BIOCHEMICAL AND STORAGE CHARACTERISTICS OF MYOFIBRILLAR PROTEIN *(SURIMI)* FROM FRESHWATER MAJOR CARPS

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BY

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2000

DEDICATED TO MY PARENTS

DECLARATION

I, T.V. Sankar, do hereby declare that the thesis entitled, "Biochemical and Storage Characteristics of Myofibrillar Protein (*Surimi*) from Freshwater Major Carps" is a genuine record of research work done by me under the guidance of Dr. A. Ramachandran, Professor, School of Industrial Fisheries, Cochin University of Science and Technology, and that no part of this work has previously formed the basis for the award of any degree, diploma, associate-ship, fellowship or other similar title of any university or institution.

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CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried by Shri. T.V. Sankar, under my supervision and guidance in the School of Industrial Fisheries, Cochin University of Science and Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy and that no part of this work thereof has been submitted for any other degree.

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Chapter – 1

Introduction

Fish is the cheapest source of good quality animal protein readily available to the masses throughout the world and its demand is always on the increase. According to the Provisional Food Balance Sheet of Fish and Fishery products of FAO the world average per capita supply is 13.3 kg/annum (Anon, 1993). For the developed countries Japan had a value of 71.9 kg / annum while for Albania the figure is 2.8 kg / annum. For the developing countries St. Helena had the highest availability of 99.3 kg / annum while Afghanistan had just 0.1 kg / annum. In India, fish provides protein enrichment to 40% of the fish eaters out of the non-vegetarian population of 56% (Kumar, 1996). This is undoubtedly true in the case of coastal population where fish is the main food. The per capita availability of fish for the Indian population is 5.13 kg / annum against the national recommendation of 11 kg / annum on the basis of nutritional requirement norms, when the population of 830 million is taken into consideration. Calculating on the basis of non-vegetarian population alone, the availability is 8.7 kg / annum and on the basis of fish eating population alone, it is 12.44 kg / annum (Kumar, 1996).

The fisheries in the world are characterised by harvesting from the wild and farming of aquatic organisms, both of which contribute to the total world production of fish. The total world fish production increased from 99.01 million tonnes in 1990 to 122 million tonnes in 1997 (Anon, 1999a). About 80% of this share was from capture fisheries sector and the remaining from aquaculture. Marine sector was

responsible for about 92% of the total capture fisheries and the contribution from inland capture fisheries was a mere 8% (Anon, 1999a). However, the production from marine sector decreased from 85% to 80% during this period while that of inland sector increased from 14% to 20% of the total production. About 95% of the total marine production came by way of capture fisheries and its share declined from 87% to 77% between 1992 and 1997 with a concomitant increase in aquaculture production. The marine aquaculture share, however, increased by 6% during the period. Similarly, considering the inland sector, the aquaculture share increased from 55% in 1990 to 69% in 1997. All these figures categorically signifies the over all importance of aquaculture production.

Considering the utilisation, the share towards human consumption has only marginally increased over the years, i.e. from 72% in 1990 to just over 76% in 1997 (Anon, 1999a). The remaining goes for reduction towards fishmeal. This marginally increased demand over the years also put pressure on the production of fisheries.

1.1. The marine scenario

The total world marine fish production (Fig 1) was to the tune of 93.3 million tons in 1997 (Anon, 1999b). The overall production showed a decreasing trend since 1994. China with its production of 15.7 million tonnes occupies the primary position (Fig. 2). The production in

the case of the secondly placed Peru was only half of that produced by China. India, with its share of 3.6 million tonnes maintains the eighth position for the past couple of years and this contributes to only 3.9% of the world fish landing. However, with 8,129 km long coast line, 0.5 million square km of continental shelf and 2.02 million square km EEZ, India is still a major marine fish producer in the world (Sugunan, 1997). The total fish production showed a four-fold increase from 1951 to 1997. Considering the landing over the past few years, the total landing increased from 97.43 million tonnes in 1990 to over 108 million tonnes in 1994 and showed a decline recording 88 million tonnes in 1996 and 1997.

China, the leading country in capture fisheries, responsible for one–sixth of world fish production, recorded about 20.7 million tonnes in 1996. The value decreased to 15.7 million tonnes in 1997. Peru with second position recorded a landing close to 9 million tonnes during 1992-97 with an exceptionally high landings over 9.5 million tonnes in 1996. The trends in other countries were similar to that of China. India's share increased from 4.2 million tonnes in 1992 to 4.5 million tonnes in 1994. The value, however, decreased to 3.4 million tonnes in 1997. Eutrophication, pollution, habitat modification, inappropriate fishing pressures and poor fishery management are some of the factors contributing to this decline in capture fisheries.

1.2. The aquaculture scenario

Aquaculture is fast becoming a major area providing anchorage to fish production. The stagnating growth rate in marine fish production, the growing uncertainties about their sustainability, and the favourable environmental conditions gave a renewed boost to aquaculture. The production of total aquatic organisms increased from 15,544,640 MT in 1988 to more than double the value of 36,050,168 MT in 1997 (Anon, 1999c). Inland aquaculture production contributed to more than 60% and the remaining was from marine sector (Fig. 3). In terms of value (Fig. 4) realized the marine production had an edge over the other and is related to the production of value yielding shrimps from the marine environment. The trend, however, switched in favour of freshwater fish production since 1996-97.

According to the latest production figures of 1997, fish and shellfish accounted for about 78% of the total production (Fig. 5) and were responsible for more than 90% of the value realized from aquaculture sector. The crustaceans and aquatic plants accounted for the remaining 10%.

The aquaculture is practiced in all the three major categories of water bodies, namely freshwater, brackish water and marine waters. The production from fresh water source was responsible for more than 50% of the value realised from aquaculture sector (Fig. 6). This is primarily due to the importance given in different countries towards fresh water

aquaculture production. About 40% of the production from marine aquaculture is from other resources including shellfish.

According to 1988 figures, Asia occupied the primary spot in aquaculture production and was responsible for 82 % of world production. Europe with a share of 9.8 % took the second position. The condition remained the same even after 10 years and Asia still occupies the first position with 89% share and the production in Europe declined to 5.7% (Anon, 1999b). Among the different countries China recorded the highest production of 19,315,623 MT in 1997 contributing to 67% of the total world production. India with 6% and Japan with 3% occupied second and third positions respectively. Though other countries like USA, Korean republic, Philippines, and France contributed substantially, the production in these countries declined in recent times.

The annual production rate (APR) is fluctuating positively over the years, with 20% APR recorded in 1991 (Fig. 7). The total production in 1997, however, showed 100% increase compared to the production in 1988 (Anon, 1999b). Considering aquaculture production by category, the freshwater fishes contributed to over 90% of the aquaculture production. The crustacean production, which contributed to 2-5 % of the total production until 1992, increased by almost two fold in the subsequent years (Anon, 1999b).

1.2.1. Aquaculture production in India

The aquaculture production over the few years showed that fresh water fish contributed to a major share of 97% of the total freshwater aquaculture in 1988 (Anon, 1999b) (Fig. 8). The share marginally declined over the years alongside the marginal increase in crustacean production. The crustacean production showed an increase upto 6% in 1994 but subsequently declined due to various reasons to mere 3.2% of the total production in 1997. Various reasons including a viral epidemic caused this decline.

Among the freshwater fishes, major carps (rohu - *Labeo rohita,* catla - *Catla catla* and mrigal - *Cirrhinus mrigala)* predominated with its share increasing from 6.9% in 1987 to 85.2% in 1997 (Fig. 9). According to the latest figures (Anon, 1999c), the production of rohu was marginally higher with 35% followed by mrigal (32%) and catla (29%). The other fish species contributing to freshwater fish production include, *Anabas testudineus*, *Clarias* spp. (cat fish), *Ctenopharyngodon idella* (grass carp), *Cyprinus carpio* (common carp) and *Hypophthalmicthys molitrix* (silver carp).

1.3. Export of marine products from India

India is one of the major exporting countries and more than 118 items are exported to more than 69 countries. Export figures for the past five years indicate that the quantity exported almost doubled, i.e. from

1,62,930 tonnes in 1991 to 3,53,676 tonnes in 1996 with a 2.5 times increase in value realisation (Anon, 1996). Among the major importers Japan occupied the primary position with 22% of the quantity and almost 50% of the value followed by South East Asia including China, USA, EU and Middle East. After Indonesia, India is the largest supplier of shrimp to Japan.

Considering the 1996 figures, about 12% of the total landing is exported. The all time record was Rs. 4697.48 crores during 1997-98. According to the latest export figures of 1998-99, the total export was about 3,02,934 tonnes with a value of 4626.87 crores (Anon, 1999d). In terms of foreign exchange it was about US $ 1,106.91million. Frozen finfish contributed to a share of 35.83% realizing 10.7% of the total value (Fig 10 & 11). This is in fact a decrease of about 7% in terms of quantity and about 5% in terms of value compared to the export figures of the previous year. Pomfret, reef cod, tuna, mackerel, seer fish etc. increased the share in the export of frozen fin fishes, while export of frozen ribbon fish and freshwater fishes decreased considerably. On the other hand the export of frozen shrimp increased by about 7% both in terms of volume (33.83%) and value (72.29%). The other major export products include, frozen squid, frozen cuttle fish contributing to 10.65% and 11.42% of the export share in volume. Blanched squid and cuttlefish, seafood and vegetable mix, fish and vegetable mix in

barbecue style are some of the products, which found markets in limited quantities.

1.4. Diversification of processing industry

One of the ways of meeting the escalating world demand for fish is through exploration and exploitation of unconventional resources, besides finding more efficient method of utilising the available catch. Development of mince-based industry provides scope for the diversification of the industry for the development of international trade in value added products.

With the adoption of Exclusive Economic Zone in the early '80, there was a marked change in the fishing area under the sovereign rights of different countries. As a consequence, major fishing countries like USA, Japan etc., increased their fishing effort to harvest from their waters. Of late, the consumer has become more health and diet conscious and products with high fish fat has become desirable (Regenstein, 1986). The decreased fondness for the red meat products also contributed largely to this trend.

Lately considerable attention was given to fish mince for the production of value added products by food manufacturers primarily because of the ready and cheap availability of fish mince from fish off-cuts at a cheaper rate (Rodger *et al.*, 1980). However, the widespread use of fish mince is limited due to the instability of the fish mince. The

loss of integrity of shape in mince in comparison to fish fillets limits its use in steak products at a very significant level. Another major deterrent to the potential industrial utilisation of minced fish is the difficulty in obtaining uniform raw material as a result of variation in species, feeding habits, harvesting and post harvest handling. Though the minced fish was initially started to recover the protein from cut-off, filleting wastes etc., it became a method for the utilisation of commercially less important fishery resources. As a result, hitherto, neglected fish species that are otherwise difficult to process by traditional methods found a market.

1.4.1. Minced fish

Minced fish, the less expensive protein and an important ingredient of the mince based industry, is fish meat separated from the skin, bones, scales and fins (Grantham, 1981). Mincing employs the method of squeezing and extruding flesh along with blood and fat through a drum of 3 – 5 mm perforations to ensure coarse particle size (Newman, 1976). The process fractionates the raw material in to a range of anatomically and physiologically significant components, which can affect the flavour, and appearance of the mince. The equipment used in the separation of minces work on three different principles (Grantham, 1981). A belt and drum combination is being used in most of the equipments. In a variation of this type the belt and drum rotate at

different speeds to increase the shear force. Machines with a screw and perforated cylinder, or two concentric cylinders with inner one rotating and perforated is the other type used for the purpose.

The apparent problem associated with mincing is the incorporation of bone in the mince. The presence of bone is aesthetically undesirable besides being the source of oxidative rancidity in the mince (Lee and Toledo, 1976; Grantham, 1981). The bone content of the mince depends on the source material with cut-off and head contributing to higher levels than fillets and whole fish. There is a positive correlation between the perforation of the drum used and the bone content (Wong *et al.*, 1978). Analyses of calcium content (Dawson *et al.*, 1978), aqueous dispersion and sedimentation (Patashnik *et al.*, 1974) and urea or alkali solubilisation (Yamamoto and Wong, 1974) are some of the different methods available to determine the bone content in the mince. The bone content of the mince can be minimised to a large extent by controlling the pressure on the drums during mincing.

The yield, composition and marketing of fish mince have been extensively studied (Menon and Samuel, 1975; Perigreeen *et al.*, 1979; Joseph and Perigreen, 1983). Though the light coloured or meat coloured mince is preferred by the industry, the colour of the material largely depends on the type of raw material used for the preparation. The mixing up of melanoid pigments (Jauregui and Baker, 1980), black belly membranes, blood (King, 1973) and head and guts (Poulter and Disney,

1978) causes dark discolouration in the comminuted flesh. Storage of such mince leads to quality deterioration. Trim waste give meat coloured mince, whole fish give pinkish mince due to the mixing up of blood, kidney etc., and the filleting wastes give dark coloured mince (Regenstein, 1986). Colour standards are available for frozen mince blocks (King and Ryan, 1977).

