiness	Gumminess (N)	Chewiness (N)
0	8.09 ±0.93^{a}	0.21±0.02^{a}	0.34±0.01^{a}	3.99±0.29^{a}	5.35±1.19^{a}
2	8.00±0.95^{a}	0.21±0.02^{a}	0.34±0.01^{a}	4.00±0.50^{a}	5.34±0.89^{a}
4	7.67±0.32^{a}	0.20±0.00^{a,b}	0.33±0.01^{a}	3.92±0.07^{a}	4.94±0.62^{a,b}
6	7.33±0.66^{a}	0.19±0.01^{a,b}	0.33±0.00^{a}	3.86±0.32^{a}	4.90±1.04^{a,b}
8	6.70±0.35^{a,b}	0.18±0.01^{b}	0.27±0.03^{b}	3.78±0.66^{a,b}	4.82±0.16^{a,b}
10	5.39±0.25^{b,c}	0.18±0.02^{b}	0.26±0.03^{b,c}	3.68±0.50^{a,b}	4.60±0.08^{a,b}
12	4.06±0.98^{c,d}	0.17±0.01^{b}	0.23±0.03^{c,d}	3.30±0.16^{a,b}	4.27±0.14^{a,b}
14	3.73±1.42^{d}	0.18±.02^{b}	0.22±0.02^{d}	3.12±0.05^{b}	3.93±0.46^{b}

Value are means ± SD, n=3, $p< 0.05$.
Values in the same column bearing unlike letters differ significantly.

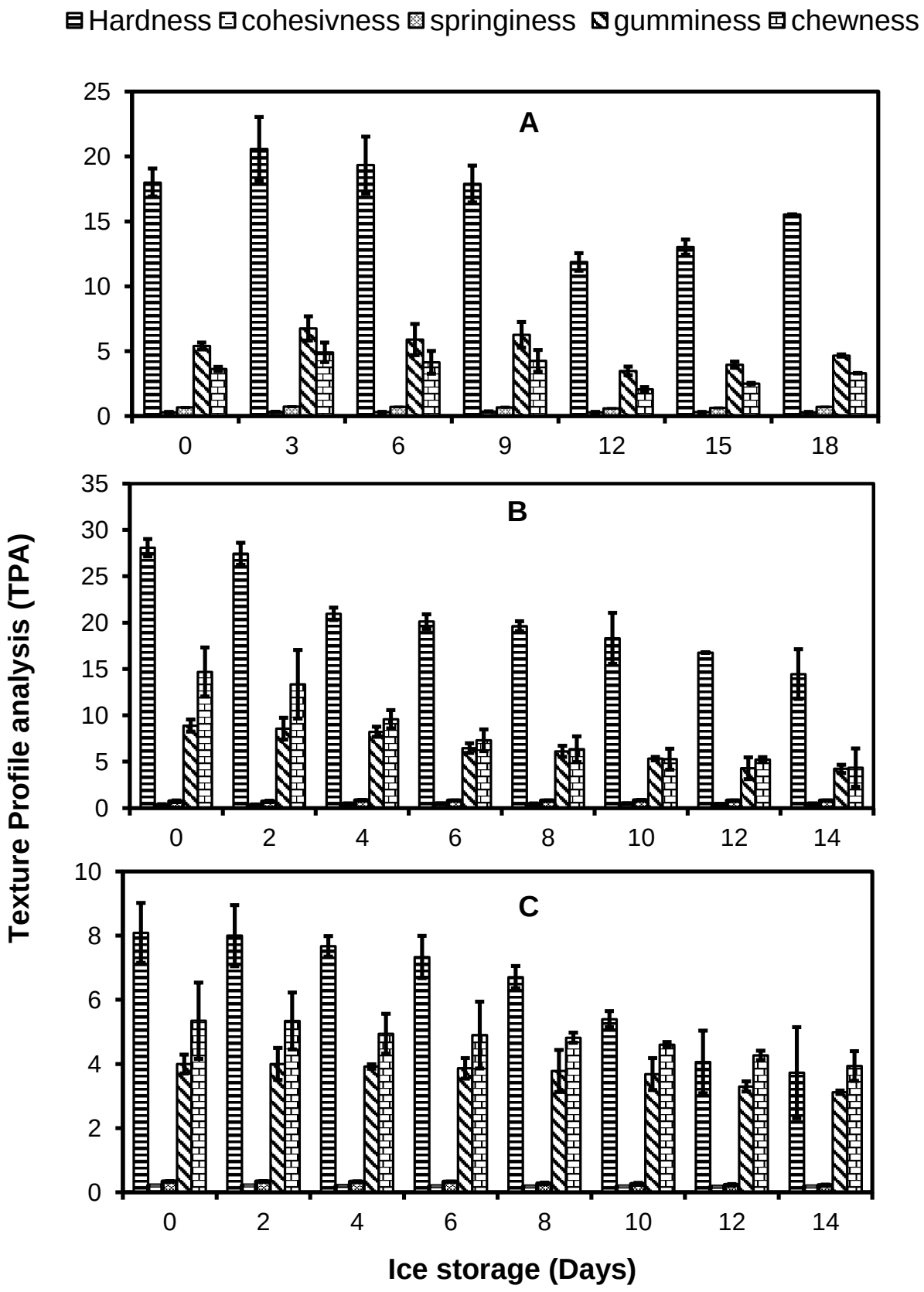


Figure 22. Changes in texture profile analysis (TPA) of heat-induced gel produced from A: dhoma fish B:Indian squid and C:white leg shrimp meat during ice storage.

4.6 Effect of ice storage on rheological properties

In this section, the results of the changes in dynamic visco-elastic properties parameterised by the measures of storage modulus (G'), loss modulus (G") and tan delta (δ) of fresh and ice stored samples of dhoma fish, Indian squid and white leg shrimp are given (Fig. 23 to 25). Storage modulus (also called elastic modulus or G'), loss modulus (also called viscous modulus or G") and tan δ (also called damping factor) of fresh and ice stored samples were measured using a temperature sweep (5 °C to 90 °C) at a heating rate of 1 °C/min.

Dhoma fish

The storage modulus of the fresh dhoma fish meat was 15.89 kPa at 5 °C which did not vary between 5 °C to 31.80 °C. However, on further heating the value increased up to 48.04 °C with relatively less rate but thereafter value took off sharply and attained maximum value of 695.84 kPa at 64.97 °C. Similarly, loss modulus was 1.75 kPa which did not vary notably up to 37.21°C but thereafter value surged sharply and attained maximum value of 132.19 kPa at 64.97 °C. The value of tan δ was 0.11 at 5 °C which did not change much up to 41.95 °C and attained a maximum value of 0.20 at 46.69 °C and further the value was more or less constant up to 90 °C.

On the 3rd day of storage, the variation in the storage and loss modulus did not occur in the similar temperature range (as in fresh meat) but on further heating a depression in the values was observed between 42.63 °C to 49.40 °C, though, on further heating value increased sharply to 76.48 °C. Interestingly, the value of tan delta in the above said range got sudden hike from 0.11 to 0.21 thereafter value more or less maintained on further heating up to 90 °C. The storage and loss modulus values during ice storage reduced continuously up to 6th day but the highest value observed on 9th day of storage further reduced to 15th day and improved again on 18th day of storage. However, the value of loss modulus was much lower than the storage modulus throughout storage study. The tan δ value of dhoma fish meat was found to be more or less unchanged up to 40-42 °C thereafter, value increased in the temperature range of 46 to 54 °C from 0.11 to 0.20 (±0.01) up to 9th day. From 12th day, temperature

range of increment in tan δ shifted from 37 to 45 °C (12^{th} to 18^{th} day). After increment of tan δ value on above said temperature ranges, the values recorded to be almost stabilized in the temperature range up to 90 °C throughout storage study.

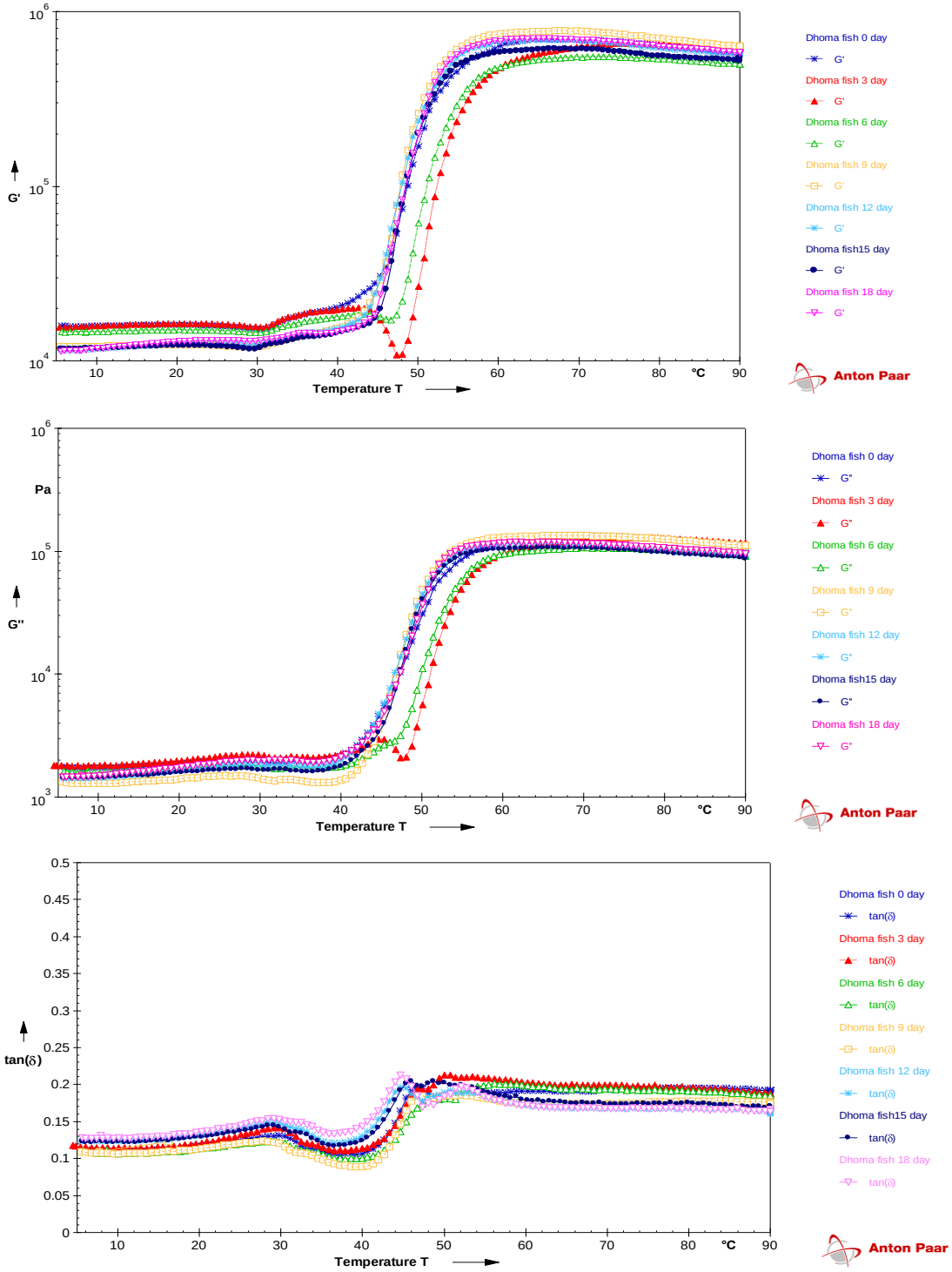


Figure 23.Changes in the dynamic visco-elastic behavior (G'-storage modulus, G"-loss modulus and tan δ-damping factor) of dhoma fish mincemeat during ice storage.