Mixing up gut and viscera pose other problems as well. Besides contaminating the mince with microorganisms they are also potential source of heavy metals and pesticides (Crabb and Griffith, 1976; Poulter and Disney, 1978). Contamination with parasites especially in fresh water species is well known (Grantham, 1981). The haemoglobin present in the fish flesh gets dispersed as a result of mincing and catalyses the reactions in dimethylamine forming species leading to the accumulation of formaldehyde (Tozawa and Sato, 1974).

The fish mince is highly unstable than intact fish muscle (Martin, 1976; Grantham, 1981; Babbit, 1986) and need to be frozen at the earliest. The mincing leads to mechanical disruption and the process itself accelerates the disconfirmation, aggregation and cross-linking of myofibrillar protein with the consequent decrease in the salt soluble protein, water holding capacity, emulsifying capacity and rheological properties (DeKoning *et al.*, 1987; Gleman and Benjamin, 1989). Hydrolysis of lipids leading to the production of free fatty acids, lipid oxidation leading to the accumulation of oxidation products, production

of peptides and free amino acids and degradation of nucleotide are some of the reactions taking place in fish post mortem (Jiang *et. al.*, 1987). Further the quality of fish mince depends on the species of fish, season, processing and handling techniques.

Most of the marine fish and shellfish produce in their digestive process trimethylamine oxide (TMAO), which plays a key role in osmoregulation. Among the marine fishes elasmobranchs contain higher amounts of TMAO. Among the teleosts, gadoid family contains highest amounts of TMAO and the lowest amount is in flat fishes. Generally, TMAO is either extremely scanty or completely absent in freshwater fishes (Shenouda, 1980).

The TMAO, in frozen fish get converted into dimethylamine by body enzymes while in fresh or iced fish gets converted by bacterial enzyme into trimethylamine, a fishy smelling substance (Castell *et al.*, 1974; Regenstein *et. al.*, 1982). As a result of tissue disruption demethylation of trimethylamineoxide to dimethylamine and formaldehyde occurs rather rapidly. These products contribute to the decreased solubility, loss of enzyme activity and functional properties of fish proteins (Sikorski and Kotakowska, 1994) and ultimately interfere with the textural properties of fish proteins leading to sponginess of fish mince (Regenstein, 1986). The formaldehyde interacts with the side chain groups of proteins affecting their stability and denatures them. The denatured proteins interact with each other through hydrophobic bonds

affecting the texture (Ang and Hultin, 1989). Babbit (1974) compared the mince from different sources and found that the mince from fillets and skin on fillets were found to be very acceptable. Fish belonging to commercially important gaddoid family are reported to contain an enzyme in their viscera, liver and kidney capable of degrading trimethylamine oxide to dimethylamine and formaldehyde (Svensson, 1980; Suzuki, 1981). The proteolytic enzymes liberated as a result of mincing from different organs degrade the texture of the meat. The cathepsin like enzymes of the muscle (Cheng *et al.*, 1979) and the enzymes from intestine and pyloric caeca (Grantham, 1981) play a role in this. An alkaline protease of the sarcoplasmic fraction was suspected to play a key role in myosin degradation during thermal processing (Rodger *et al.*, 1980; Lanier *et al.*, 1981).

Fish is a good source of long chain polyunsaturated fatty acids (Ackman *et al.*, 1976), which are highly essential to the system from the nutritional point of view. They are highly unstable and are susceptible to both enzymatic hydrolysis or non-enzymatic oxidation. The mixing up of haemoglobin and the metal ions present in the fish accelerates the oxidation reactions. Action of the lipolytic enzymes on triglycerides and phospholipids generate free fatty acids. The lipolytic enzymes namely lipases, esterases and lipoxygenases found in the intestinal contaminants and in the dark muscles play roles in these reactions. The action of these enzymes is accelerated in chilled and fresh mince while in frozen stored

mince the low temperature inhibits their action. These products of oxidation and hydrolysis ultimately interfere with the flavour and texture of the mince (Crawford *et al.,* 1972; Lee and Toledo, 1977; Tsukuda, 1978; Sankar and Nair, 1987; Nair and Sankar, 1990).

During frozen storage, the textural properties of the proteins undergo change as a result of dimethylamine and formaldehyde produced (Jahncke *et al.*, 1992) or by the action of free fatty acid formed as a result of fat degradation or their oxidation products (Hiltz *et.al.*, 1976; Gill *et. al.*, 1979; Crawford *et al.*, 1979). The role of formaldehyde in these reactions has long been elucidated (Sikorski *et. al.*, 1976; Ang and Hultin, 1989; Ragnarsson and Regenstein, 1989). These effects are more pronounced in the case of minced fish as a result of the contaminants and they are to be removed to make the mince more stable. Actomyosin is considered to be the protein responsible for gelling properties of mince-based products and the insolubilisation of the same increases as a result of storage.

The comminuted fish mince, however, can be stabilised by adding certain chemicals. Antioxidants like Butylated Hydroxy Anisole, TBHQ (Bligh and Reiger, 1976), Butylated Hydroxy Toluene (Miyauchi *et. al.*, 1975; Poulter and Disney, 1978), Vitamin C (Moledina *et. al.*, 1977) and several other chemical and natural antioxidants commonly used for the purpose (Grantham, 1981). However, some of the antioxidants while preventing the oxidation reactions accelerate the non-

enzymatic degradation of phospholipids leading to the accumulation of free fatty acids (Mai and Kinsella, 1979). Similarly a large number of chemicals like polyphosphates, sugars and higher polysaccharide stabilise proteins during frozen storage (Grantham, 1981). The production of dimethylamine and formaldehyde was reduced by 50% in hoki mince on frozen storage by the addition of sugars (Lanier, 1994). Acceptable quality surimi could be made from Pacific whiting mince stabilised by the addition of 6% sucrose, though the colour of the surimi was slightly dull compared to the control (Simpson *et. al.*, 1995). Sugars and starches enhance the functionality of the protein as well.

1.4.2. Myofibrillar protein or Surimi

The inherent property of minced fish is the unique texture forming ability, which makes it a suitable base for the manufacture of a variety of seafood-based products. The mince whether produced in simple form or in formulated products is susceptible to rapid bacterial and enzymatic spoilage. The pressurised extrusion of flesh through the drum permits the mixing up of bacteria and undesirable components throughout. The fine particulate nature of the mince provides nutrients for the bacteria to multiply.

The technologies developed with a view to prevent or reduce the freeze denaturation encountered in the frozen storage paved way for the development of surimi processing. In fact, surimi is one of the oldest

traditional preparations of Japan. The first commercial production of surimi was started in 1960 using the then, low value fish Alaska Pollock (*Theragra chaleogramma*). The utilization of surimi increased drastically in recent times because of its unique texture, high protein and low fat content. Because of this increased demand, substantial efforts are being made in many countries to study the suitability of other species for surimi production (Gopakumar *et al.*, 1992; Kim *et. al.*, 1996).

Besides Alaska Pollock, croakers, lizardfish, sharp-toothed eel, cutlass fish, horse mackerel, sharks and flounders (Suzuki, 1981), Chum salmon (Saeki *et al.*, 1995) and Northern Squafish (*Ptychocheilus oregonensis*) (Lin and Morrissey, 1995) and Pacific whiting (Pipatsattayanuwong *et al.*, 1995) are some of the fishes, which have been used for the preparation of myofibrillar protein concentrate. The list includes some of the New Zealand fishes like Sea perch (*Helicolems percoides*), red ginnard (*Gurrupiscis kunu*), grouper (*Polyprion onygeneios*), red cod (*Physiculus bachus*), etc. However, two fishes Barracouta (*Thyrsitesmatun spp.*) and elephant fish (*Callorhynchus milli*) did not have the kamaboko forming ability. Similarly a number of Argentine, Chilian and Philipino fishes including the brackish water fishes like milk fish (*Chanos chanos*) and tilapia have been utilized for the preparation of surimi. Red hake (Lee, 1986), silver hake, white hake, Atlantic croaker, Atlantic menhaden, Pacific whiting (Chang - Lee *et al.*, 1989; Pacheoco-Aguilar *et al.*, 1989; Chang - Lee *et al.*, 1990; Morrisey

et al., 1993) arrowtooth flounder (Green and Babbit, 1990) also have been tried for surimi preparation but have not been successful due to higher proteolytic enzymes and higher fat content besides the dark meat and limited stock. Special processing techniques for the production of surimi from oil sardines have been developed by Nishioka (1993).

The barracuda (*Sphyraena* spp.), threadfin bream (*Nemipterus japonicus*), ribbon fish (*Trichiurus savala, Trichurus lepturus)*, kalava (*Epinephilus diacanthus*), lizard fish (*Saurida tumbil*) tilapia, and *Priacanthus* spp. from the Indian waters were found to have utilisation for surimi production (Gopakumar *et al.*, 1992; Muraleedharan *et al.*, 1996a; Muraleedharan *et al.*, 1997).

Surimi or myofibrillar protein concentrate is a mechanically deboned, minced fish, washed to remove blood, fat, soluble pigments and other odouriferous substances and stabilised by the addition of cryoprotective agents to increase their frozen shelf life (Lee, 1986; Ofstad *et al.*, 1990). As it is the frozen myofibrillar protein, emphasis is given on protecting the unique texture forming and water holding characteristics. Further the absence of flavour permits its utilisation as a vehicle for flavour addition in the preparation of analogue products. Basically, colour, gel forming ability and stability during frozen storage are the important factors of the washed mince responsible for the development of mince-based products.

As the idea behind the preparation of surimi is to utilise fish protein as an ingredient in the preparation of value added products, it is mandatory to have the material in an exceptionally good form with high stability. The retention of gel forming ability and water holding capacity of actomyosin are essential in the manufacture of surimi-based products (Lee, 1984). Denaturation and aggregation of myofibrillar protein play a dominant role in changing the functional quality of frozen fish meat (Shenouda, 1980). The stabilisation hence requires the removal or inactivation of denaturants and protection of protein from the remaining denaturant action. Water washing or leaching the fish meat accomplishes the first part and addition of cryoprotectants play a role in stabilising the washed meat. Low molecular sugars, sugar alcohol, phosphates etc. have been used for the purpose.

1.5. Present status of surimi industry

The world production of surimi is approximately 5,30,000 MT / year. The consumption of surimi and the production of surimi based products are 5,03,000 MT and 11,22,000 MT / year respectively (Ishikawa, 1996). Among the major producers USA ranks first followed by Japan, Thailand and Korea. According to the latest figure, about 88% of the surimi produced in USA is exported to Japan (Anon, 1997). Production of surimi in Russia pinnacled during 1998 posing a contender in the market. Among the consuming countries, Japan

contributes to 75% of the world's consumption and Korea and USA ranks 2nd and 3rd positions. The consumption of surimi based products increased tremendously in China and Thailand in 1998 creating a price rise and demand (Holmes, 1998). Japan contributes to about 73% of the production of total world's surimi based products. In recent times the trend is towards diversification of fish species for the production of surimi. USA, New Zealand, European countries and some of the developing countries are engaged in exploring the possibilities of utilising locally available species for surimi production. Developing countries like India has recently taken efforts to utilise the trawl by catch for conversion to surimi. Though the production and consumption of surimi and surimi based products have been spread to different countries, Japan still occupies the primary position and the world surimi market fluctuates with the economy of Japan.

During 1996 and 1997, the import of Alaska pollock surimi to Japan decreased slightly to 1,25,000 tonnes. However, the import of hake or cod surimi increased enormously with United States contributing to more than 95%. Threadfin bream surimi production in Thailand and its export to Japan also increased during this period (Anon, 1999a).

In India the production of surimi started in the early 1990s. During 1992, the production of surimi was a mere 1540 kg and the total quantity was exported to Japan and in 1993 the export rose to 95,612 kg

(Anon, 1997). The production increased to 8,27,440 kg in 1994, 20,11,760 kg in 1995 and 58,32,445 kg in 1996 (Fig. 12). The total worth of surimi export for 1996 was Rs. 30,45,57,786. During 1994 the export was restricted to Rep. of Korea and Japan but subsequently, Indian surimi found markets in China, Taiwan, Malaysia, Singapore, Thailand, Kuwait and Australia. During 1996, more than 55% of the surimi produced was exported to Rep. Korea and Japan imported only 20%. The Indian surimi is mainly produced from *Nemipterus japonius*, *Priacanthus hamrur* and *Otolitus* spp..

1.6. Surimi based products

The characteristic ability of surimi to form gels makes it an excellent base in fabricated seafood products like imitation crabs, shrimps, scallops, lobster, etc. The uniqueness of surimi is attributed to its structural proteins, which in turn is responsible for its functional characteristics. The sophistication of infrastructure is related to the extent of simulation required. The products are classified according to the method of production or fabrication and structural features, as moulded, fiberised, composite moulded and emulsified (Lee, 1984).

Moulding the chopped surimi into desired shapes and allowing it to gel produces the ***moulded products***. The extrusion may be single extrusion or co-extrusion and the former gives a rubbery mouth feel while the latter meat like texture. The ***fiberised products*** are prepared by

extruding surimi of top quality as thin sheet through a rectangular nozzle. The sheets are heat set and cut in to stripes of desired width. The stripes are rolled and further processed into final products. Simulated crab leg products and shellfish products belong to this category. In ***composite moulded products***, surimi sheets of required lengths are mixed with or without surimi paste and extruded into desired shapes. These products give better bite than other moulded products. Fish ham is one type of composite moulded product. The surimi is emulsified with fat of animal or vegetable origin upto 10% level depending on the final product and processed to make ***emulsified surimi products***. The emulsified surimi paste is packed into casings and steamed to make the final products. Sausages belong to this type of product.

Surimi has been used in the production of a number of kneaded products, which are classified according to the manufacturing processes. The list include kamaboko (steamed), chikuwa (tube shaped boiled or baked), fried kamaboko (deep fried), hampen (boiled), fish ham, satsumaage (fried fish product) and fish sausages (Miyake *et. al.*, 1985; Kano, 1992). Texturised products are prepared by modifying the elasticity of the surimi to the desired texture by incorporating ingredients like, starch and egg white. The flavour of the final product was modified by addition of extracts of natural seafood. Further, surimi is more flexible than fish block for product development and hence surimi can be considered as a major alternate fish source for the future.

1.7. Aim and objectives

The information available on the utilisation of freshwater fish for the preparation of mince-based products is scanty. The fresh water fish contribute to about 15% of the over all fish production in India. The share of freshwater fish is only on the increase, like, 10 % in 1990, 13.6% in 1992, 13.5% in 1994, 15.2 in 1996 and 14.6 % in 1997 (Anon, 1999a; Anon, 1999b). This figure might be doubled in the near future with the present rate of expansion and development of aquaculture.

In India, several species of freshwater fishes are widely cultivated and are popular among the population in the interior places. The coastal population displays an inherent preference for marine fish. Generally, freshwater fish is consumed fresh and information on the utilisation of the same for other purposes including product development is scanty. Further, the lack of proper marketing strategy for freshwater fishes cripples its utilisation quite often. Most often, the catch is taken to a distant place and sold at throwaway prices. This necessitates the requirement for a better utilisation technique.

There are reports available on the utilisation of mince from tilapia for the preparation of spiced mince (Zain, 1989) and for production of smoked products (Obileye and Spinelli, 1978). Mince from silver carps have been utilised for the production of sausages comparable to commercial sausages (Gleman and Benjamin, 1989) and emulsion-based products (Angel, 1979). In India, the ice storage studies on the major

carps demonstrated that they have excellent keeping quality upto 16, 15 and 17 days for rohu, mrigal and catla respectively (Joseph *et al.*, 1990).

With this background, this study aims to evaluate the possibilities of utilising freshwater major carps – rohu (*Labeo rohita),* catla *(Catla catla)* and mrigal *(Cirrhinus mrigala)*- for the preparation of fish mince and myofibrillar protein concentrate. Hence, the objectives include

1. Study the complete biochemical composition of the Indian major carps - *Labeo rohita, Cirrhinus mrigala* and *Catla catla.*
2. Study the characteristics of myofibrillar proteins, the essential components responsible for the gelling properties of surimi, from major carps.
3. Standardise a washing schedule for the preparation of myofibrillar protein concentrate (surimi) from Indian major carps.
4. Study the compositional changes associated with carp proteins as a result of washing during the production of myofibrillar protein concentrate.
5. Study the storage characteristics of myofibrillar protein concentrate from major carps.