Indian squid

In case of fresh Indian squid meat, value of storage modulus was 5.26 kPa at 5 °C which on heating gradually increased to 8.42 kPa at 28.41 °C. On further heating, the value continuously dipped and reached to a minimum (0.98 kPa) at 41.27 °C. Similarly, loss modulus at 5 °C was 0.96 kPa which increased up to 2.60 kPa at 34.51 °C. Thereafter, the value continuously decreased to 0.58 kPa (minimum) at 41.95 °C. However, on further rise in temperature, the values of storage and loss modulus augmented continuously and attained the highest values of 350.94 kPa and 75.48 kPa at 71.74 °C and 90 °C respectively. The tan δ value was 0.18 at 5 °C which relatively remained more or less similar up to 28.41 °C but on further increase in temperature consequently the value shot up tremendously and achieved its maxima (0.74) at 41.27 °C and reduced then to be almost it's original.

On storage, a clear progression in the value of storage modulus was also observed in heating range of 5 to 32.50 °C but on further heating value dipped and hit the lowest at 39 °C on most of days of sampling during whole ice storage study. The range of temperature in which value reduced was 24 to 44 °C during storage study. The highest value attained by storage modulus of the squid meat found to be increased from 350.94 kPa to 3731.81 kPa between 0 to 12^{th} day however on last day of sampling value reduced to 551.83 kPa. More or less similar type of changes were registered for loss modulus, though with lesser values. The tan δ values of the day 2^{nd} (0.95) and 12^{th} day (0.98) were almost similar. The value varied from 0.35 to 0.98 during ice storage.

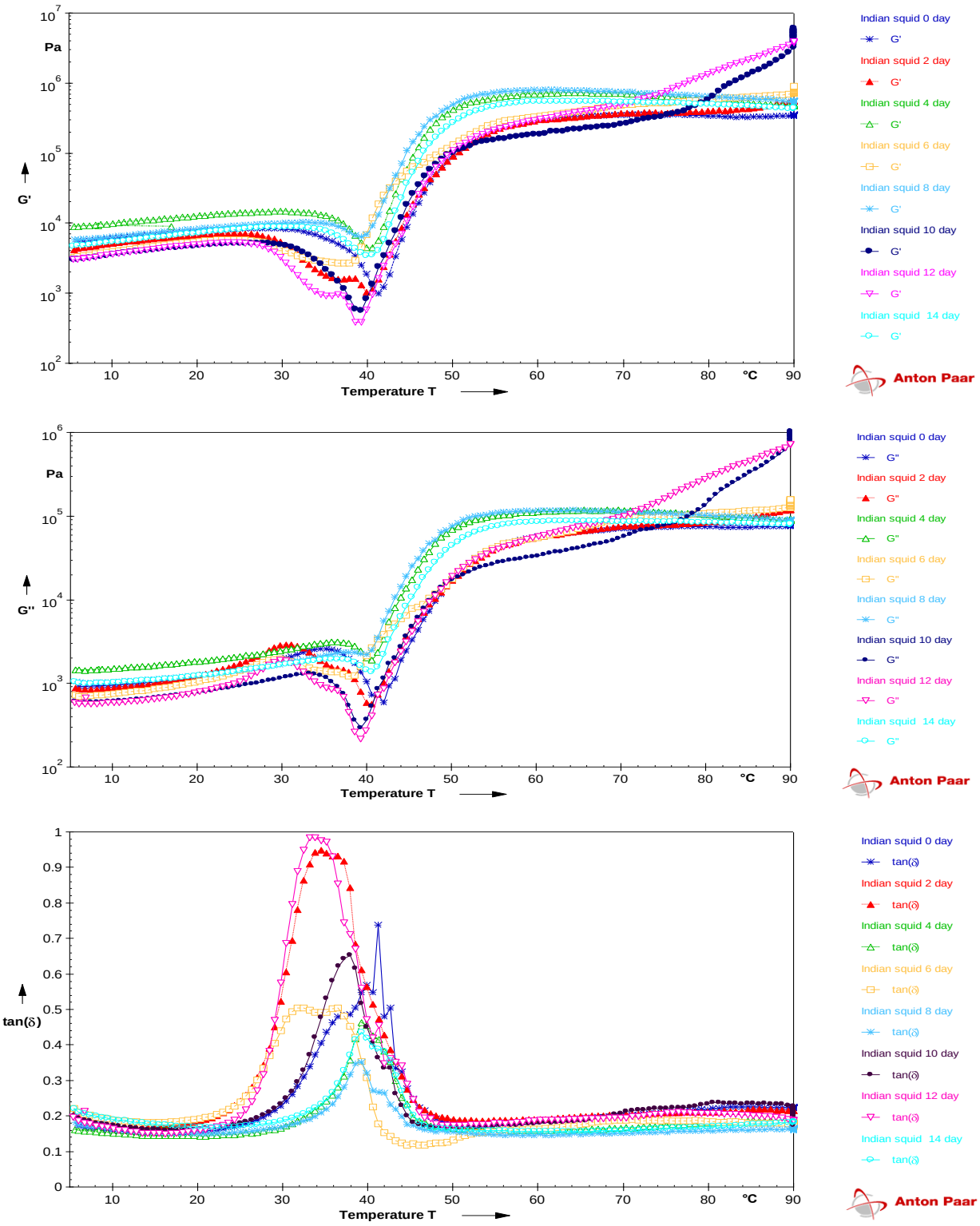


Figure 24. Changes in the dynamic visco-elastic behavior (G'-storage modulus, G"-loss modulus and tan δ-damping factor) of Indian squid mincemeat during ice storage.

White leg shrimp

The storage modulus of fresh meat of white leg shrimp was 14.40 kPa at 5 °C. On heating, the value decreased continuously to 10.68 kPa at 35.86 °C. Similarly, loss modulus at 5 °C was 3.01 kPa which on further heating decreased to 2.45 kPa at 35.86 °C. However, tan delta value was found to be increased from 0.21 to 0.25 in the temperature range of 5 to 39.93 °C. Thereafter, the values of storage and loss modulus propelled continuously and achieved maximum values of 535.77 kPa and 101.02 kPa at 64.30 °C and 68.36 °C respectively. The value of tan delta dipped to 0.18 at 43.98 °C and almost maintained up 71.07 °C further return to near the original value.

The maximum value (535.77 kPa) of the storage modulus of fresh meat of white leg shrimp was obtained on 0^{th} day and at the end of the 14^{th} day of storage value decreased to 246.56 kPa. The similar behaviour with lower values was observed for loss modulus during ice storage. However, in both moduli, the decrement was not uniformly distributed. In the fresh condition, temperature range of transition phase of storage modulus (sol to gel) was 35.86 °C to 64.30 °C which become widest on 8^{th} day (38.57 to 81.22 °C). However, at the end of the storage period the range narrowed to 37.89 to 65.95 °C.

Tan delta value showed an upward shift in the temperature range of decrement of storage modulus while behaviour was reverse on further heating especially during transition phase. The lowest value of tan delta was attained on 2^{nd} day during transition phase (43.31 °C) of storage modulus. After this transition phase, the value stabilizes during throughout storage study.

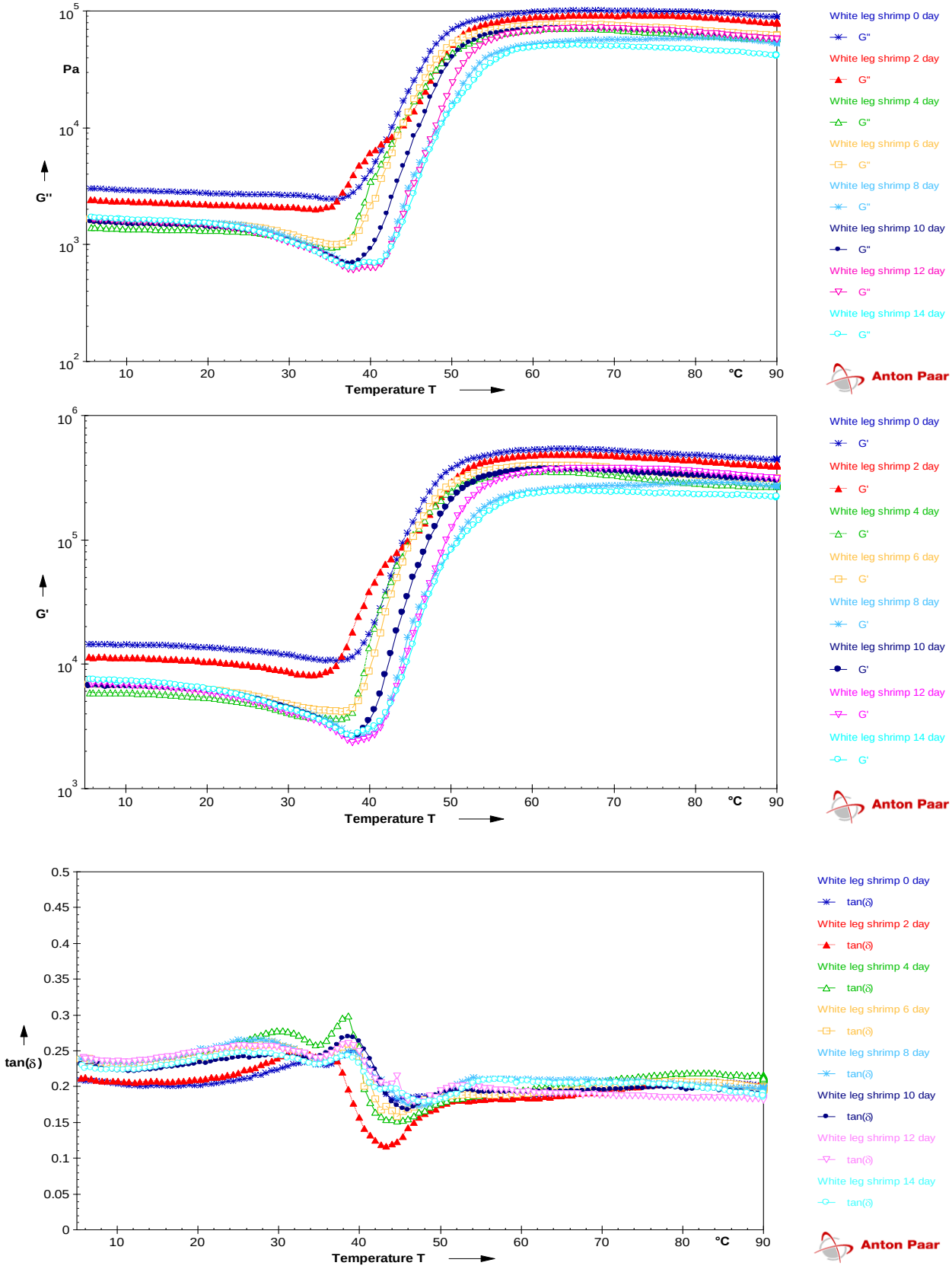


Figure 25. Changes in the dynamic visco-elastic behavior (G'-storage modulus, G"-loss modulus and tan δ-damping factor) of white leg shrimp mincemeat during different period of ice storage.