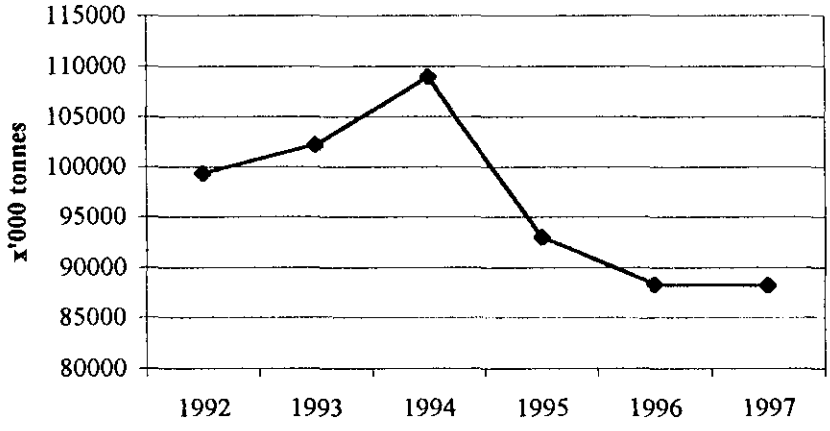


Fig 1. Total world marine production for the past six years

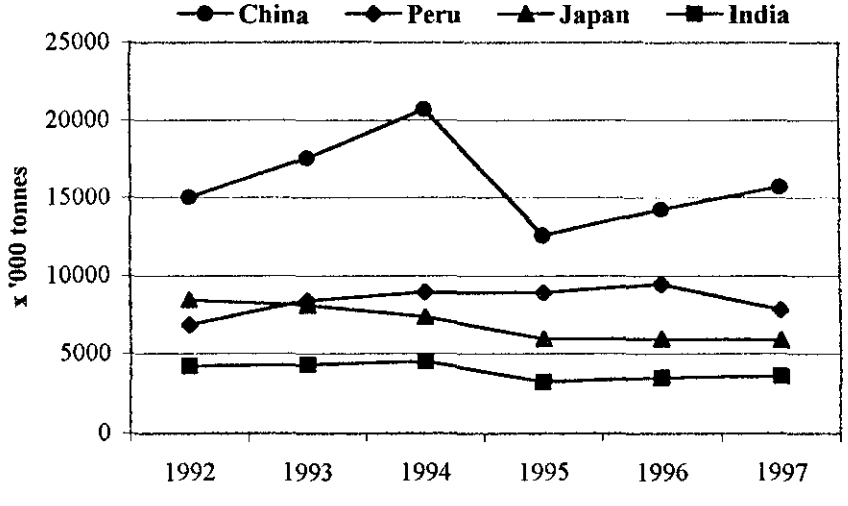


Fig 2. Marine production in four top countries for the past six years

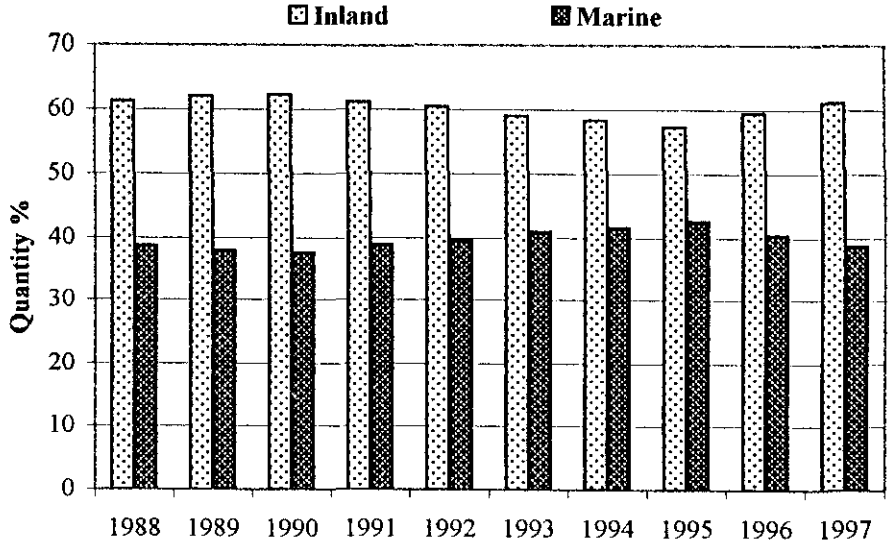


Fig. 3 World aquaculture production from 1988 to 1997

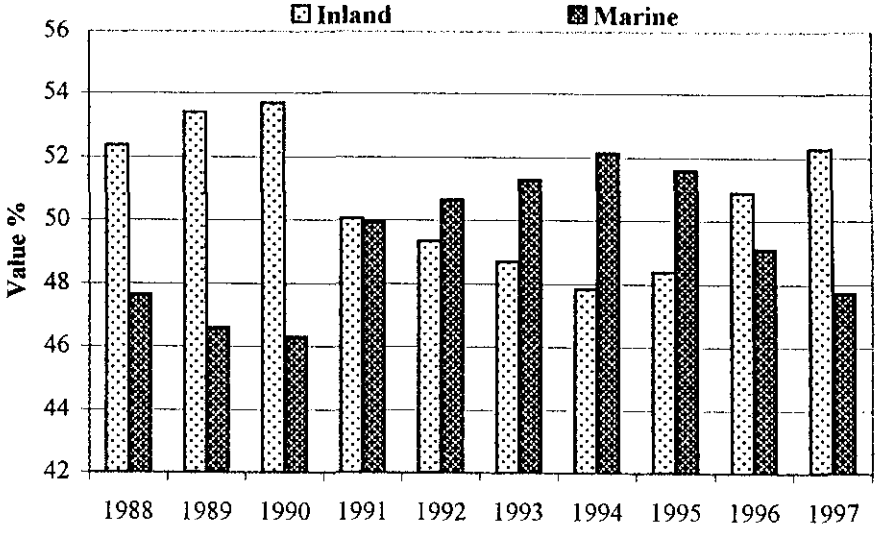


Fig. 4 Value realised from aqualculture production by category

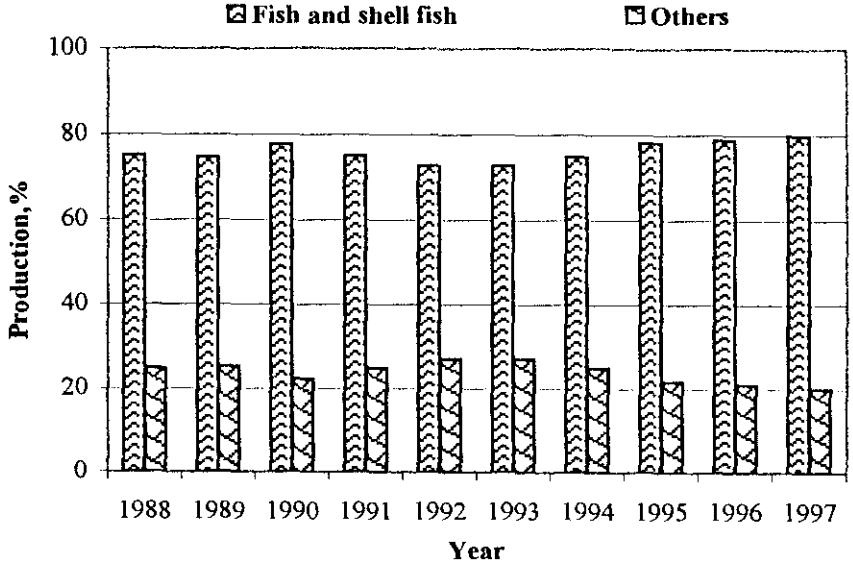


Fig 5. Total world aquaculture productionof fish, shelfish and others

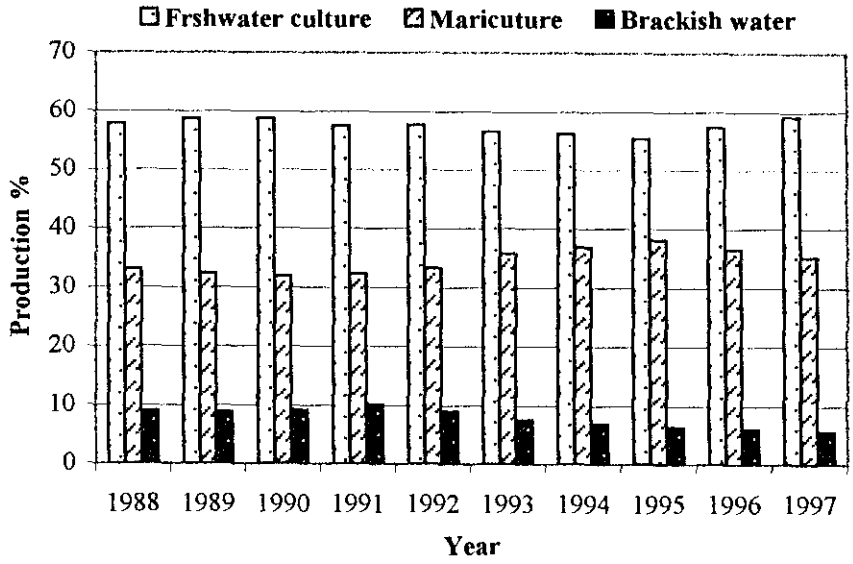


Fig 6. Total aquaculture production in different culture systems

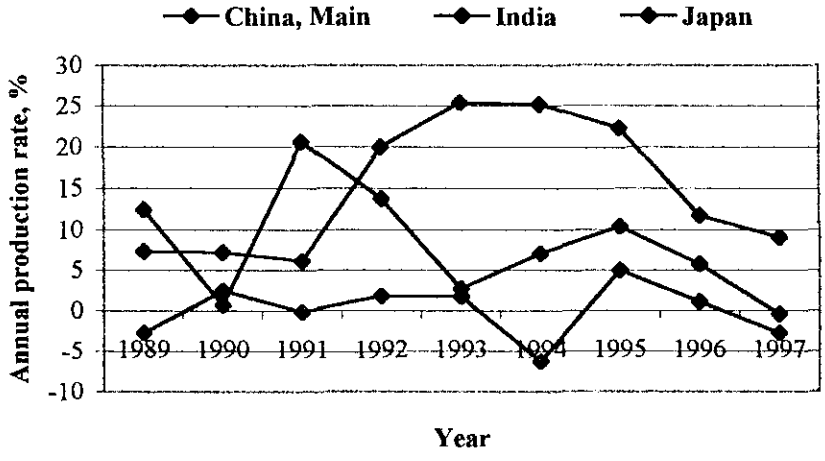


Fig 7. Annual production of three major producers of Asia

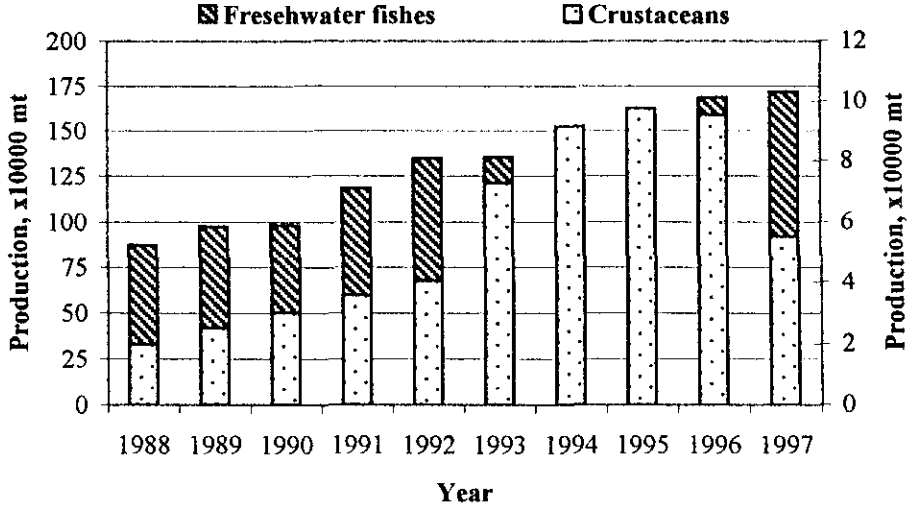


Fig 8. Category wise production in India for the past ten years

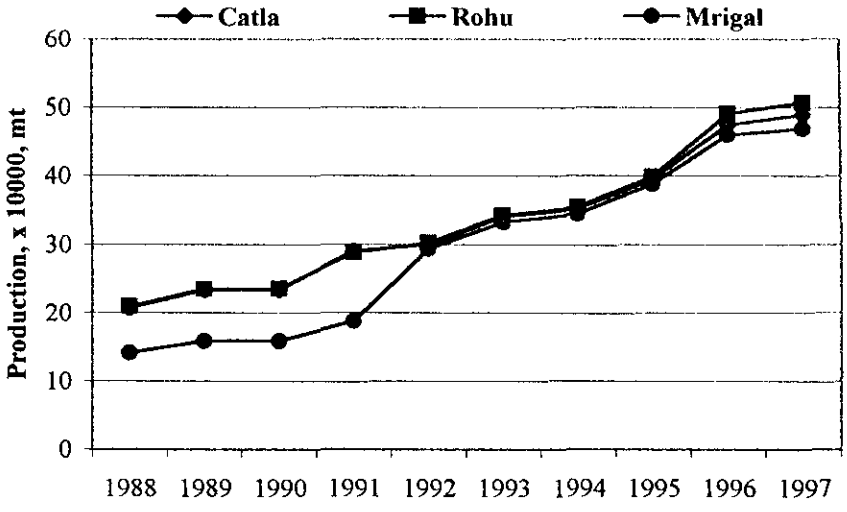


Fig 9. Production of Indain major carps in India over the past ten years

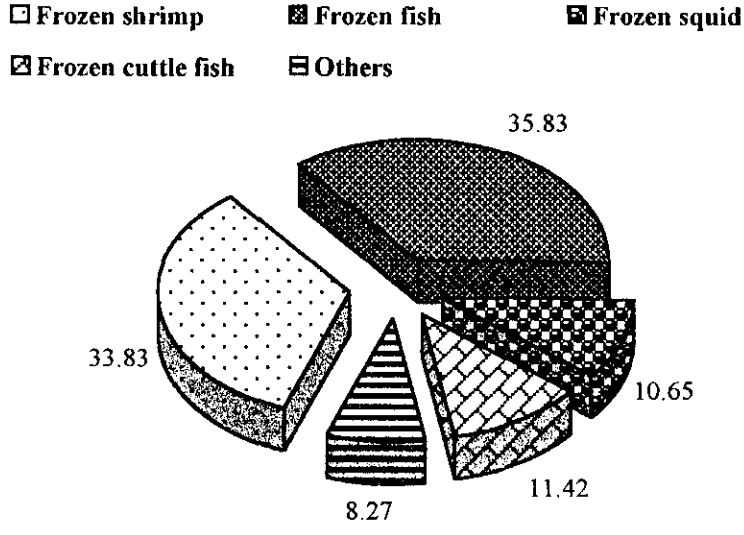


Fig 10. Export share of diferent commodities during 1998-99

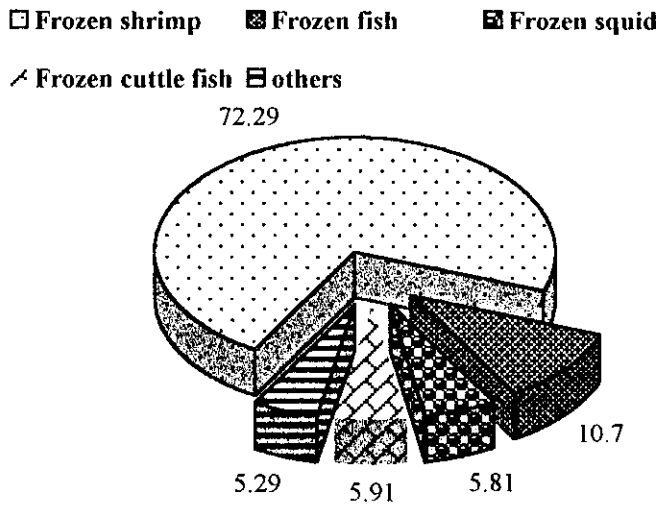


Fig 11. Export share value (%) of different commodities during 1998-99

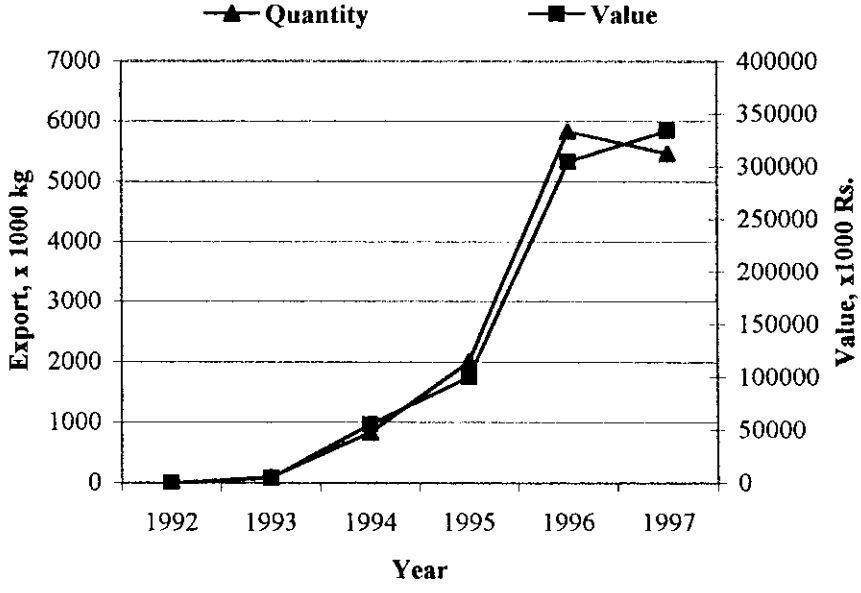


Fig. 12 Export of surimi from India during 1992-97

Chapter – 2

Materials and Methods

2. 1. Raw materials

The raw materials for the study are the three fish belonging to the family *Cyprinidae*, namely rohu (*Labeo rohita* – Hamilton-Bachanan), catla (*Catla catla* – Hamilton - Bachanan), and mrigal (*Cirrhinus mrigala* - Hamilton – Bachanan).

2. 2. Description and Biology of the fish

The three fish fall into the category of Indian major carps and are extensively found in the inland water bodies of the country. The morphological and other details are as described by Talwar and Jingran (1981).

2. 2. 1. Rohu (*Labeo rohita*)

Rohu (Fig 1) is characterised by a moderately elongate body with dorsal profile more elongated than the ventral profile. Snout fairly pressed, projects beyond mouth and devoid of lateral lobe. Eyes large; mouth small and inferior; lips thick and fringed, with distinct inner fold to each lip. Barbels either absent or small maxillary barbels concealed in lateral lobe. Dorsal fin inserted midway between snout- tip and base of caudal fin. Pectoral fins shorter than head. Caudal fin deeply forked. Scales moderate; lateral lines with 40-44 scales. The live fish is bluish in colour along back, becoming silvery on the flanks and beneath, with a reddish mark on each scale during breeding season. They have reddish eyes and grayish or dark fins except the pectoral fins, which are dusky.

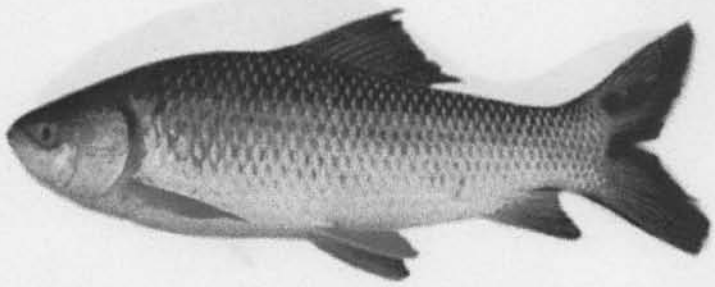


Fig 1. Rohu (*Labeo rohita*)

The body colour tends to vary in fish living among weeds, exhibiting greenish black on back.

This graceful reverine fish, a natural inhabitant of freshwater sections of the rivers of North India, is extensively cultured in India along with catla and mrigal. It is a bottom feeder and feeds on plant matter including decaying vegetation. It attains maturity towards the end of second year and the spawning season generally coincides with the Southwest monsoon. The fish attains a maximum length of one metre.

2. 2. 2. Catla (*Catla catla*)

One of the renowned and fastest growing Indian major carps, catla (Fig 2) is characterised by a deep body and enormously large head. Mouth wide and upturned, with a protruding lower jaw. They have long pectoral fins extending upto pelvic fins. Scales are conspicuously large and lateral line has 40 – 43 scales. The live fish is grayish on back and

Fig 2. Catla (*Catla catla*)

flanks and silvery white below. The fins are dusky. Generally those inhabiting in weedy or turbid ponds have a darker colour.

The fish was originally confined to the plains north of Krishna, but has been extensively introduced to practically all river systems and many of the tanks and reservoirs. It is non-predatory and its feeding is restricted to the surface and mid waters. It grows to a length of 40 - 45 cm in the first year and under favourable environmental condition grows to 120 cm in three years. The spawning season coincides with Southwest monsoon in Northwest India and in the rivers it is somewhat variable.

2. 2. 3. Mrigal (*Cirrhinus mrigala*)

The fine fish with elegant appearance, mrigal has been transplanted into waters of peninsular India for aquaculture. The fish has a streamlined body with its depth equal to that of its head. It has a blunt snout and often with pores. It has a broad mouth with entire upper lip and indistinct lower lip. The dorsal fin is as high as body. The pectoral

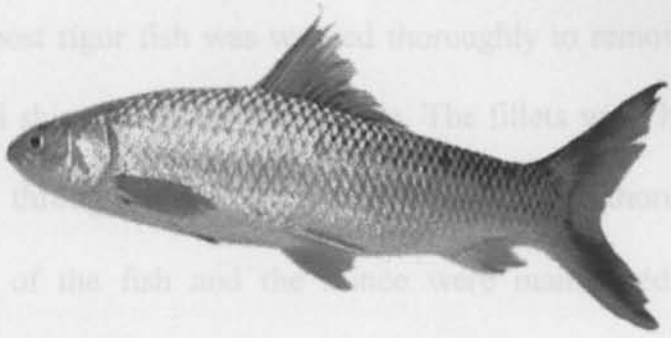


Fig 3. Mrigal (*Cirrhinus mrigala*)

fins are shorter than head and the caudal fins are deeply forked. The lateral line has 40 – 45 scales. Live fish is dark gray along the back often with a coppery tinge and the flanks are silvery with a yellow tinge. The belly is silvery white and the eyes are golden. The pectoral, pelvic and anal fins are orange tipped while dorsal and caudal fins are dusky. It is bottom feeder subsisting on decayed vegetation and breeds during southwest monsoon in shallow pockets.

2. 3. Sample preparation

Fish was collected from the culture ponds in absolutely fresh condition and brought to the laboratory in partially iced condition. The fish was kept overnight to resolve the rigor and the post rigor fish was taken for processing.

2. 3. 1. Sample preparation for biochemical analysis

The post rigor fish was washed thoroughly to remove the slime, dirt etc., and skin free fillets were made. The fillets were homogenised by passing through a hand extruder and mixed thoroughly. The temperature of the fish and the mince were maintained below 5°C through out. From this, samples were taken for the analysis of proximate composition.

2. 3. 2. Protein studies

Fresh major carps, rohu, catla and mrigal were harvested and brought to the laboratory in prime condition. They were stored in partially iced condition to resolve rigor and the post rigor samples were taken for the study. As the aim here was to compare the differences, if any, between the differently sized fish, fish of two different sizes – a smaller size weighing around 500g and a commercial size weighing above 1000g- were selected based on the gonadal maturation stage of the fish. The experimental details are given in Chapter - 4 (Characteristics of myofibrillar proteins).

2. 3. 3. Washing experiments

The washing experiments were conducted to study two major aspects viz. the efficiency of water and brine (0.2% NaCl) in the extraction of soluble proteins and the changes in the composition of fish

muscle as a result of washing. The detailed schedule followed is discussed in the respective chapter.

2. 4. Analytical methods

2. 4. 1. Proximate composition

The dressed fish was homogenised and the mince samples were taken for different analytical experiments. Moisture, protein, fat and ash were determined according to the methods of AOAC (1990). The values were expressed as g per 100g.

2. 4. 2. Fatty acid profile

The muscle lipids were extracted by cold extraction (Folch *et al.*, 1957) using 2:1 mixture of chloroform and methanol. The quantitative derivatisation of fatty acids to fatty acid methyl esters (FAME) for the ultimate analysis using Gas Liquid Chromatography was carried out using Boron triflouride-methanol (BF_3-CH_3OH) reagent (Matcalfe *et al.*, 1966). Approximately 150 mg of fatty acid material was refluxed over a water-bath with 4 ml of 0.5 N methanolic sodium hydroxide. To the saponified sample 5 ml of BF3-CH_3OH reagent was added and refluxed for another 2 min. To the mixture sufficient saturated sodium chloride was added to separate the fatty acid methyl esters, which was then extracted into ether layer. The ethyl layer was dried over sodium sulfate and injected into GC for analysis.

2. 4. 2. 1. Gas Chromatographic Analysis

The FAMEs were analysed by a Chrompack CP 9001 gas chromatograph equipped with a flame ionisation detector (FID). The column used was 10% OV 275 on Chromosorb HP (6 feet by 1/8 inch outer diameter). The column temperature was programmed from 100°C to 160°C at a rate of 3°C per minute and from 160°C to 220°C at a rate of 5°C per minute. The injector and detector temperatures were kept at 240°C and 250°C respectively. Nitrogen was used as mobile gas at a flow rate of 13.5 ml per minute. The flow rates of hydrogen and air were 60 and 250 ml per minute respectively.

2. 4. 3. Amino acid profile

2. 4. 3. 1. Sample preparation

About 100 mg of fish mince with 10 ml of 6N HCl was digested at 110°C in sealed tubes for 24 hours. The solution from the tubes were filtered and flash evaporated thrice using distilled water to remove HCl and taken in a buffer (Sodium citrate tribasic, perchloric acid, n-caprylic acid, pH 2.2).

2. 4. 3 .1. 1. HPLC Analysis

The HPLC analysis was carried out according to the method of Ishida *et al.,* (1981). The sample thus prepared was filtered using a membrane filter of 0.45µm and 20 µl was injected into Shimadzu HPLC – LC 10 AS, fitted with a packed column (ISC-07/S1504-Na). The

column material was a strong acidic cation exchange resin i.e. Styrene divinyl benzene copolymer with sulfinic acid. The elution buffers used were as described in the HPLC manual. Oven temperature was kept at 60°C. The amino acid identification was done by non-switching flow method and fluorescence detection after the post column derivatisation of amino acids with O-phthaladehyde.

2. 4. 3. 2. Estimation of Tryptophan

Tryptophan was estimated as per the method of Sastry and Tummuru (1985) after alkali hydrolysis of the sample using 5% sodium hydroxide at 110°C for 24 hours.

2. 4. 4. Muscle protein composition

The homogenised mince was fractionated by a procedure (Hashimoto *et al.*, 1979) shown in Fig 4 with some modifications. In place of phosphate buffers prescribed, Sodium bicarbonate solution (I=0.05) was used to extract sarcoplasmic nitrogen fraction and NaCl - bicarbonate solution, 0.48M NaCl and 0.02M $NaHCO_3$ (I= 0.5), was used to extract the myofibrillar protein fraction. All the operations were performed at 3-4°C as quantitatively as possible.

Six grams of muscle was homogenised with 60 ml of $NaHCO_3$ buffer of pH 7.5 (I=0.05) using a polytron homogeniser at 10000 rpm for 90 seconds. The homogenate was centrifuged at 5000 x g for 15 min. To the residue was added, 60 ml of the same buffer and the mixture

homogenised and centrifuged again. These two supernatants were combined and to it trichloroacetic acid was added upto 5% level. The resulting precipitate was collected by filtration and used as **sarcoplasmic protein fraction**. The filtrate was used as **non-protein nitrogenous compound fraction**.

The above residue was homogenised with 10 volume of NaCl-NaHCo3 buffer of pH 7.5 (I=0.5) and centrifuged as above. The residue was similarly homogenised and centrifuged again and the combined supernatant was used as **myofibrillar protein fraction**.

The residue obtained consisting of mostly denatured actomyosin was subjected to exhaustive extraction with 0.1N NaOH. The mixture was centrifuged and the supernatant was collected as **alkali-soluble protein fraction**. The final residue was used as **stroma protein fraction**.

The protein and non-protein fractions collected as above were analysed for nitrogen by micro-kjeldahl method and the protein composition of the muscle evaluated.

2. 4. 5. Autolysis

The mince was blended with water in the ratio of 1:4. The autolysis methodology described by Green and Babbit (1990) was adopted. 2 ml of the homogenate with 4 ml buffer of different pH (2 – 10) was incubated at 55°C for one hour as described. The reaction was

stopped by adding 11% trichloroacetic acid (5 ml). The Folin positive material in the supernatant was determined by the method of Herriot (1955). The activity was expressed as μmol tyrosine released per ml test sample per minute.

2. 4. 6. Total Salt Soluble proteins

The total salt extractable protein (King and Poulter, 1985) was obtained by homogenising 3 g meat or washed meat with 60 ml of 5% NaCl containing 0.02M $NaHCO_3$ (pH - 7.5), for 2 min. The homogenate was centrifuged for 20 min. at 5000 X g, in a refrigerated centrifuge at 3-5° C, to collect the salt soluble fraction.

2. 4. 7. Sarcoplasmic protein

The sarcoplasmic proteins were extracted by homogenising 3 g fish meat or washed fish meat in 0.02M $NaHCO_3$ (pH 7.0 to 7.5), instead of plain water. The homogenate was centrifuged at 5000 X g, in a refrigerated centrifuge at 3-5°C, for 20 minutes to collect the water-soluble sarcoplasmic proteins.

2. 4. 8. Myofibrillar protein

The difference between total salt soluble protein (2. 4. 6) and water-soluble sarcoplasmic protein (2. 4. 7) was taken as total myofibrillar protein.

2. 4. 9. Total SDS soluble protein

The method described by Lin and Park (1996) was adopted. 3 g mince, washed mince or filtrate was homogenised with 5% (W/V) sodium dodecyl sulphate (SDS) solution to a final volume of 30 ml. The homogenates were incubated at 80°C in a water bath for one hour so as to dissolve all sarcoplasmic and myofibrillar proteins. Supernatant after centrifugation (7,500 x g for 20 min.) was taken as soluble protein.

2. 4. 10. Preparation of actomyosin

Actomyosin was prepared according to the method of Jiang *et. al.*, (1987). 10-g fish muscle was blended using a warring blender, subjoined with a baffle plate for 2 min. with 90 ml of chilled 0.6M KCl, pH 7.2. The extract was centrifuged at 5000 x g, for 20 min. The supernatant was diluted with 2 vol. of chilled distilled water. The precipitated actomyosin was separated by centrifugation at 5000 X g, 0° C, for 20 min was then suspended in chilled 0.10M KCl, pH 7.2, an ionic strength almost equivalent to that of fish muscle and mixed with a magnetic stirrer for 1 hour at 0° C.

2. 4. 11. Estimation of soluble proteins

2. 4. 11. 1. Biuret method

The total salt soluble protein and actomyosin were estimated by biuret method (Gornall *et al.*, 1949). Bovine serum albumin was taken as standard.

2. 4. 11. 2. Lowry method

The SDS soluble proteins in the case of washing experiments were determined by the method of Lowry *et al.*, (1951) using Bovine serum albumin as standard.

2. 4. 12. Adenosine Tri Phosphatase (ATPase -Ca^{2+})

The ATPase activity of actomyosin was estimated as per the method of Jiang *et.al.*, (1987). To 1 ml of actomyosin solution (1 mg/ml) were added 0.5 ml of 0.5 M Tris- maleate buffer (pH 7.0), 0.5 ml of 0.1 M $CaCl_2$, 7.5 ml of distilled water, and finally 0.5 ml of 20 mM ATP (Na- salt) pH 7.0 in that order. The reaction was stopped by adding 5 ml 15 % trichloroacetic acid, 3-min after the addition of ATP at 25°C. The inorganic phosphate liberated was determined according to the method of Fiske and Subbarow (1925).

2. 4. 13. First order rate constants of Adenosine Tri Phosphatase

The first order rate constant (K_D) for inactivation of myofibrillar Ca-ATPase was calculated using the relation,

$K_D = (\ln C_0 - \ln C_t)\ 1/t$

where,

C_0 and C_t are ATPase activities before and after exposure to a temperature for "t" seconds (Tsai, *et al.*, 1989). The rate constants for all the three fish were compared to establish the stability of actomyosin.

2. 4. 14. Sulfhydryl group

The total reactive sulfhydryl group in the protein molecule were estimated based on the method of Sedlak and Lindsay (1968). The actomyosin solutions treated at various temperatures were centrifuged at 10,000 rpm for 15 minutes. The supernatant was taken for the analysis of total sulfhydryl group, as they are reported to give an indication on the extent of protein denaturation.

2. 4. 15. Surface hydrophobicity

The surface hydrophobicity of the native and heat-treated actomyosin solutions was estimated by the fluorescence technique using Cis-parinaric acid (9,11,13,15 –cis.trans.trans.cis-octadecatetraenoic acid) as fluorescence probe (Kato and Nakai, 1980).

2. 4. 16. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis

To establish the characteristics of the different protein fractions and for investigation of the changes in electrophoretic separation of proteins during different processing conditions, SDS-PAGE was performed according to Laemmli, (1970).