5. DISCUSSION

In this section, the results obtained have been analysed to explain the mechanism of changes in physico-chemical, functional and rheological properties of the myofibrillar proteins from dhoma fish, Indian squid and white leg shrimp. The relevant inferences were also drawn based on earlier work.

5.1 Proximate composition of fresh meat obtained from dhoma fish, Indian squid and white leg shrimp

Moisture content was the principal constituent in fresh meat obtained from dhoma fish, Indian squid and white leg shrimp (Table 1). The highest moisture content among the experimental species was found in Indian squid (84.58 %) followed by dhoma fish (81.42 %) and white leg shrimp (73.45 %). Usually moisture content remains inversely proportional to the fat content indicating that lean fishes have more moisture content than fatty fishes. All the selected species for this study have been known to contain low fat.

The crude protein content of dhoma fish was 15.38 % which is slightly less than reported for other croaker species i.e.*Pseudotolithus typus* (Ondo-Azi *et al.*, 2013). The protein content was 14.17 % in squid and nearly 20 % in white leg shrimp meat. In the present investigation, protein content was more or less similar to that obtained for other squids (Abugoch, 1999; Marquez-Rios *et al.,* 2007) and shrimp species (Turan *et al.,* 2011, Puga-López *et al.,* 2013). The fat content was higher in white leg shrimp (1.82 %) in comparison to dhoma fish (1.28 %) and Indian squid (0.72 %) among the experimental species.

The proximate composition of dhoma fish indicates that the moisture content of 81.42 % is on higher side compared to many marine species. The high moisture content, relatively good amount of proteins and low fat content (1.28%) categorises dhoma fish as lean fish. This fish, like any other croaker species therefore, is an ideal candidate for surimi preparation. The ash content was 1.01 % which was expectedly low as the analysed meat was devoid of fins and bones.

The proximate composition of dhoma fish was in the range of those reported for from analyses of 23 marine fish that suggested that the average moisture, protein, fat and ash content ranged from 67.23 to 80.48%, 15 to 20% (Majority of fishes), 0.24 to 14.72 and < 2% (87% of fishes) respectively(Kumar *et al.,* 2014).In our study, the proximate composition of white leg shrimp *was* within the range that are reported for other shrimp species like brown shrimp (*Crangon crangon*) and white shrimp (*Fenneropenaeus indicus)*, (Diler and Ata, 2003; Oksuz *et al.,* 2009; Turan *et al.,* 2011; Puga-lópez *et al.,* 2013).

5.2 Yield of edible portion of dhoma fish, Indian squid and white leg shrimp

The yield of edible portions and meat from the experimental species were estimated as they provide the key information, a consumer should possess before buying the fish. Therefore, the yield percentages were obtained in three different stages as per common consumption pattern of different consumers. Given the whole edible parts, dhoma fish has higher yield than other two experimental species. The yields of mincemeat, edible portion without head and edible portion with head from dhoma fish were 37.41 %, 57.99 % and 75.49 % of the total weight of the fish respectively (Table 2). However, obtained values were lower than those reported for the moon fish (Kolade, *et al.,* 2010) and tiger tooth croaker (Sahar *et al.,* 2014). In the present investigation, yield from white leg shrimp was 52.93 % (Peeled & Deveined) which was higher than yield reported for fresh water prawn (41.13 %) (Haq and Quddus, 1995). The yield of edible parts of the Indian squid was 64.35 % which is found in the range reported for oceanic and inshore squid species (60–80%) (Sikorski and Kołodziejska, 1986).

5.3 Fatty acid profile of dhoma fish, Indian squid and white leg shrimp

It was observed that the fatty acid composition of total lipids varied greatly in the muscle of all three experimental species (Table 3 and Fig. 3). In all three experimental species C:16 (palmitic acid) was the dominant saturated fatty acid. The highest saturated fatty acid (SFA) content was found in dhoma fish whereas monounsaturated fatty acid (MUFA) content was more in white leg

shrimp. The differences in fatty acid composition may be influenced by environmental and nutritional conditions (Polvi and Ackman, 1992). In the present study, the major portion of the fatty acids of dhoma fish, Indian squid and white leg shrimp was constituted by saturated fatty acids which are much higher than the several commercially important fish species from the Sinop region of the Black Sea (Kocatepe and Turan, 2012). Among SFA, palmitic acid (C16; 49-51% of total fatty acids) was the major saturated fatty acid while the oleic acids was the main component in monounsaturated fatty acid (MUFA) in the present experimental species.

Polyunsaturated fatty acid (PUFA) contents in dhoma fish and Indian squid were almost similar (18-20 %) while the content was 14.55 % of total fatty acids in the white leg shrimp. The nutritionally important PUFA content of all experimental species was less than reported for puffer fish (Eswar *et al.,* 2014). The n-3 fatty acids (EPA and DHA) in all experimental species were found in the noticeable quantity (13-15 % of the total fatty acids). In case of dhoma fish, DHA (11.36% of the total fatty acids) was the prominent fatty acid among the n-3 fatty acids while EPA (9.96 % of the total fatty acids) was high in white leg shrimp. The quantity of DHA and EPA contents were almost similar in Indian squid. The fatty acid composition of two puffer fish species were observed and PUFA like n-3 fatty acids were reported to be 31.17 % & 31.19% while n-6 PUFA were 7.26 %, 7.29 % in *Lagocephalus lunaris* and *Lagocephalus inermis* respectively (Eswar *et al.,* 2014). Eicosapentaenoic acid (EPA) and DHA display several properties which are beneficial for human health. In addition to reducing the risk of some cardiovascular diseases (Ness *et al.,* 2002)and cancers (Norat *et al.,* 2005) they also can improve various functions in the human organs (Berbert *et al.,* 2005).

It is suggested that the dietary intake of food with a high ratio of n-3/n-6 would be beneficial. FAO experts have recommended that the ratio of n-3/n-6 in the diet should be higher than 0.2 (FAO/WHO, 2003), while UK Department of Health recommends it to be higher than 0.25 (HMSO, 1994). In the present study, this ratio in the fats extracted from dhoma fish, Indian squid and white leg shrimp were 3.11, 2.76 and 8.51 respectively. In all experimental species, the ratio is well above the recommended levels suggesting the

goodness of the fats in these species. The ratios found for the all the three experimental species are more than the reported for many fresh water fishes like rohu (1.04) pangas (0.74) magur (0.70), catla (6.54*)* (Jakhar *et al.,* 2012). Hence, it can be concluded that the dhoma fish, Indian squid and white leg shrimp are excellent sources of n-3 fatty acids, thereby adding to their value as species of commercial and nutritional importance in India.

5.4 Changes in the physico-chemical properties during ice storage

5.4.1 Changes in pH

The pH of fish flesh influences the rate of deterioration because of its control on bacterial growth; the lower the pH, the slower bacterial decomposition will be. Normally, the pH of the live fish muscle is close to 7; however, *post mortem* pH can vary from 6.0 to 7.0 depending on the season, species, and other factors (Simeonidou *et al.,* 1998). In the present study, pH values of the fresh meat obtained from dhoma fish, Indian squid and white leg shrimp were 7.29, 6.73 and 6.62 respectively (Table 4 and Fig.4). Dileep *et al.* (2005) reported pH of 6.92 and 23 % NPN content in fresh meat of ribbon fish. Higher amount of NPN content leading to production of volatile bases upon degradation may be the cause of high pH in fresh marine fish meat. NPN content in the fresh meat of dhoma fish, Indian squid and white leg shrimp were 26 %, 13.66 % and 9.46 % of total nitrogen respectively.

The pH value of fresh dhoma fish meat was 7.29 which decreased marginally by 3rd day and increased thereafter during storage period. Though, a marginal fall of pH (by 0.05 unit) could not be related to rigor, the increase of pH steadily to 7.58 by the end of storage can be related to the production of volatile bases (Bahuaud *et al.,* 2008). The pH values of all the experimental species recorded an increase throughout storage study. A steep pH increment indicates accumulation of alkaline metabolites such as TMA and TVBN because of increased bacterial activity (Huss, 1995). In case of white leg shrimp 1 unit pH change was registered while the value of pH for Indian squid meat did not change much during 14 days of storage. The increment in the pH was very slow

in Indian squid in comparison to dhoma fish and white leg shrimp during storage. This might be due to the fact that squid muscle contains more buffering compounds such as TMAO-N (75–250 mg N/100 g; Sotelo and Rehbein, 2000). Kelley and Yancey (1999) have shown the influence of the depth of living on the TMAO content increased significantly with the depth at which the animal was caught - in order of shallow < bathyal < abyssal. In general the highest TMAO-N content is found in skates (85-340), squids (30-466 mg/100g) (Kelley and Yancey, 1999) and dogfish (175-217 mg/100g) (Oehlenschläger, 1996). Gadoids, hakes and redfish have somewhat less (60-120 mg/100g) while flatfish and pelagic fish have the least, normally herring, mackerel or horse mackerel do not exceed 30 mg/100g (Huss, 1995; Oehlenschläger, 1996).Previous reports on marine water fishes (Erkan and Ozden, 2007; Reza *et al.,* 2009), squid (Tantasuttikul *et al.,* 2011) and shrimp (Martínez *et al.,* 2008, Canizales-Rodríguez *et al.,* 2015) have also showed similar changes in pH values during storage in ice.

5.4.2 Changes in TVBN

TVBN contents represent protein degradation products and include trimethylamine (TMA), dimethylamine (DMA), monomethyl amine (MMA) and ammonia (Seibel and Walsh, 2002). The TVBN contents of fresh dhoma fish, Indian squid and white leg shrimp were 4.67, 3.22 and 4.20 mg/100 g meat respectively (Table 5). It is now well known that TMA and TVBN contents of excellent quality fish meat is known to remain below 5 mg/100 g and 20 mg/100 g meat respectively (Sen, 2005). The TVBN values of the fresh experimental species indicate that the samples collected from the landing centre were in excellent condition at the time of start of experiment. The TVBN contents of all the experimental species increased gradually during the ice storage study. TVBN value of 35 mg/100 g has been considered as the upper limit for acceptability for the fishery products beyond which product is considered to be spoilt (Ludoff and Meyer, 1973; Schormüller, 1969).Ke *et al.* (1984) proposed a classification to typify the quality of the cephalopod in accordance with TVB-N content, where a value higher than 45 mg/100 g is considered unacceptable.

However, in the Japanese market, a TVB-N value above 15 mg/100 g in cephalopods is unacceptable (Ruiz-Capillas *et al.,* 2002).

In the present study, TVBN values were found to increase constantly and reached beyond the acceptability limit on 9th day in case of dhoma fish (Fig. 5A) and on 10th day for Indian squid (Fig. 5B) and for white leg shrimp (Fig. 5C) (see Table 5). As soon as species exceeded the acceptability limit, the increment in the TVBN values became quite sharp which is possibly because of the high load of muscle degrading bacteria towards that phase of storage. Though bacterial counts have not been recorded, it is known that following initial lag phage and establishment of psychrotrophic bacteria, they tend to grow rapidly. More pronounced formation of TVB-N compound was also found in cuttlefish after 12 days of ice storage (Tantasuttikul *et al.,* 2011).

5.4.3 Changes in TMA

TMA is also considered as a quality parameter related to chemical quality of marine fishery products (Gram and Huss, 1996). The pungent odour of spoiling fish in many marine species has often been correlated with tissue TMA levels as well as with the number of spoiling microorganisms present. In the fresh meat of dhoma fish, Indian squid and white leg shrimp, TMA values were 3.50, 1.61 and 2.80 mg/100 g respectively (Table 6) indicating their prime quality at the time of start of the experiment. At the initial stages, the TMA contents in red mullet and goldband goatfish were 1.17 and 1.50 mg/100 g, respectively (Özyurt *et al.,* 2009). The values for the cuttle fish (~1 mg) and squid (~1 mg) (Tantasuttikul et *al.,* 2011) were also comparable to those from the present study in the fresh condition. The upper limit of the acceptability of TMA for fish and fishery product is 15-20 mg/100 g (Gopakumar, 2006). In all the cases, TMA values shot up significantly ($p<0.05$) during storage and crossed the limit of acceptability after 6th day in dhoma fish and after 8th day in Indian squid and white leg shrimp. This is due to the fact that in the muscles *post-mortem*, trimethylamine oxide (TMAO) is reduced into TMA in the presence of TMAO reductase mostly produced by microorganisms (Adams and Moss, 2000). However, the rate of increment in TMA was slightly higher in dhoma fish than rest of the experimental species. In case of dhoma fish, the value increased

significantly ($p<0.05$) and reached to 51.33 mg/100g of fish meat by the end of the storage study (Fig. 6A).

5.4.4 Changes in NPN

The NPN contents in fresh meat of dhoma fish, Indian squid and white leg shrimp were 614 mg %(26 %of total nitrogen), 309.66 mg %(13.66 %of total nitrogen) and 297.67 mg % (9.46 %of total nitrogen) respectively (Table 7). Dileep *et al.* (2005) also reported high content of NPN (23 %) in fresh meat of ribbon fish. Overall, the content was found to be reduced significantly ($p<0.05$) during ice storage period in all the cases. The NPN fractions of dhoma fish, Indian squid and white leg shrimp reduced to 30.26 %, 12.49 % and 67.71 % of the original values at the end of the study. The reduction in the NPN content of fresh water prawn (Akintola and Bakare, 2014), squid muscle (*Loligo plei*) (Lapa-Guimaraes *et al.,* 2005) and ribbon fish (Dileep *et al.,* 2005) during ice storage has also been reported. The NPN comprises of free amino acids and trimethylamine oxide, which are water soluble, and hence a reduction could be observed. This decrease may also be due to release of low molecular weight non-protein nitrogenous substances along with drip, which were removed during continuous replenishment of ice for melted water during storage. Raghunath (1984) reported that the NPN content increased in the water derived from ice during 8 h of storage. Aforesaid statement appeared to be justified as our experimental species were also kept directly in the contact of ice during ice storage study.

5.4.5 Changes in Ca^{2+}ATPase enzyme activity

To evaluate denaturation pattern of myofibrillar proteins, Ca^{2+} ATPase activity was examined on each sampling day during the ice storage study periods. Ca^{2+} ATPase activity considered as an indicator of myosin integrity (Benjakul *et al.,* 1997). The highest intensity of Ca^{2+}ATPase enzyme activity was observed in Indian squid (0.138µmol Pi/min./mg MFP) compared to dhoma fish (0.114 µmoles Pi/min./mg MFP)) and white leg shrimp (0.118 µmoles Pi/min./mg MFP). The values obtained in the present study were comparable with those of three species of mackerel (Chaijan *et al.,* 2010) that ranged between ~0.12 µmol

Pi/min./mg MFP and ~0.22 µmol Pi/min./mg MFP. The initial values of the ATPase activity of white leg shrimp, dhoma fish and Indian squid sharply decreased by almost 80 %, 70 % and 58 % by 6th day of storage respectively (Table 8, Fig.8 A,B and C). Reduction in ATPase activity due to conformational changes and oxidation of sulfhydryl groups of actomyosin have been reported for threadfin bream during ice storage (Yongsawatdigul and Park, 2002). *Post mortem* storage affects the Ca^{2+} ATPase activity of fish muscle proteins (Benjakul *et al.,* 1997). In fish muscle rigor-mortis is induced with a decrease in the ATP level of muscle (Partman, 1965). The intensity of rigor-mortis depends on the amounts of ATP decomposed per unit time (Yamanaka *et al.,* 1978). Heber *et al.* (1973) reported that the cause for the rapid disappearance of ATP in the fish muscle stored below freezing point might be due to the activation of adenosine triphosphatase by Ca^{2+} released as a result of cellular destruction caused by ice crystal formation. In case of dhoma fish, a drastic fall was observed up to 6th day of storage. The results were found to be in the conformity with lizard fish (Benjakul *et al.,* 2003) where it was observed that myosin underwent some changes in native conformation after 6 days. The major shift in the rates of changes in many of the functional properties coinciding with reduction in ATPase activity, probably indicates degradation of native form of myosin occurs rapidly after death, though its concentration may remain same when seen from band intensity in SDS-PAGE analysis.

5.4.6 Changes in Surface hydrophobicity

The surface hydrophobicity (SH), a measure of protein denaturation, indicates the release of hydrophobic amino acid residues buried inside secondary and tertiary structures of un-denatured proteins. The release of hydrophobic amino acid residues reduce polarity of protein and have direct impact on the WHC. In case of dhoma fish and Indian squid, the SH remains more or less constant till 6th day while in white leg shrimp up to 8th day of ice storage, after which the values steadily increased as the storage continued (Fig. 9 A,B,C and Table 9). Native myosin has hydrophobic residues strongly concentrated in the core of the helix (Maclachlan and Karn, 1982) and the surface of the helix is essentially devoid of hydrophobic groups (Borejdo, 1983).

In this way, the lower surface hydrophobicity of white leg shrimp up to 8th day may indicate greater stability of this protein compared to the proteins from dhoma fish and Indian squid during storage. This may indicate actual release of hydrophobic amino acid residues from 6th day onwards in case of dhoma fish and squid, and 8th onwards in shrimp.

In case of dhoma fish, it is very clear that the increment in solubility was recorded where hydrophobicity did not change significantly ($p<0.05$) up to 6th day. Further, there was a significant ($p<0.05$) decrease in solubility of myofibrillar proteins on 9th day which could be related to the possible unfolding of MFP exposing the hydrophobic groups to the exterior similar to the phenomenon suggested by Sarma *et al.,*1999. However, prior to 6th day, surface hydrophobicity probably did not contribute to the reduction in water holding capacity.

In the case of Indian squid and white leg shrimp, the correlations between the values of solubility and SH of MFP were significantly negative correlation I.e. -0.87 and -0.96 respectively. In the present study, the cause of increment in SH of MFP may be due to increment of pH during ice storage as reported in previous section, supported by the findings of Liu *et al.* (2014) who have reported that the enhancement of SH of tuna myofibrillar proteins happened with the increment of pH. Mignino *et al.* (2008) studied the relationship between SH and various functional properties of squid meat suggested that the changes in the functionality reflect on the amount of SH. They reported that SH of actomyosin from squid mantle also registered significant ($p<0.05$) rise in 24 h during storage at 2-4°C (Mignino *et al.* 2013). In this study, the decrement in the ATPase activity and decrease in MHC band intensity further confirmed the changes in the conformation of the MFP extracted from Indian squid during ice storage.

5.4.7 Changes in SDS-PAGE pattern

To assess the changes in the MFP extracted from dhoma fish, Indian squid and white leg shrimp SDS-PAGE pattern during storage was performed. The degradation and/or digestion of proteins by proteolysis as a

consequence of *post-mortem* changes have been monitored by SDS-PAGE (Munasinghe *et al.,* 2005; Bonnal *et al.,* 2001). In case of dhoma fish, SDS-PAGE pattern revealed that the concentration of myosin heavy chain (MHC) was not much altered during different periods of ice storage although intensity of some bands decreased during the storage period. A possible explanation for this might be that polypeptides formed during proteolysis could not bind to the dye used in electrophoretic staining (Tejada *et al*., 2002) and they did not appear on the gel electrophoretic banding pattern. Similar observations are reported for common sole during ice storage (Özoğul *et al.,* 2011). The actin fraction appeared to be unchanged during 18 days of storage.

In the case of Indian squid, myosin heavy chain (MHC 200 kDa), paramyosin (110 kDa), and actin (45 kDa) bands were observed in the fresh mantle muscle at the beginning of storage (day 0) which is in conformity with the results of Tantasuttikul *et al.* (2011) with the banding patterns of of squid (*Photololigo duvaucelii*) and cuttlefish (*Sepia aculeata*) MFP. The changes in the banding pattern of myosin (200 kDa fraction) were very clear where degradation can be seen on 2nd day itself. However, by the 10th day, the band recovered, but thereafter the gradually disappeared by the end of the storage. The degradation product of myosin can also be clearly seen just below the myosin band in the gel picture (Fig. 11). Similar types of degradation products have been observed in other squid species stored at 2-4°C (Mignino, 2013). This phenomenon showed a clear bearing on squid MFP of ice storage. Several authors suggested that fish muscle possess high proteolytic activity which in turn affects the integrity of MHC (Ayensa *et al.,* 2002; Gómez-Guillén *et al*., 2002; Ruiz-Capillas *et al*., 2003; De La Fuente-Betancourt *et al.,* 2009). The result of the present study are in agreement with Benjakul *et al.* (1997)who reported that MHC was more prone to proteolytic degradation compared to other muscle proteins such as actin, troponin and tropomyosin. The paramyosin band is quite clear in squid gel which showed a continuous recovery during storage period while actin remain intact throughout ice storage period. A significant increase in the paramyosin percentage has been observed in the previous storage study (Mignino, 2013). The presence of paramyosin has also been observed in other species like mollusc (*Perna viridis*) (Binsi *et al.,* 2006).