The protein extracts prepared were mixed with Laemmli sample buffer, containing buffer, 1% SDS, 25% glycerin, 2% mercaptoethanol and 0.2% bromophenol blue (pH 7.2), in the ratio of 1:1 and boiled for 2 min and kept frozen pending electrophoresis.

The concentration of separating polyacrylamide gel used was 10% and a stacking gel concentration of 4% was employed to stack the sample. After the electrophoretic run (200V,current) in 0.375M Tris-Glycine-SDS buffer, the gels were stained with 0.12% Coomassie blue in methanol for 30 min. They were destained with 7% acetic acid.

2. 4. 17. Water retention (WR)

Water retention of mince and washed mince was carried out by the method of Kocher and Foegeding (1993) with slight modification. Instead of micro centrifuge tubes with separator, the mince or washed mince (approximately 1 g) was taken in two layers of Whatman No.1 filter paper and centrifuged at 2500xg for 10 min in a Remi cooling centrifuge at 25°C. Water retention was determined as:

$$WR = \frac{\text{(Total water in sample)- (g water released)}}{\text{Total g protein in sample}}$$

2. 4. 18. Gel forming ability

2. 4. 18. 1. Preparation of heat induced gels

Heat induced gels were prepared from both mince and surimi by grinding the myofibrillar protein with 3% NaCl for 20 minutes using a kitchen mixer. During grinding, the temperature of the surimi gel was kept below 10°C to preserve the functionality of the actomyosin (Lee,

1984). The resulting paste so obtained was stuffed manually into polypropylene tubing of 5.0 cm diameter, taking care to eliminate the trapped air as much as possible. The ends of the tubes were tied and cooked by immersion in a water bath maintained at 90°C for 30 minutes. The gels were, then, cooled at once in ice and then kept at 5°C over night and analysed. The rheological characteristics were studied by following folding test, gel strength and compressibility of the gels prepared.

2. 4. 18. 2. Folding test

The grade of the surimi formed according to the extent of crack when a 3mm thickness gel is cut and folded between thumb and index finger (Lee, 1984). Then different grades were given according to the following breaking characteristics of the gel formed, such as,

AA - No crack occurs after folding twice,

A - Crack occurs after folding twice, but no crack occurs after folding once

B - Crack occurs gradually after folding once,

C - Crack occurs immediately after folding once, and,

D - Breakable by finger press without folding.

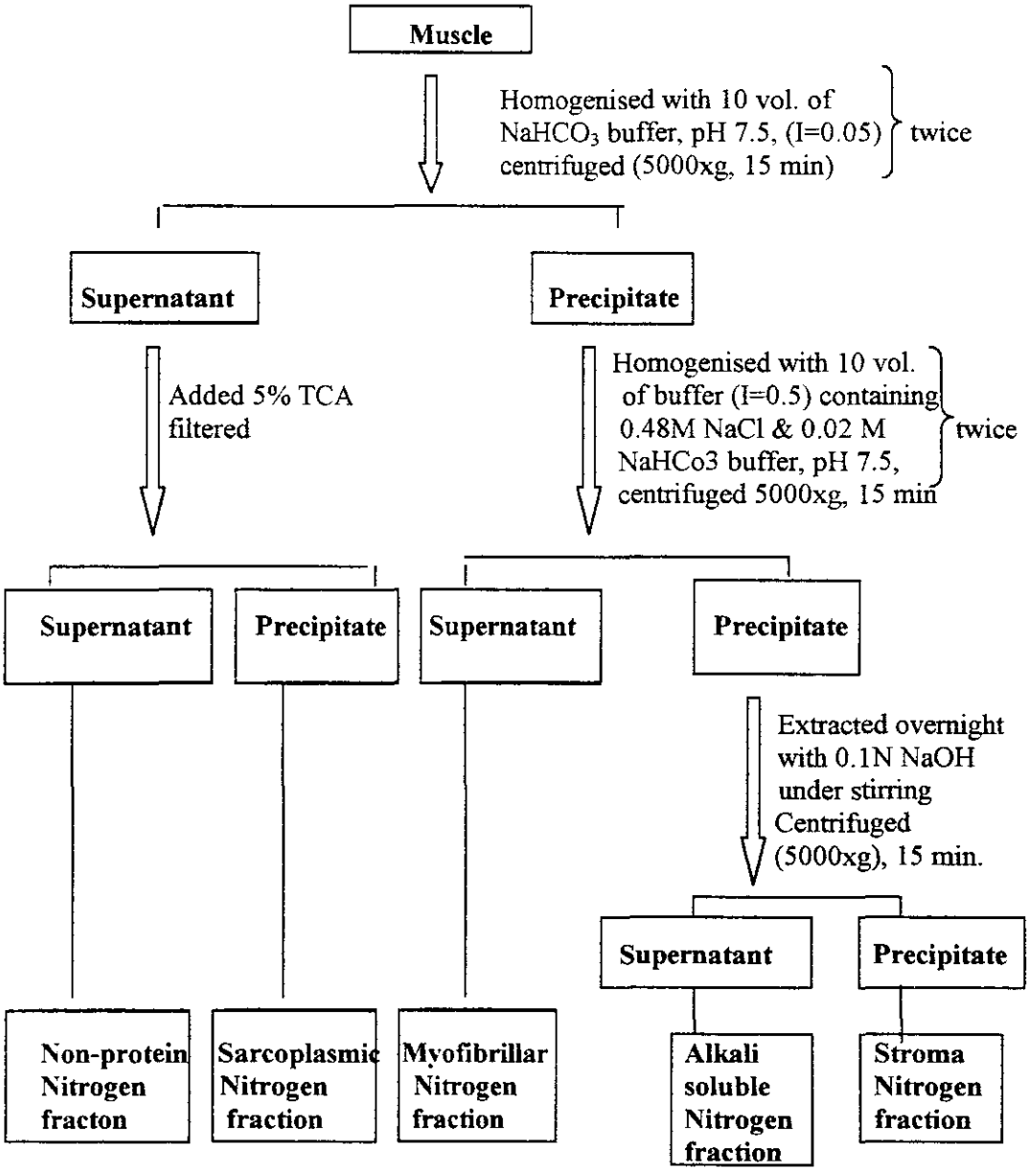
2. 4. 18. 3. Gel strength and Compressibility

The gel strength and compressibility of the gel, the attributes that defines the gel characteristics of the surimi paste, were measured by a "SUN" rheometer using a cylindrical specimen of defined dimension (2.5 cm long and 3.7 cm in diameter) and a 5mm diameter plunger with a round head (Lee, 1984). The gel strength (g-cm) is defined as the product of breaking force (load-g) and deformation.

Gel strength: breaking force (load-g) **x** Deformation

The **compressibility** is related to the force (g) required to compress the sample by a known distance (4-mm).

Fig 4. Fractionation procedure of muscle proteins



Chapter – 3

Composition

3. 1. Introduction

Amongst the different animal foods, fish plays a primary role in human nutrition. The different characteristics of the fish flesh, which is almost entirely different from other sources, provides a unique position to the fish as far as nutrition is concerned. The uniqueness associated with various biomolecules particularly proteins, lipids, vitamins make fish a highly nutritious commodity.

The edible portion of the fish varies according to age, shape and maturity (Suzuki, 1981) and it is normally 40-50% of the total body weight. However, for eliptical shaped skipjack and salmon it is over 60% while for bellied and flat fish it is somewhat lower (30-40%).

Table 1 shows that fish meat is having an edge over the live stock meat in having higher protein and lipid levels and the content of lipid is highly related to season. The amino acid composition of the fish almost remained same and there was not much difference within the same habitat. Fish contains slightly higher vitamin content and it varies with species. Eel and lamprey are reported to contain relatively higher contents of vitamins (Suzuki, 1981). This unique combination of all nutrients makes fish a nutritionally important commodity.

The composition differs from fish to fish within the species and the average composition for one species differs from that of the other. The fat content particularly depend upon the season of the year, feed, age, sex besides other unidentified reasons. The water content varies

with season and there is a clear relation between the water and fat content. Higher water content is associated with low fat seasons and *vice versa*. The presence of unsaturation at ω-3 and ω-6 positions in the fatty acids of fish origin makes them even more nutritionally significant as such acids play efficient role in growth and maintenance of the normal body functions. The proximate composition of a number of fish belonging to marine, freshwater and brackish water fish has been reported (Gopakumar, 1997; Mukundan *et al.*, 1986; Devadasan *et al.*, 1978).

Fish skeletal muscle contains many different proteinases, which participate in *post mortem* muscle softening. The rate of softening, however, varies with species and depends on the physiology of the muscle. An extensive softening of muscle tissue was noticed in Chum salmon during spawning period and is related to high proteolytic activity in the muscle during the period (Yamashita and Konagaya, 1991a). This reaction is reported to be due to the action of certain cysteine proteinases belonging to cathepsin group present in the muscle and acting on the myofibrillar proteins (Yamashita and Konagaya, 1990). Proteolytic activities of several endogenous proteins act on myofibrillar proteins and include cathepsisns (Yamashita and Konagaya, 1991b; An *et. al.*, 1994) and myosin heavy chain binding proteinases (Kinoshita *et. al.*, 1990). Cathepsin L is particularly active in this because of its remarkable

endopeptidase activity for a variety of proteins (Yamashita and Konagaya, 1991b).

3. 2. Objectives

There is not much information available in fish growing to big size as far as composition is concerned. Hence, this chapter aims to study the,

- total biochemical composition of freshwater major carps – rohu, catla and mrigal –as a function of different size, and
- autolytic activity in the muscle of major carps.

3. 3. Materials and Methods

The fish mince was prepared from fresh fish as per method (2 .3. 1) given in Materials and Methods and samples were taken for different analytical experiments.

3. 3. 1. Proximate composition

The proximate composition of the sample was carried out by the methods (2. 4. 1) described in Chapter 2 (Materials and Methods).

3. 3. 2. Fatty acid profile

The extraction of fat from fish muscle and analysis of fatty acid profile are elaborately dealt with (2. 4. 2) in Chapter 2.

3. 3. 3. Amino acid Profile

The preparation of sample for amino acid analysis and estimation by high performance liquid chromatography are given under (2. 4. 3) of Chapter 2 (Materials and Methods).

3. 3. 4. Muscle protein composition

The homogenised mince was fractionated by a procedure elaborated under Section 2. 4. 4 in Chapter 2 (Materials and Methods).

3. 3. 5. Autolysis

The proteolytic activity of the fish muscle was studied following the autolytic activity of fish muscle as described under Section 2. 4. 5 in Chapter 2 (Materials and Methods). The autolysed samples were subjected to electrophoresis as per the procedure given under Section 2. 4. 16 in Chapter 2.

3. 4. Results and Discussion

3. 4. 1. Moisture

Average moisture content (Table 2) noticed for fresh rohu, catla and mrigal flesh were in the range of 77 -81%. The higher moisture content of the fish flesh noticed in this case was due to the partial icing of the fish for 24 hours to resolve rigor prior to processing. Earlier reports have shown moisture content in the range of 76 to 77% for the same fish (Devadasan *et al.*, 1978; Mukundan *et al*, 1986; Joseph *et al.*,

1990; Gopakumar, 1997). The moisture contents were in comparison with that from marine, brackish water and deep-sea fish except that the marine fish eel (*Muraenesox spp.*) and Bombay duck (*Harpodon nehereus*) were reported to have very high moisture contents (Gopakumar, 1997). Increase in moisture in deboned fish was reported during the processing of iced Pacific herring (Chang Lee *et al.*, 1990).

3. 4. 2. Protein

Protein is expressed as crude protein (Table 2), after multiplying the total Kjeldahl nitrogen content by 6.25. 16-19% protein content was noticed in the case of rohu, catla and mrigal, with a lower value of 16.13 for rohu - big, and a higher value of 19.25 for mrigal - small. Calculating on dry weight basis, the protein content remained the same, the exception being catla – small and mrigal – big which showed slightly higher values. The protein contents are in agreement with that reported earlier (Gopakumar, 1997). The total protein is the sum of different protein fractions – water-soluble sarcoplasmic proteins including the non-protein nitrogen, the salt soluble myofibrillar protein and the stroma proteins- and these individual proteins that determine the texture, nutritional value and other functional properties of the proteins.

The water-soluble nitrogen fraction (Table 3) constitutes about 21% of total nitrogen in all the cases except rohu – big and catla – small, which showed lower values. In these two cases the alkali soluble

denatured protein content showed an increase, suggesting the possible denaturation of water-soluble proteins during the handling process. These results are comparable to the reports in these fish (Joseph *et al.*, 1990). The salt soluble fraction (Table 2), reflect the actual content of structural proteins, i.e. the myofibrillar proteins which are of significance as far as the production of surimi and fabricated seafood products are concerned. The actual salt soluble fraction (SSN) constituted 55 – 60% of total nitrogen except catla-small and mrigal-big where the contents were marginally lower. This could be due to the insolubilisation of SSN as a result of the handling condition and is explained by the increased content of alkali soluble nitrogen.