The PAGE pattern of white leg shrimp MFP between 2nd to 4th day,showed fading of the bands (Fig. 12). The actin fraction remained relatively unchanged. Myosin (MHC) and actin bands diminishing during storage period was recorded in salt soluble protein of prawn (*Macrobrachuim rosenbergii*) (Pornrata *et al.,* 2007). According to Martinez *et al.* (2001) the particular species of shrimp affects the protein muscle degradation during ice storage. There were no appreciable changes in SDS-PAGE of water and salt soluble proteins from *Penaeus japonicus* and *Penaeus monodon* after 4 days ice storage (Martinez *et al*., 2001). The actin fractions of MFP of all experimental species in the present study were found to be most stable during ice storage.

5.4.8 Changes in Histological observation

The results of histological changes of fresh and ice stored experimental species are presented in Fig. 13, 14 and 15. Interestingly, the arrangements of myofibrils of all three experimental species under study were quite different from one another. Myofibrils of dhoma fish were thick but shorter compared to that of squid and shrimp. The arrangement of myofibrils of Indian squid was very ordered. They were very fine and long and were further supported by a thick and fixed parallel structure (cord like structure) keeping them bundled. This unique arrangement may possibly be the cause for higher hardness of the squid muscle compared to fish and shrimp muscles in general. The arrangement of myofibrils in white leg shrimp was also ordered but the fibrils were relatively thicker and shorter than that in squid.

On day 0, myofibrils were well attached to each other and were showing good homogeneity in all the experimental species (Fig. 13A, 14A and 15A). In the case of dhoma fish and Indian squid, no major detachments was documented in myofibrils up to 6 days of storage as evident from Fig. 13 A-C and Fig. 14 A-D. But, in case of shrimp, fibrils remained intact only up to 4th day of storage period (Fig. 15 A-C), after which, the detachment started and progressively increased during subsequent storage. Taylor *et al.* (2002) also found that the fibres were detached from each other after the 5 days of ice storage. Our results are supported by evidences from the study of Taylor *et al.* (2002) that myofibre-myofibre detachments as well as myofibre-myocommata

detatchment lead to muscle softening. It is interesting to note that small increase of percentage of detachments in muscle might be associated with loss of hardness during first few days in ice storage as reported by Taylor *et al.* (2002). The loss of fibre-fibre attachment might be due to disconnection of cell cytoskelton from sarcomeres to endomysium (Taylor *et al.,* 2002) which also confirmed by Olga (2014). On further storage in ice (after 6 days), the compact structure of myofibrils started disintegrating with visible gaps in between the fibrils becoming wider. (Fig. 13 D to F). Though, the widening of the gaps in tissue was perceptible, they were not uniform within the tissue of a particular days sampling. In case of dhoma fish, the major changes in the tissues also coincided with bio-chemical changes as chemical quality indices (TMA and TVBN) as stated earlier on 9th day sampling indicated the TMA and TVBN values were found above the acceptable limits. The myofibre-myofibre detachments might be associated with softening i.e. due to loss of hardness (Taylor, 2002). The effect of chilled storage on the tissue structure of smoked salmon fish (Løje *et al.,* 2007), grouper fillets (Sharifian *et al.,* 2011), cultured Pacific bluefin tuna (Roy *et al.,* 2012) and salmon (Olga, 2014) depicts similar findings.

5.5 Changes in the functional properties of myofibrillar protein during ice storage

In this section, changes in the functional properties of MFP extracted from dhoma fish, Indian squid and white leg shrimp during ice storage have been discussed.

5.5.1 Changes in solubility profile of MFP

In the fresh condition, the solubility of MFP of dhoma fish (55.32 %) was much less than that of Indian squid (89.58 %) and white leg shrimp (86.76 %) (Table 10). The initial solubility of MFP from dhoma fish muscles was low (Fig. 16 A) as compared to other marine fishes that have been reported such as queen fish, sea bass and sea bream (Hossain *et al.,* 2005; Cardoso *et al.,*2012).In the fresh sample solubility was 55.32 % which increased up to 82.26 % on 6th day of storage. Zayas (1997) stated that increase in the protein solubility during initial period of ice storage is attributed to weakening of fibrous linkages in

muscle structure. Above said phenomenon was also observed in many fresh water fishes like Indian major carps (Mohan *et al.,* 2006; Mehta *et al.,* 2014). However, after 6th day solubility decreased which may be correlated with significant ($p<0.05$) increase in surface hydrophobicity of MFP as the ice storage period increased. The inverse relation between protein insolubility and hydrophobicity has been reported by Sankar and Ramachandran (2005) while studying thermal denaturation of fish protein.

In case of Indian squid and white leg shrimp, the solubility value registered a continuous decrease throughout the period of ice storage. However, solubility of MFP extracted from white leg shrimp did not change significantly ($p<0.05$) up to 8th day, after which reduced to 77.06 % on 14th day of storage. Solubility is considered as one of the very important factors affecting other functional properties (viscosity, gelation, foaming and emulsification) due to pH, concentration of salt, temperature and duration of extraction (Hall, 1992). In a study on ice-stored farmed and depot *Macrobrachium rosenbergii* (fresh water prawn) revealed almost similar mean initial myofibrillar protein solubilities which was found to be reduced during 10 days of ice storage (Haider *et al.,* 2011). In a study on jumbo squid (Ramirez-Suarez *et al.,* 2008), a significant reduction in mantle muscle protein solubility was observed on 4th day of ice storage. In general, the muscle protein solubility decreased during the storage period. The MHC band was also found to be slightly faded between 2nd to 4th days of storage (Fig. 11). Negative correlation values of -0.87 and -0.96 between SH and solubility were recorded for Indian squid and white leg shrimp respectively.

5.5.2 Changes in apparent reduced viscosity

The values for apparent reduced viscosity of MFP at the concentration of 5 mg/ml from dhoma fish, Indian squid and white leg shrimp during ice storage are depicted in Fig. 17A, B and C and presented in Table 11. The apparent reduced viscosity values of MFP extracted from fresh Indian squid (0.63 dl/mg) and shrimp (0.60 dl/mg) were almost similar. On the other hand, the value for dhoma fish (0.36 dl/mg) was lowest among the all experimental species. In the present study, the value of viscosity obtained for dhoma fish was higher than that of ribbon fish (Dileep *et al.,*2005), lesser than pre and post spawned

flounder (Paredi and Crupkin, 2007), and comparable with tilapia (Murthy and Rajanna, 2011). In the case of dhoma fish, the values of viscosity were found to be reduced significantly ($p<0.05$) at the end of ice storage when compared to that of fresh fish. Any change in the myofibrillar proteins due to denaturation as a result of dissociation or association is reflected in the viscosity. Suzuki (1981) attributed this change to the decrease in particle axis ratio. A steep rise in the viscosity of dhoma fish MFP on 15th day may be associated with high instability of the actomyosin during ice storage, a phenomenon very similar to the ribbon fish actomyosin during ice storage (Dileep *et al.,*2005).

The viscosity values of MFP from Indian squid and white leg shrimp recorded continuous and ordered decrease during whole period of storage study. The value of MFP from Indian squid and white leg shrimp reduced to 55 % and 60 % respectively of the original values at the end of the storage. This reduction indicates that the major myofibrillar proteins denatured during storage in ice similar to the report on pre-and post-spawned flounder (*Paralichtys patagonicus*) (Paredi and Crupkin, 2007). However, viscosity did not change much up to 8 days in case of white leg shrimp, corroborated by the small changes seen in the solubility and electrophoresis bands intensity, Murthy and Rajanna (2011) stated that the decrease in solubility of the proteins of tilapia meat in high ionic strength buffer could have different viscosity profile. As other than solubility, viscosity also depend on the size and shape of the molecules in the solution.

5.5.3 Changes in the water holding capacity

Measuring the water holding capacity (WHC) becomes a useful tool for describing the quality of fish protein. WHC of fresh meat is an important property as it affects both the yield and the quality of the end processed product. Initially, the WHC of Indian squid was 57.83 % being the highest, followed by dhoma fish (37.79 %), while the lowest WHC was observed for white leg shrimp (22.63%). The highest WHC recorded for squid could be due to the fine and long fibrils or well organised muscles architecture (Fig. 14A) compared to those in dhoma fish and shrimp. The strong and compact nature of myofibrils might resist the leakage of the water from muscles during the experiment.

The trends of changes in the WHC of all three experimental species are found to be different during ice storage. In this investigation, the WHC of fresh meat from dhoma fish decreased to 24.9 % on the 6th day. Ocaño-Higuera (1999) stated that during storage in ice, fish muscle generally becomes tougher, accompanied by a progressive loss of fluid and reduction of water holding capacity. A reduction in WHC of shrimp muscle was also reported to happen between 0 to 7 days (Zeng *et al.,* 2005). However, in the present investigation, the values of WHC increased to near original value by the 9th day (40.19 %) (Fig. 18A) of ice storage and thereafter, the values remained steady till the end of the experiment. The increase of the WHC during ageing of meat is due to reduced water content described as the ‘‘leaking out’’ effect as explained by Moeseke and De Smet (1999).

The WHC of Indian squid muscles registered a continuous decrease during whole period of storage study. The initial value of 57.83% reduced to 35 % at the end of the study (Fig. 18 B and Table 12). Decreased WHC of muscle has often been described as an effect of structural alterations in the muscle *postmortem*. Such alterations could be due to shrinkage of the myofilament lattice, myosin denaturation (Offer and Knight, 1988) or increased extracellular space (Offer and Cousins, 1992; Guignot *et al.,* 1993). Similar results have been recorded in the previous ice storage study of shrimp (Tantasuttikul *et al.,* 2011). In case of white leg shrimp, the WHC value increased significantly by 2nd day itself which further continued to increase significantly ($p<0.05$) up to 6th day and thereafter, the values decreased at a lower rate (Fig. 18 C and Table 12). Similar observations have been made for halibut fish (Olsson *et al.,*2003) and pork meat (Moeseke and De Smet, 1999) during storage.

5.5.4 Changes in emulsion capacity

The emulsion capacity (EC) of MFP extracted from dhoma fish, Indian squid and white leg shrimp as a function of ice storage is given in Table 13 and Fig.19 A, B & C. The EC of MFP extracted from dhoma fish, Indian squid and white leg shrimp were 2-3 times higher than that of the total proteins of ribbon fish (Dileep *et al.,* 2005), Indian major carps (Mehta *et al.,* 2014), green mussel

(Binsi *et al.,* 2006) and cephalopods (Ozalp and Karakaya, 2009). Yapar *et al.* (2006) reported that the variations in EC could be due to the type of meat, dissimilarity of the protein fraction, protein conformation, physicochemical properties and functional groups of the proteins. If one compares, the EC value of fish myofibrillar protein is usually higher than MFP from beef and poultry, as it contains less connective tissues and higher myofibrils than the latter (Gogus and Kolsarici, 1992; Yapar *et al.,* 2006).

In general, the EC of MFP extracted from dhoma fish and white leg shrimp registered an increasing trend during ice storage in the present study (Table 13 and Fig. 19 A & C). This increase could be attributed to the increase in the surface hydrophobicity which is evident in the present investigation. The increment of surface hydrophobicity coupled with increasing pH probably enhanced the amount of hydrophobic amino acid residues which were available to bind more oil as the ice storage progressed. The increase in EC may also be due to exposed hydrophobic groups which enhanced the interactions between proteins and lipids. Studies have indicated a good correlation between surface hydrophobicity and emulsifying properties (Li-Chan and Nakai, 1991). Mignino *et al.* (2013) stated that a higher content of degradation products and/or flexible peptides produced by proteolytic activity in actomyosin extracted from squid mantle permit to these peptides diffuse fast to the interface, which led to improvement of emulsifying properties. The fall in the EC of dhoma fish between 15 to 18 days despite uniform increase in pH and SH could not be explained.

However, in case of Indian squid, a continuous and significant ($p<0.05$) decrease was observed (barring 12^{th} day) during the period of ice storage, which is concurrent with a continuous decreased in solubility of MFP. Previous studies have also reported high correlation between emulsion capacity and myofibrillar proteins concentration (Knipe, 2004, Sarma *et al.,* 2000; Venugopal, 1997).

5.5.5 Changes in gel forming ability

Breaking force and deformation values of fresh and ice stored heat-induced gel from dhoma fish, Indian squid and white leg shrimp mince are shown

in Table 14,15 and 16 and Fig. 20 A,B and C respectively. The highest gel strength value was obtained for dhoma fish mince among all three experimental species. However, in case of Indian squid (189.55 g), breaking force of the gel was higher than other two experimental species. The least deformation value was obtained for white leg shrimp (6.99 mm) when compared to squid and dhoma fish.

The breaking forces of Indian mackerel, short-bodied mackerel and frigate mackerel (Chaijan *et al.,* 2010) were higher while deformation was lower than that of dhoma fish. The gel strength value of heat induced gel obtained from fresh dhoma fish mince was higher than Indian mackerel while lower than those of Indian major carp (Mehta *et al.,* 2014) and striped catfish (*Pangasianodon hypophthalmus*) (Tanuja *et al.,* 2014).

With the progression of storage period, both breaking force and deformation force of heat induced gel from dhoma mince decreased continuously up to 18 days of storage barring minor increase on 12th day. The gel strength value was reduced to 68 % on the day of chemical rejection (i.e. 6th day) which further remained almost 28 % of the original value at the end of the study. The decrease in the gel forming ability of heat-induced gel was concomitant with the increased solubility, decrease in WHC and Ca^{2+} ATPase activity (Fig.16, 18 and 8). In addition to this, slight decrease in MHC band intensity occurred over the storage period (Fig. 10). This can be very well correlated with decrease in breaking force and deformation as the storage progressed. This result was in agreement with the increase in protein denaturation and degradation with increasing storage of lizardfish (*Saurida tumbil*) andtwo species of scad (Benjakul, 2003; Wongwichian *et al.,* 2013). Myosin integrity is of paramount importance for gelation (An *et al.,* 1996). Denatured proteins can form aggregates off setting the protein-protein and protein-water interactions. The reduced gel strength and reduced deformation force could be an outcome of these changes as suggested by Wongwichian *et al.,* 2013 while comparing the ice stored scad fish to fresh fish. The gel strength value of unwashed meat of *Labeo calbasu* decreased to almost 25% of the initial value in 48 h of ice storage (Yathavamoorthi *et al.,* 2010).The degradation of myosin resulted in an inferior

gel network formation causing a lower elasticity with poor water holding capacity in gel matrix. Kurokawa (1979) reported that gel strength of kamaboko made from lizard fish stored in ice for 3 days was less than 50 % of that made from fresh fish. Yean (1993) also found a decrease in gel strength of surimi produced from threadfin bream stored in ice for more than 2 days. It appears that though decrease in gel strength is expected, the degree to which the gel strength reduces depends on the type of fish.

In the fresh condition, the gel strength value of the Indian squid was 257.49 g.cm which decreased to 48 % on the day of chemical rejection (8^{th} day), while at the end of storage period, the value reduced to almost 29 % of the original value. The gel strength of other squid species (*Illex argentinus*) mantle paste prepared in fresh condition (Suarez *et al.,* 2014) was comparable with the value obtained in the present study. The gel produced from fins is reported to have more gel strength value than the gel produced from mantle only (De La Fuente-Betancourt *et al.,* 2009). A continuous reduction in the breaking force and deformation was also observed throughout the period of ice storage. Endogenous enzymatic activity has been demonstrated in mantle of several squid species (*Loligo vulgaris, Illex coindetii, Toradores eblanae* and *Dosidiucus gigas*). This proteolytic activity promotes loss of texture and protein functionality during storage or processing (Ayensa *et al.,* 2002; Gómez-Guillén *et al.,* 2002; Ruiz-Capillas *et al.,* 2003 De La Fuente-Betancourt *et al.,* 2009). If enzymatic activity is not controlled, most of the protein in muscle tissues will be degraded to small peptides causing the loss in the functional properties needed for the muscles to be useful food ingredients. However, a poor gel forming ability was found in Indian squid in squid when compared with fish muscle even in fresh conditions. This could be attributed to weak protein–protein and protein–water interactions in squid. It is still unknown why muscle proteins of cephalopods have such characteristics A more realistic explanation can be based on the fact of the squids have high paramyosin content in its muscle and the role of paramyosin in the gelation is still unknown. Conversely, Sanchez-Alonso *et al.* (2007) also have attributed the problem to high endogenous enzyme activity present in muscle, mainly of the group metalloproteases.

The gel strength value of 80.62 g.cm in the fresh white leg shrimp muscle was reduced to 77.77 % of the original value by 8th day of storage. The value of breaking force was also found to decrease during the ice storage. However, the reduction in the deformation force was not significant ($p<0.05$) during ice storage. The gel produced from white leg shrimp was bearing pink color and was very fragile. The strength of the gel was very low as compared to dhoma fish and Indian squid. The gel produced from pink shrimp also reported to have weaker gel strength (Takahashi *et al.,* 2014) and it is deduced that this low strength may be because of the high cysteine protease activity. This also could be correlated to weak protein–protein and protein–water interactions as evident by the lowest water holding capacity among the all experimental species.

5.5.6 Changes in colour parameter of the heat induced gels

The results of colour parameters analysis of the gel produced from dhoma fish, Indian squid and white leg shrimp have been depicted in Table 17, 18 & 19 and Fig. 21 A, B &C respectively. In the present study, lightness value (L) was higher for experimental shrimp species when compared to squid and dhoma fish in the fresh condition. Higher lightness of the gel may be because of the brightness developed as a result of pigment oxidation in the shrimp meat. Almost similar values of colour parameters were obtained for the one step heated gels produced from Pacific white shrimp. However, the values of redness and yellowness were slightly higher in the gel produced from Pacific white shrimp (Eakpetch *et al.,* 2008) than the shrimp species in this study. The lightness and yellowness of the squid gel were slightly less than those reported for jumbo squid gels, but value of redness was higher in white leg shrimp gels (De La Fuente-Betancourt *et al.,* 2009). The redness value of the gels obtained from white leg shrimp was much higher (13.40) than obtained for Indian squid (-1.64) and dhoma fish (-1.88). This is because of the presence of carotenoids including astaxanthin and canthaxanthin in the shrimp muscle as reported in the previous studies (Armenta-Lopez *et al.,* 2002. Okada *et al.,* 1998).

In all animals studied, the lightness values were found to decrease significantly ($p<0.05$) as the storage progressed. The redness and yellowness of the gels produced from dhoma fish recorded a decrease during whole storage

study. Similar trends of the yellowness and redness were observed for the gels produced from Indian squid during storage. The yellowness values also got decreased but the rate of the reduction was relatively less. Contrary to this, the value for the gels from Indian squid was found to be increased continuously up to 8^{th} day followed by fluctuation. This phenomenon may be because of oxidation of pigments in the squid.

The lightness and redness of the gels produced from white leg shrimp did not change significantly ($p<0.05$) up to 4^{th} and 6^{th} days respectively, after which they decreased. The yellowness continuously reduced up to 10^{th} day followed by a marginal increase. The decrease in lightness and redness may be attributed to the oxidation of carotenoids present in the shrimp muscle.

5.5.7 Changes in texture profile analysis

Texture profile analysis of heat induced gels prepared from fresh and ice stored dhoma fish, Indian squid and white leg shrimp are shown in Table 20, 21 & 22 and Fig. 22 A, B &C respectively. In the fresh condition, the gel produced from Indian squid showed higher value for textural parameters such as hardness, springiness, gumminess and chewiness than dhoma fish and white leg shrimp. The hardness of the gel produced from squid was more than 2 times to gel produced from dhoma fish and 3 time that of the gel produced from white leg shrimp. Similarly, the value of chewiness of the gel produced from Indian squid was more than 3 time to that of the gels produced from dhoma fish and shrimp.

In case of the gels produced from dhoma fish, hardness, springiness, gumminess and chewiness values decreased during 18 days of storage while there were no noticeable changes in the values of cohesiveness during storage. The changes in hardness are comparable to the changes in breaking force, whereas, chewiness value showed changes after 9^{th} day. Gumminess is directly related to hardness, hence similar changes were observed between the two parameters throughout the ice storage period. Similar findings have been reported for gels prepared form weakfish, squid and their various meat ratios (Suarez *et al.,* 2014). However, it is apparent that some textural parameters started changing after 9^{th} day. Such changes depend on complex

biochemical changes taking place in the muscle and are difficult to attribute to any particular parameter.

The hardness of the Indian squid under study (28.09 N) was slightly higher than the reported for gels produced from jumbo squid (22 N) (De La Fuente-Betancourt *et al.,* 2009). The hardness of the gels reduced significantly on 4th day but thereafter the value remained steady up to 10th day and then subsequently reduced to 14.45 N. The cohesiveness of squid gels was almost similar to the values reported for gels made up of jumbo squid mantle (Galvez-Rongel *et al.,* 2012). Chewiness is one the most important textural characteristics which has profound influence on products acceptance. The chewiness of the gels produced from Indian squid was 14.69 N which and with the progression of storage period value decreased to 4.35 N.

The hardness value of white leg shrimp under study was comparable to that of Northern shrimp (Qingzhu, 2003), while the values for springiness and cohesiveness were lower. The textural parameters like hardness cohesiveness, springiness, gumminess and chewiness of the gel produced from white leg shrimp species did not vary significantly up to 8th day of ice storage. This indicates that although the gelling properties of shrimp proteins are not superior, the textural parameters remained desirable all through storage period. This could be attributed to the fact that the proteases responsible for denaturation of the shrimp proteins may be getting inactivated during cooking of the gels.