The other major nitrogenous component is the non-protein nitrogen and this represented 12% (Table 3) of the total nitrogen in all the cases excepting mrigal-small (9.36%). Generally the non-protein nitrogen constitutes about 9 - 18 per cent in teleosts and 33 - 38 per cent in elasmobranchs (Haard *et al.*, 1994), and includes free amino acids, peptides, guanido compounds, urea, nucleotide, quaternary ammonium compounds etc. These water-soluble fractions are of significance to food technologists because of their association to the taste of the seafood and contribution to spoilage. Joseph *et. al.*, (1990) reported similar results in the same fish during their ice storage studies. The insoluble connective tissue contributed to 2-3 per cent of total nitrogen, which is well within

the range of 2-5 per cent reported for bony fish (Sikorski and Borderas, 1994).

3. 4. 3. Fat

The fat content of rohu, catla and mrigal were in the range 1.0-2.5, and hence they can be put in the category of low to moderately fatty category. Even though, not much change was noticed on, as is basis, calculating in terms of dry weight basis, showed a clear distinction between small and big fish.

3. 4. 4. Ash

The ash content and hence the mineral content of the fish showed only a marginal variation (Table 2). However, calculating on dry weight basis, showed a clear variation in the ash content between the fish species. The big fish showed a higher ash content with mrigal heading the list followed by catla and rohu. Higher ash content in mrigal over rohu has already been reported (Mukundan *et. al.*, 1986).

3. 4. 5. Fatty acid composition

The fatty acid composition was slightly different from that of marine fish (Table 4). Generally saturated fatty acids form 15 – 35% of total fatty acids (Ackman, 1989). Saturated acid constituted 30 – 36 % in different fish with highest being in rohu-small and the lowest in catla-small (Table 5). This includes about 2-5% of odd numbered saturated

fatty acids. Among the saturated fatty acids, palmitic acid (C16) content was 20-25% of the total fatty acids and is comparable to that in marine fish (Nair, 1998). The palmitic acid accounts for upto 60% of saturated fatty acids in marine fish (Ackman and Eaten, 1966) and in freshwater major carps C16 constituted 60-80% of the total saturated fatty acids, the exception being mrigal –small where it was just 58%.

The monounsaturated fatty acids (MUFA) formed 31-39% of the total fatty acids (Table 5) in major carps and its content was higher than that reported for marine fish (Nair and Gopakumar, 1978). 60-68% of the MUFA in freshwater carps was C18: 1 (oleic acid). Fish from Indian waters were reported to contain about 13% of C18: 1 (Nair, 1998) against the more than 20% seen in freshwater major carps. Hence the C18: 1 content of freshwater carps was higher than that of marine fish. The other constituents of the group C20: 1 and C24: 1 were noticed in minor quantities.

The polyunsaturated fatty acid (PUFA) occupied 23 – 28% of the total fatty acids among the different fish, with maximum content in mrigal-small and minimum in mrigal-big. 68-71% of the PUFA was constituted by C18: 2 (n-6), C20: 4 (n-3) and C22: 6(n-3) with C22: 6 predominating in all the cases. The C22: 6 content, however was comparatively less than that of marine fish. The PUFA content in the case of marine fish range from 28 to 57% with C20 : 5 and C22: 6 predominating and constituting to about 50% in most cases (Nair and

Gopakumar, 1978). The other principal fatty acid from marine source C20: 5 (EPA) content was very low in fresh water fish. The C20 : 4 (n-3) was marginally higher but the C22 : 6 was very much lower than that of marine fish.

3. 4. 6. Amino acid composition

Glutamic acid is the major amino acid occupying 15 – 20% of total amino acids and this result is in accordance with earlier report in rohu and mrigal (Mukundan *et.al.*, 1986). Maximum content was in rohu - small and minimum in catla - big (Table 6). The glutamic acid content was marginally higher than that in marine fish (Gopakumar, 1997). Aspartic acid with about 10-12% occupied the second position. Marginal decrease in glutamic acid and a similar increase in aspartic acid were noticed with growth.

The essential amino acids contributed to 41 to 51% of the total amino acids. Catla showed a marginally higher content of essential amino acids. Similarly the content of histidine, an amino acid of significance in children was higher in mrigal. The lysine content in the major carps was lower than that in marine fish. However, there was no change in amino acid pattern during growth. The aromatic amino acids formed 8 to 10% of the total amino acids and found to be slightly higher than that of marine fish. The increase noticed is mainly due to the higher tyrosine content associated with these fish. Proline content of freshwater

carps was lower than that of marine species indicating the lesser connective tissue content of freshwater carps.

3. 4. 7. Autolytic activity

The autolytic activity was significantly higher in small fish in all the three major carps (Fig 1, 2 and 3). Among the smaller fish, catla showed several times higher activity at alkaline, neutral and acid pH ranges. Mrigal followed with activities at almost all pH ranges while rohu showed lowest activity among the three fish with maximum activity in the alkaline region. Rohu-small showed low activity at lower pH ranges (pH 2-5) but rohu - big showed marginally higher activity. However, above pH 5, there was an increase in autolysis and at pH 8 the activity increased by more than double the activity at pH 5 and then decreased marginally. Catla showed high activity at pH ranges 3, 4, 7, 9 and 10, moderate activity at pH 5 and 8 and low activity at pH 6. In big fish, high activity was noticed in the acid and neutral pH ranges. The SDS-PAGE electrophoresis (Fig 4) showed that rohu had high activities at pH 7, 8 and 9 as shown by the decrease in the intensity of the major band, possibly myosin. The big fish (Fig 5) showed marginal activity at all pH ranges. Between the two types in catla, small fish (Fig 6) showed activities at pH 4 to 10 with higher activities at pH 5, 6 and 7 while for big fish (Fig 7) autolytic activities were noticed at pH 3 to 9 with higher activities at 5 and 8. For mrigal, small fish (Fig 8) showed increasing

activities with increase in pH while big fish (Fig 9) showed activities at pH 4, 5 and 9. Such enzymes present in the water-soluble extract catalyzing the degradation of actomyosin has been reported in many species (Nike, *et al.*, 1984a; Nike *et al.*, 1984b; Stoknes *et al.*, 1995). This necessitates the requirement of washing process during surimi preparation.

Thus, in major carps, the water-soluble nitrogen fraction constitutes about 21% while salt soluble fraction constituted 55 – 60% of total nitrogen. Non-protein nitrogen and connective tissue nitrogen represented 12% and 3% of the total nitrogen respectively. C16 palmitic acid was the major saturated fatty acid and its content was marginally higher than that of marine fish. The monounsaturated fatty acids (MUFA) formed 31-39% of the total fatty acids and 60-68% of the MUFA in freshwater carps was C18:1. The polyunsaturated fatty acid (PUFA) occupied 23 – 28%. C20:5 (EPA) content was very low in fresh water fish. The essential amino acid contributed 41 to 51% of the total amino acids in freshwater major carps. Aromatic amino acid content was slightly higher compared to marine fishes while proline showed a lesser content. The autolytic activity was significantly higher in small fish in all the three categories. Catla showed several times higher activity at alkaline, neutral and acid pH ranges.

Table 1. Comparison of live stock meat and fish meat (Suzuki, 1981)

	Live stock meat	Fish meat
Water (%)	65 – 80	66 – 84
Protein (%)	16 – 22	15 – 24
Lipid (%)	1.5 – 13	0.1 – 22
Carbohydrates (%)	0.5 - 13	1 – 3
Inorganic substances (%)	1.5 or less	0.8 - 2

Table 2. Comparison of proximate composition* of rohu, catla and mrigal

	Rohu		Catla		Mrigal	
	Small	**Big**	**Small**	**Big**	**Small**	**Big**
Weight, g / length, cm	560 / 39	1200 / 57	560 / 36	1350 / 45	560 / 38	1600 / 56
Weight of Gonad, g	2.25	4.07	2.21	3.98	2.78	4.35
Moisture, %	79.26	81.75	80.72	79.23	77.82	80.00
Crude protein,% (N x 6.25)	18.41	16.13	16.98	16.54	19.25	16.29
Fat, %	1.01(4.87)	2.43 (11.72)	1.12 (5.81)	2.09 (10.84)	1.25 (5.64)	2.13 (10.65)
Carbohydrate,%	0.85 (4.10)	1.82 (8.78)	1.12 (5.81)	1.89 (9.80)	1.12 (5.05)	1.95 (9.75)
Ash, %	1.02 (4.92)	1.15 (5.54)	1.17 (6.09)	1.32 (6.85)	1.56 (7.03)	1.97 (9.85)
Calorie value	86.13	93.67	82.48	92.53	92.73	92.13

*Values are average of triplicate analysis
Value in parenthesis denote dry weight basis

Table 3. Comparison of different protein fractions* in rohu, catla and mrigal

	Rohu		Catla		Mrigal	
	Small	**Big**	**Small**	**Big**	**Small**	**Big**
Water soluble nitrogen fraction	21.69	18.61	19.81	25.77	21.42	21.16
Salt soluble nitrogen fraction	58.31	55.43	50.94	54.64	54.36	51.54
Non protein nitrogen fraction	12.88	12.79	13.84	12.72	9.36	14.33
Alkali soluble nitrogen fraction	1.69	10.08	10.89	6.56	8.35	8.92
Stroma nitrogen fraction	2.37	3.49	2.63	2.09	2.82	2.68

*Per cent of total nitrogen

Values are average of triplicate analysis

Table 4. Comparison of fatty acid composition* of fish meat from rohu, catla and mrigal (area %)

	Rohu		Catla		Mrigal	
Fatty acid	Small	Big	Small	Big	Small	Big
C12	0.52	0.26	0.00	0.25	0.83	0.26
C13	0.15	0.00	0.40	0.26	0.26	0.00
C14	5.12	2.86	2.20	3.05	4.39	2.86
C14:1	0.59	1.45	0.77	1.91	0.97	2.86
C15	2.75	1.34	1.63	1.21	2.39	1.34
C16	21.58	25.08	23.10	25.65	19.43	27.28
C16:1	9.64	8.36	8.95	9.12	9.24	8.28
C17	1.84	3.66	2.25	4.05	2.10	096
C18	3.65	0.44	0.21	0.31	4.21	0.44
C18:1	22.35	24.88	26.36	26.17	19.54	25.56
C18:2	6.25	5.77	5.00	6.12	7.34	4.99
C18:3	2.56	2.45	3.10	2.25	2.35	1.90
C18:4	1.95	1.79	2.04	1.95	1.42	1.36
C19	0.00	0.30	0.48	0.52	0.00	0.30
C20:1	0.18	0.01	0.25	0.18	0.23	0.00
C20:2	0.84	0.79	0.80	0.94	0.75	0.89
C20:4	5.28	4.32	4.88	4.57	6.23	4.58
C20:5	2.64	1.41	2.80	2.75	2.56	1.50
C22:1	0.62	0.49	0.92	0.88	0.51	0.56
C22:6	8.12	7.96	6.00	6.12	7.46	7.86
C24:1	1.51	1.20	1.95	1.45	1.48	1.20

*Values are average of triplicate analysis

Table 5. Comparison of fatty acids in the fish meat from rohu, catla and Mrigal (area per cent)

	Rohu		Catla		Mrigal	
	Small	Big	Small	Big	Small	Big
Total SFA	35.61	33.94	30.27	35.3	33.61	33.44
Total MUFA	34.89	36.39	39.20	39.71	31.97	38.46
Total PUFA	27.64	24.49	24.62	24.70	28.11	23.08

Table 6. Comparison of amino acid composition* of fish meat from rohu, catla and mrigal (g/100g protein)

	Rohu		Catla		Mrigal	
Amino acid	Small	Big	Small	Big	Small	Big
Aspartic	10.99	11.29	10.26	11.21	11.59	12.25
Threonine	3.95	4.55	4.37	4.18	4.25	4.56
Serine	4.00	4.24	4.24	4.03	4.05	4.26
Glutamic	20.06	18.98	18.21	15.63	17.88	18.02
Proline	2.73	3.03	3.48	3.91	3.47	3.52
Glycine	4.99	4.95	3.31	3.19	5.00	5.02
Alanine	7.80	7.07	8.79	6.76	7.07	7.25
Cysteine	0.76	0.00	2.01	2.13	0.84	0.52
Valine	4.05	5.07	5.21	4.07	4.80	4.92
Methionine	1.79	2.18	2.25	1.86	1.91	2.14
Isoleucine	3.94	4.56	4.91	4.33	3.68	2.58
Leucine	8.58	8.89	9.33	8.45	7.09	7.98
Tyrosine	3.56	3.48	3.82	3.27	3.51	3.66
Phenylalanine	3.41	4.49	4.54	3.92	4.20	4.32
Histidine	3.78	5.09	3.95	3.83	4.97	5.28
Lysine	3.01	5.40	7.40	8.20	5.34	3.21
Trp	1.12	1.49	1.27	1.18	1.39	1.45
Arginine	5.58	6.24	7.89	12.07	5.58	5.78