5.6 Effect of ice storage on rheological properties

5.6.1 Dynamic viscoelastic properties of dhoma fish

Gelation of muscle proteins yields from the transformation of an amorphous viscous phase to a three dimensional elastic network. Therefore, the process of formation of gel can be monitored by rheological parameters (Egelandsdal *et al.,* 1995). The storage modulus (G') is a good index for gel forming ability of food proteins (Karthikeyan *et al.,* 2006). The G' value of the fresh dhoma fish meat proteins was 15.89 kPa at 5°C which did not vary in the temperature ranges of 5 °C to 31.80°C (Fig. 23). This indicates that dhoma fish proteins were stable up to this temperature (31.80 °C) without any appreciable

change. However, on further heating, the values increased up to 48.04°C with relatively less rate but thereafter value took off sharply and attained maximum value of 695.84 kPa at 64.97 °C. A similar sharp increase in G' value has been reported in Alaska pollock (48.20 °C), pacific whiting (48.20°C), bigeye snapper (48.30°C) and threadfin bream (48.60°C) (Esturk and Park, 2014). The relatively lower rate of the increment in G' value between 31 to 48 °C suggests that the transformation from viscous sol to elastic network started in this temperature range which geared up intensely after 48 °C. Probably, the actual gel formation process starts after aforesaid temperature. The gel formation can be ascribed to a structure building reaction from the ordered aggregation and gel network formation effected by the application of thermal energy (Ziegler and Foegeding, 1990; Karthikeyan *et al.,* 2006). This transition may be mainly because of the opening up of head region of myosin molecule leading to hydrophobic interaction and possibly disulphide bond formation (Binsi *et al.,* 2006). The viscous modulus or G" also followed the same trends as that of G' but the intensity of ordered aggregation was much higher leading to lower G" value. The lower value of the G" than that of G' was also reported for green muscle (Binsi *et al.,* 2006), silver carp (Liu *et al.,* 2007) and many other marine fish species (Karthikeyan *et al.,* 2006; Cardoso *et al.,* 2012). The tan δ is the measure of energy lost as a result of viscous flow compared with the energy stored from elastic deformation in a single deformation cycle (Chan *et al.,* 2011). A change in tan δ indicates the type of network formed; lower tan δ values represents formation of better 3-dimensional network (Sun and Arntfield, 2010). The value of tan δ of fresh meat from dhoma fish was 0.11 at 5 °C which did not change much up to 41.95 °C (0.12) and attained a maximum value of 0.20 at 46.69 °C which retained up to 90 °C. Even though, the slight increase was observed in the tan δ elsewhere in the temperature sweep, the value remains well below 0.20 indicating that the elastic component was dominant during gelation process giving reasons for the good gelling ability of the dhoma fish meat. However, the increment in tan δ value in the temperature range 41.95 - 46.69°C may be due to unfolding of myosin molecules. On the whole, it can be inferred that the gel formation for dhoma fish meat takes place in two phases first between 29 to 40 °C and subsequently after 50 °C.

On the 3rd day of storage the trends of G' and G" were similar to that of the fresh sample (0 Day) with slightly lesser intensity but a decrease in the G' and G" was recorded in the temperature range of 42.63 to 49.40 °C while lowest value observed at 47.37 °C (10.84 kPa and 2.09 kPa respectively). Similar depression in both the values in the temperature range of 40 to 50 °C was reported in meagre and gilthead seabream temperature sweep (Cardoso *et al.,* 2012). However, the phenomenon was also recorded for hake and sea bass but not as clearly as meagre and gilthead seabream (Cardoso *et al.,* 2012). In the temperature range where G' decreased and tan δ increased significantly has been attributed to gel breakdown. Similar phenomenon also ascribed to unfolding of light meromyosin (tail) (LMM) and heavy meromyosin (HMM) chains of myosin (Sano *et al.,* 1988; Esturk and Park, 2014). Barring 3rd day, the behaviour of the G' and G" have been same during temperature sweep during throughout the storage study. However, such intense depression was not observed on subsequent storage days though, the intensity (value) of G' and G" reduced continuously up to 6th day while highest value recorded on 9th day (776.54 kPa at 67.01 °C). Furthermore, the value found to be decreased up to 15th day while slightly increased at the end of the study. The tan δ value found to be increased between the temperature range of 37.22 to 50 °C during whole ice storage study except 9th day where it increased up to 54 °C. This increment more or less recorded in the similar temperature range where G' value decreased which further supports the gel breakdown between 37.22 to 50 °C during ice storage study. Further, the value found to be stabilised which ensuring the strong gel formation.

5.6.2 Dynamic viscoelastic properties of Indian squid

The changes in the fresh and ice stored Indian squid meat during temperature sweep are depicted in Fig. 24. In case of fresh Indian squid meat, value of G' was 5.26 kPa at 5 °C which on further heating gradually increased to 8.42 kPa at 28.41 °C. On subsequent heating, the value continuously dipped and reached to a definite minimum (0.98 kPa) at 41.27 °C. Similar dip in the G' value of meat of another squid species (*Loligo vulgaris*) was reported around at 30 °C, whereas the value reached a definite minimum between 40 to 50 °C (Gomez-

Guillen *et al.,* 2003). The fall observed in G' value after 28.41 °C is probably due to the proteases (calpains) getting optimum thermal energy to be in action and hence gel degradation occurs in the temperature range of 28 to 45 °C. Endogenous enzymatic activity was successfully monitored in mantle of other squid species (*Loligo vulgaris, Illex coindetii, Toradores eblanae* and *D. gigas*). This proteolytic activity promotes loss of texture and protein functionality during storage or processing (Ayensa *et al.,* 2002; Gómez-Guillén *et al.,* 2002; Ruiz-Capillas *et al.,* 2003). Thereafter, the G' value triggered and reached to 350.94 kPa at 71.74 °C as a consequence of strong protein aggregation and indicating the completion of gel formation.

In conclusion, gel formation (increase in G') of squid meat progressed mainly in two steps: the 1st step at a low temperature range of 5 to 28.41 °C, and the 2nd step at temperature beyond 45 °C. Along with this, the gel breakdown occurred in the range of 28 to 45 °C with decrease in G'. This result demonstrated that the squid meat gelation properties could be enhanced by incubating squid meat at low temperature before cooking at high temperature (Torley and Lanier, 1992). This kind of behaviour has also been recorded in temperature sweep of silver carp paste (Liu *et al.,* 2007). In the present study, the trends of the G" value were similar to that of G' during temperature sweep but with lower values.

The tan δ value of the fresh Indian squid meat was 0.18 at 5 °C which relatively remained more or less similar up to 28.41 °C but consequently the value shot up tremendously and achieved its maxima (0.74) at 41.27 °C. The tan δ is the measure of energy lost as a result of viscous flow compared with the energy stored from elastic deformation in the single deformation cycle (Chan *et al.,* 2011). A change in tan δ indicates the type of the network formed where lower tan δ values represent formation of better 3-dimensional network (Sun and Arntfield, 2010). In conclusion, the values recorded for squid were very high suggesting that the squid protein did not have proper gelling ability. It can also be deduced that the tan δ value hiked in the similar temperature range (28 to 45 °C) which further confirmed that gel breakdown occurred in aforesaid

temperature range. On subsequent heating, value reduced to be almost its original value indicating formation of strong gel.

The overall trends of the dynamic viscoelastic properties (G', G" and tan δ) were found to be uniform during the storage period though there were slight shifts in the peaks of the G', G" and tan δ values. The G' value showed a continuous increase up to 12th day of storage. Although an increase in the elastic modulus on heating is often considered as a useful functional property of proteins (in gelled products), an increase in the G' value of raw materials such as fish fillets is related to protein-protein and protein-lipid interactions leading to aggregation into undesirable, tough products (Badii and Howell, 2002). There was a shift observed in the temperature range where G' decreased though it was between 24 to 42 °C during whole storage study. Moreover, it is interesting to note that the maxima of the tan δ recorded at the temperature where the value of the G' attained the lowest during throughout storage study. The temperature, where G' is at the lowest and tan delta at highest might be the temperature where proteolytic activity achieved its maxima resulting in the gel breakdown. The tan δ values of the day 2nd (0.95) and day 12th (0.98) were almost similar. The value varied from 0.35 to 0.98 during ice storage. The value was very high compared to that of reported for many fish species such as threadfin bream (0.20) (Karthhikeyan *et al.,* 2006), ribbon fish (Dileep *et al.,* 2005) and green mussel (0.30) (Binsi *et al.,* 2006).

5.6.3 Dynamic viscoelastic properties of white leg shrimp

The changes in dynamic visco-elastic properties of fresh and ice stored white leg shrimp meat have been depicted in Fig. 25. The G' value of fresh meat of white leg shrimp was 14.40 kPa at 5 °C. On further heating, the value decreased continuously up to 35.86 °C and reached to a minimum (10.68 kPa). This decrease indicates that proteins from the white leg shrimp are highly sensitive even at low temperature resulting in the unfolding of the tail region of the myosin. Similarly, loss modulus at 5 °C was 3.01 kPa, which on further heating continuously decreased to 2.45 kPa at 35.86 °C. Thereafter, the value of

G’ propelled continuously and achieved maximum value (535.77 kPa) at 64.30 °C. An increase in G’ value during thermal gelation process is indicative of elastic structure development (Binsi *et al.,* 2009). However, tan δ value was found to be increased from 0.21 to 0.25 in the temperature range of 5 °C to 39.93 °C. Subsequently, the value of tan δ dipped to 0.18 at 43.98 °C and almost maintained up 71.07 °C and on further heating return to near the original value. This further confirmed that the process of transition of sol to gel starts from 40 °C and completion of formation of elastic network at 71.07 °C where the tan δ value got stabilised. In conclusion, it can be stated that the behaviours of tan δ and G’ are inverse to each other especially during actual gelation process. Tan δ values of *Nemipterus japonicus* (Karthhikeyan *et al.,* 2006), bigeye snapper (Binsi *et al.,* 2009) and green mussel (Binsi *et al.,* 2006) showed similar kind of trends during temperature sweep.

The maximum value of G’ of the experimental shrimp recorded a continuous decrease up to 4th day of storage and remained low compare to fresh meat throughout the period of storage. Similar observations were obtained for G” but with the lesser intensity during whole period of storage study. The drift in the temperature range of increase of G’ value was not much as it remained between 35.85 °C to 37.89 °C during storage. This lesser drift in the temperature demonstrated that storage did not have much impact on the starting of the gelation process of the shrimp meat. Similarly, the temperature where, G’ value achieved maxima between 61 to 66 °C on various days of sampling during the storage period. The lowest value of tan delta was attained on 2nd day during transition phase (43.31 °C) of storage modulus. After this transition phase, the value stabilizes which was seen on all sampling days.

In general, the value G” is lower than G’ value for the fish and shellfish meat. The ratio of G” and G’ is represented by tan δ. In the present investigation, the higher values of tan δ were documented for Indian squid, compared to dhoma fish and Indian fish, indicating that Indian squid has low gelling ability. According to findings of present investigation, the temperatures of starting of actual gelation process for cephalopod, crustaceans and finfish are also different.

From the above discussion it is evident that the physico-chemical properties (viz. pH, TMA, TVBN, NPN, Ca^{2+} ATPase enzyme activity and SDS-PAGE) and histological observations are different for the three animals i.e. the fish, the squid and the shrimp on ice storage. Hence, it can be inferred that the spoilage pattern is also species specific. Functional properties also showed the same trend. The histological observations clearly showed the differences in structural arrangement of muscle architecture among all three experimental species. The rheological studies (dynamic visco-elastic properties) clearly demonstrated that behaviour of the myofibrillar proteins from all three experimental species during gelation process is entirely different. Therefore, for producing gel based products from different kind of raw materials, species wise temperatures models should be studied to achieve best product quality.

6. SUMMARY

In the present investigation, the effect of ice storage on physico-chemical, functional and rheological properties of the myofibrillar proteins from dhoma fish, Indian squid and white leg shrimp was assessed. The salient features of the study are given in this section.

- Dhoma fish (*Johnius dussumieri*), Indian squid (*Loligo duvaucelii*) and white leg shrimp (*Litopenaeus vannamei*) were used in the present study. Dhoma fish and Indian squid were purchased from the boat owners who go for single day fishing soon after they arrived at Versova landing centre in the north-west part of Mumbai city while shrimps were purchased from cultured pond in Raigarh district of Maharastra. The experimental species were transported to the laboratory in iced condition in poly- urethane boxes. Thereafter, the items were washed with chilled water and kept in flake ice (1:1) in an alternate layer in the thermacole boxes. The ice was replenished every 24 h after draining the melt water. The samples were drawn at regular interval from the thermocole boxes for analysis.
- The highest moisture content was recorded in Indian squid (84.58 %) followed by dhoma fish (81.42 %) and white leg shrimp (73.45 %) in the fresh condition. The crude protein content of all three experimental aquatic species was in the range of 14.17 to 19.90 %. The fat contents of the three aquatic species were found to be less than 2% while ash content varied from 0.87 to1.56 %.
- The highest yield of edible portion was recorded for dhoma fish (75.49 %) followed by Indian squid (64.35%) and white leg shrimp (63.64%) in the fresh condition. However, mince meat yield was higher in white leg shrimp compared to dhoma fish and Indian squid.
- The poly unsaturated fatty acid (PUFA) contents in the three experimental animals were found to be in the range of 14.55 % -19.71 %. The proportions of total n-3 fatty acids and total n-6 fatty acids were slightly higher in dhoma fish compared to those in Indian squid and white leg shrimp.

Ice storage studies

Physico-chemical properties

- Increase in the pH of the all the three experimental was observed during the ice storage.
- On the basis of chemical quality indices (TVBN and TMA) the dhoma fish, Indian squid and white leg shrimp were rejected on 6^{th} and 8^{th} day of the storage.
- The NPN content of dhoma fish and Indian squid decreased almost linearly up to the end of the ice storage, while in case of white leg shrimp the values increased on 4^{th} and 8^{th} day of storage decreased thereafter.
- The Ca^{2+} ATPase enzyme activity decreased significantly in all the three species during storage. The decrease was faster in dhoma fish followed by white leg shrimp and Indian squid during storage.
- The surface hydrophobicity of the MFP extracted from dhoma fish, Indian squid and white leg shrimp was less than 19 µg in fresh condition. The hydrophobicity increased as the ice storage progressed indicating denaturation of MFPs. The rate of increment in surface hydrophobicity of MFP was higher in Indian squid followed by dhoma fish and white leg shrimp during storage.
- The SDS-PAGE pattern of MFP extracted from all three experimental species showed mutually different patterns. The squid MFP are relatively more susceptible to ice storage compared to MFP from dhoma fish and white leg shrimp.
- The histological observation showed that the musculature of the Indian squid was very different (compact and strong) form that of the shrimp and dhoma fish. The disruptions of the muscle (gaping) was observed after 6^{th}, 6^{th} and 8^{th} day of ice storage for dhoma fish, white leg shrimp and Indian squid respectively.

Functional properties

- The initial solubility of the MFP extracted from dhoma fish was less compared to Indian squid and white leg shrimp. In case of dhoma fish,

solubility showed an increase up to 6th day while a decreasing trend was observed for white leg shrimp and Indian squid during storage.

- The apparent reduced viscosity of the MFP (5 mg/ml) as a function of ice storage showed a marginal decrease in Indian squid and white leg shrimp while that in dhoma fish increased markedly on 15th day.
- Indian squid has the highest water holding capacity followed by dhoma fish and white leg shrimp. In case of dhoma fish and Indian squid, the WHC were found to be decreased while the value increased for white leg shrimp during ice storage.
- The emulsion capacity of the MFP extracted from Indian squid was higher than dhoma fish and white leg shrimp in the fresh condition. The emulsion capacity of MFP extracted from both dhoma fish and white leg shrimp increased while that of MFP from Indian squid decreased during ice storage.
- Gel strength values of heat-induced gel prepared from the fresh meat obtained from dhoma fish, Indian squid and white leg shrimp were 313.45 g.cm, 257.49 g.cm and 80.62 g.cm respectively. In all the cases, the gel strength values recorded a significant and continuous decrease during ice storage.
- The higher lightness of the heat-induced gels produced from the fresh white leg shrimp followed by Indian squid and dhoma fish. The redness and yellowness values for the gel produced from fresh white leg shrimp were much higher than those of Indian squid and dhoma fish. The changes in the colour parameters showed different trends during storage.
- Initially, the values of the TPA parameters of the heat-induced gels produced from fresh Indian squid were higher than the dhoma fish followed by white leg shrimp. The values of TPA in all the cases were found to be decreased during ice storage.

Rheological properties

Dhoma fish

- In the fresh condition, the G' value did not vary in the temperature range of 5 to 31.80 °C while actual gelation took place between 48 to 65 °C. The G" value also followed trends of that of the G' value.
- The value of tan δ of fresh meat from dhoma fish was 0.11 at 5 °C which did not change much up to 41.95 °C (0.12) and attained a maximum value of 0.20 at 46.69 °C.
- The intensity (value) of G' and G" reduced continuously up to 6th day while highest value was recorded on 9th day (776.54 kPa at 67.01 °C). However, the values were found to decrease up to 15th day followed by a slight increase towards the end of the study.
- The tan δ value of meat found to be increased between the temperature ranges of 37.22 to 50 °C during whole ice storage study except on 9th day when it increased up to 54 °C.

Indian squid

- The G' value of the fresh Indian squid meat increased slowly up to 28°C later the value decreased and reached the minimum at 41 °C further value raised. The G" also registered similar behaviour during the said temperature range.
- The tan δ value of the fresh Indian squid meat was 0.18 at 5 °C which relatively remained more or less similar up to 28.41 °C but consequently the value shot up steeply and achieved its maxima (0.74) at 41.27 °C.
- The overall trends of the dynamic viscoelastic properties (G', G" and tan δ) have been similar during whole ice storage study but there were minor shifts in the peaks of the G', G" and tan δ values as the storage progressed.

White leg shrimp

- Initially, the G' value of fresh meat of white leg shrimp was 14.40 kPa. On further heating, the values decreased continuously up to 35.86 °C and

reached to minimum (10.68 kPa). Thereafter, the value of G' propelled continuously and achieved maximum value (535.77 kPa) at 64.30 °C.

- The tan δ value was found to be increased slightly up to 39.93 °C. Subsequently, the value of tan δ dipped to 0.18 at 43.98 °C and almost maintained up 71.07 °C and further returned to near the original value.
- The peak values of G' of the experimental shrimp recorded a continuous decrease up to 4th day of storage and remained low compared to fresh meat. Similar observations were obtained for G" but with the lesser intensity during whole period of storage study.

In the nutshell, the three species understudy were low fat, high protein and high moisture product and can be categorised as lean fish. The bio-chemical indicators suggest that fish, squid and shrimp could be kept in prime quality for 6 to 8 days in ice. On the storage, the overall quality deteriorates but fish could be kept longer. The functional and rheological properties suggest that fish is best material for surimi whereas others showed poor gelling properties. However, shrimp is easier to chew but microscopic analysis suggest that tough texture has something to do with muscle architecture and thus defining difference in eating quality.

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8. APPENDIX

Full length peer reviewed papers in the referred journals

1. Naresh Kumar Mehta, Amjad K. Balange and Binaya Bhusan Nayak, 2016. Dynamic visco-elastic behaviour and gel forming ability of Indian squid during ice storage. ***Journal of Food Processing and Preservation.*** doi:10.1111/jfpp.12891 (Accepted for Publication January 26, 2016)
2. Naresh Kumar Mehta, Snehal S. Shitole and Binaya Bhusan Nayak, 2015. Proximate composition and fatty acid profile of commercially important sin croaker fish caught along the Mumbai coast. ***Journal of Environment and Bio-science****.* 29 (2):301-304.
3. Naresh Kumar Mehta, Amjad K. Balange and Binaya Bhusan Nayak, 2015. Application of rheology in fish product development. ***Aquaculture Times,*** *1(3):46-48.*
4. Naresh Kumar Mehta, Chauksey M. K, Tripathi, G., Amjad K. Balange and Binaya Bhusan Nayak, 2015. Physico-chemical and gel properties of myofibrillar protein from sin croaker (*Johnius dussumieri*) fish during ice storage**. *Journal of Aquatic Food Product Technology.*** *(*Accepted on 27 July 2015)
5. **Naresh Kumar Mehta** and Binaya Bhusan Nayak**, 2015**. Comparative study on dynamic visco-elastic behaviour and gel forming ability of fresh meat from different finfish and shellfish. *(communicated)*
6. **Naresh Kumar Mehta** and Binaya Bhusan Nayak**, 2015**. Effect of ice storage on Dynamic visco-elastic behaviour and functional properties of the proteins from *L. vannamei*. ***Journal of Food Science and Technology*** (manuscript ready).

Conference paper

1. **नरेश कुमार मेहता एवं बिनय भूषण नायक, 2013. धोमा मछली (जोनियस दुसुमुरै) की मयोफिब्रिल्लर प्रोटीन के टेक्सचराल एवं फंक्शनल गुणो में बर्फ़ भण्डारण के दौरान परिवर्तन.** 14वी राष्ट्रीय कृषि विज्ञानं संगोष्ठी-14-17 दिसंबर, 2013, pp-57.

2. **Naresh Kumar Mehta,** A. K. Balange and Binaya Bhusan Nayak**, 2014**. Bio-chemical properties and gel forming ability of fresh Indian squid (*Loligo duvauceli*) mincemeat. In: National conference on strategies for bridging the yield gap in fisheries and aquaculture' jointly organized by PFGF India, NFDB, AFSIB, COFAA Mangalore, March 24-25, 2014, Mangalore, India,pp.117

3. **Naresh Kumar Mehta,** M.K. Chouksey, A. K. Balange and Binaya Bhusan Nayak, **2014**. Functional and Textural properties of myofibrillar protein from Dhoma fish (*Johnius dussumieri*) during ice storage. In 10th Indian fisheries and aquaculture forum (10ifaf) organized by Asian fisheries society, Indian branch (AFSIB) in collaboration with ICAR-NBFGR, 12-15 Nov., 2014, Lucknow, India.pp-499.

4. **Naresh Kumar Mehta** and Binaya Bhusan Nayak, **2016.** Functional properties and dynamic visco-elastic behaviour of proteins from white leg shrimp (*L. vannamei*) during ice storage.in symposium organised by north-east society for fisheries and aquaculture on 21-22 Jan, 2016 at Agartala, Tripura.