*Values are average of triplicate analysis

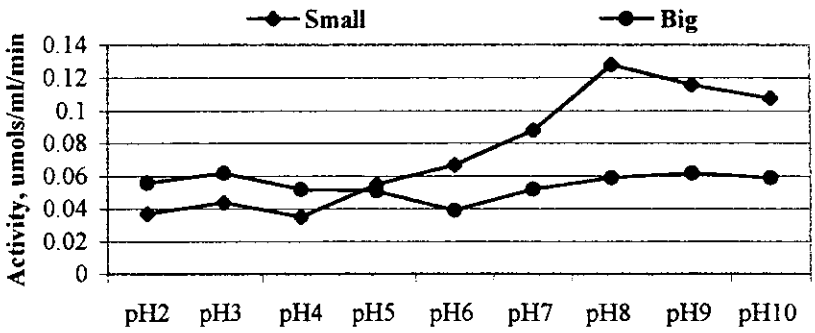


Fig 1. Changes in autolytic activity with pH in small and big size rohu

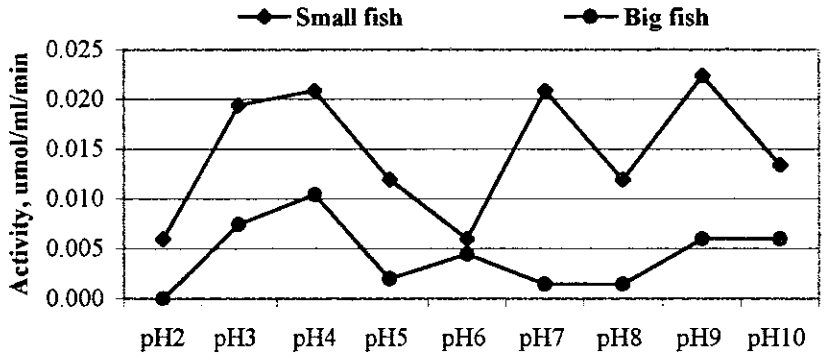


Fig 2. Changes in autolytic activities with pH in small and big size catla

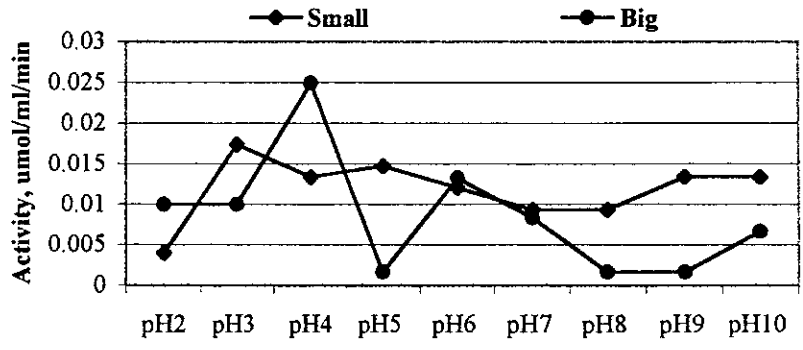


Fig 3. Changes in autolytic activity with pH in small and big size mrigal

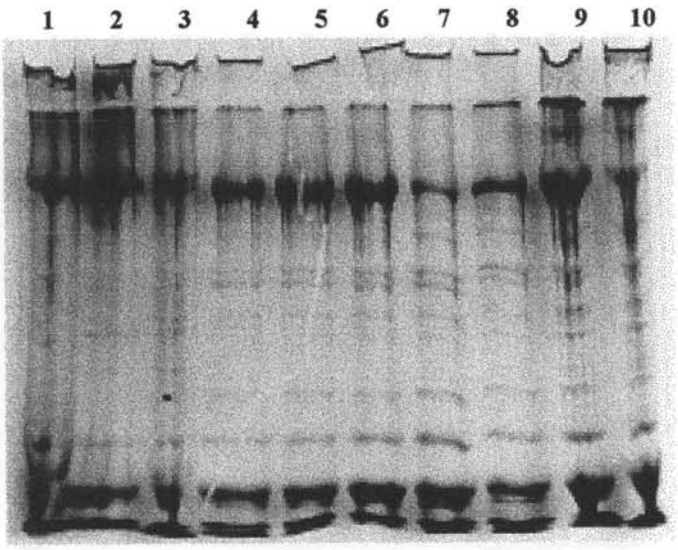


Fig. 4. SDS-PAGE profiles of the autolysed rohu (small) meat at different pH; Lanes 1 – 9 samples at pH 2 to 10; 10- control

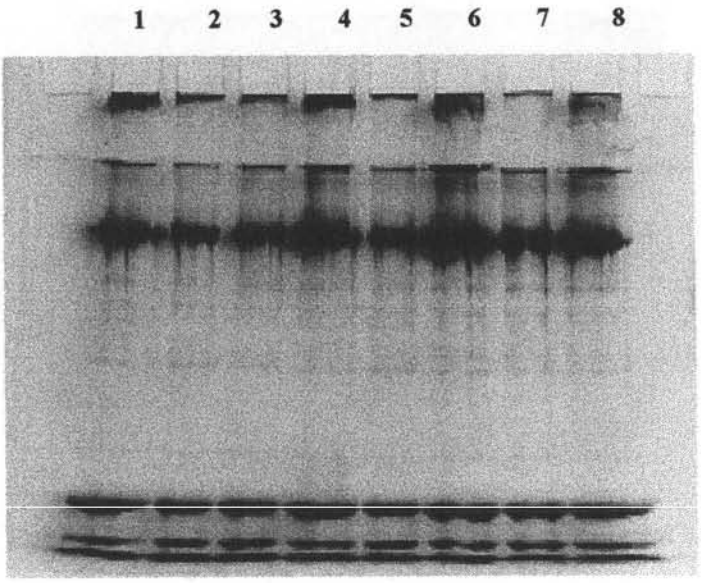


Fig. 5. SDS-PAGE profiles of the autolysed rohu (big) meat at different pH; Lanes 1 –8 samples at pH 2 to 9

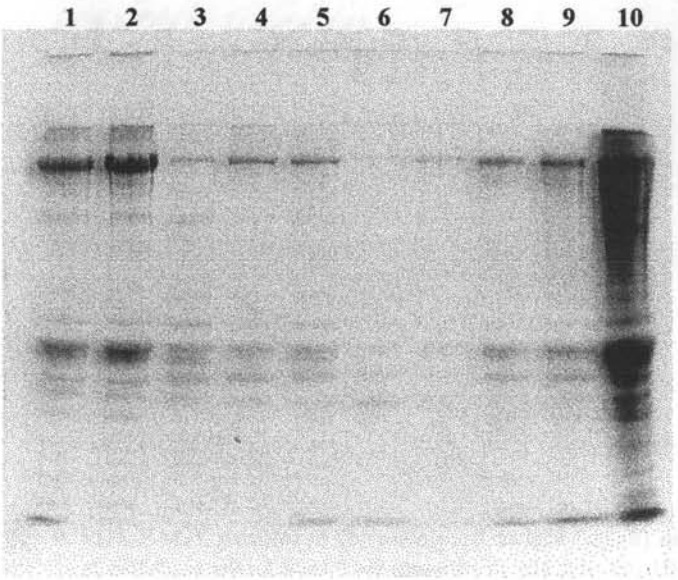


Fig. 6. SDS-PAGE profiles of the autolysed catla (small) meat at different pH; Lanes 1 –9 samples at pH 2 to 10; 10- control

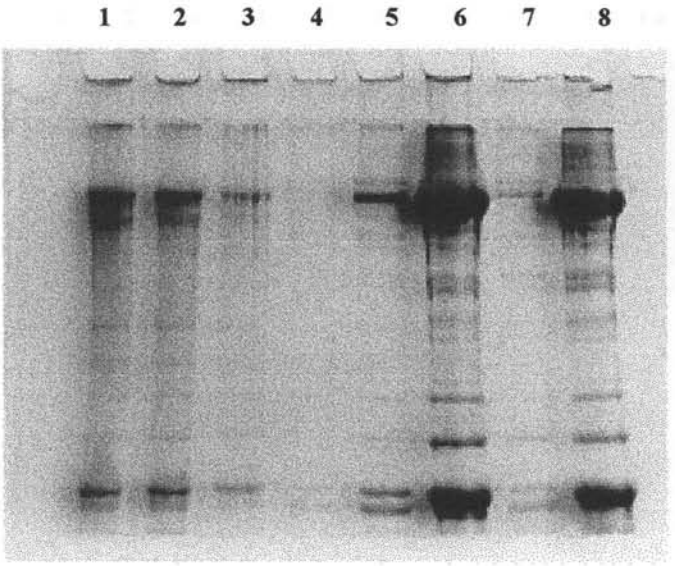


Fig. 7. SDS-PAGE profiles of the autolysed catla (big) meat at different pH. Lines 1 to 8 - pH from 2 to 9

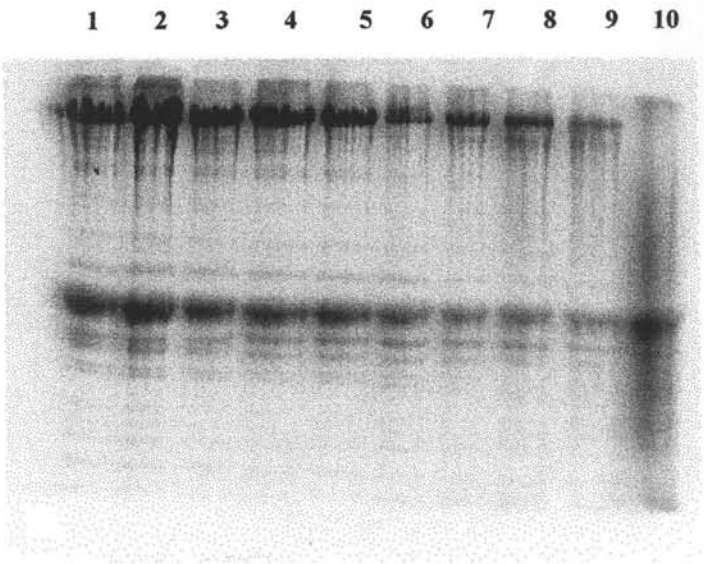


Fig. 8. SDS-PAGE profiles of the autolysed mrigal (small) meat at different pH; Lanes 1 –9 samples at pH 2 to 10; 10- control

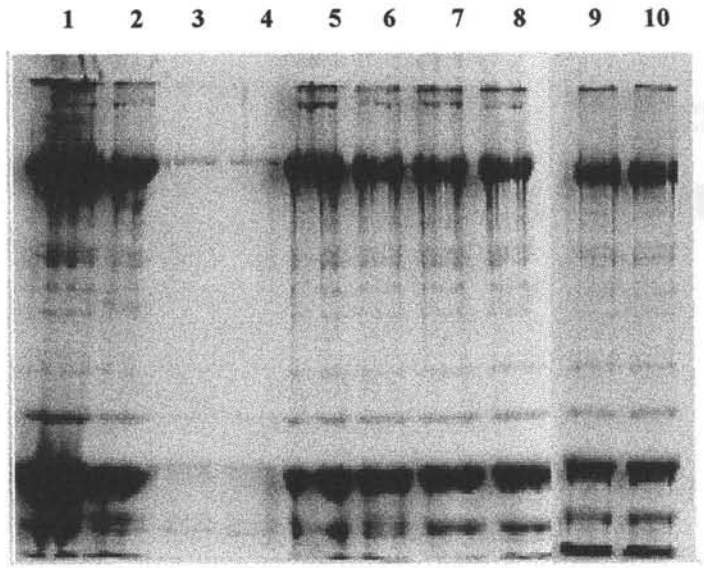


Fig. 9. SDS-PAGE profiles of the autolysed mrigal (big) meat at different pH; Lanes 1 –9 samples at pH 2 to 10; 10- control

Chapter – 4

Characteristics of Myofibrillar protein

4. 1. Introduction

The major proteins of the fish muscle are classified as sarcoplasmic proteins, myofibrillar proteins and connective tissue proteins. These proteins are present in different meats in different proportions and are responsible for the varied functional characteristics of the meat.

The muscle protein comprises of different types of nitrogenous compounds ranging in molecular weight from more than 100,000 to less than 100 Daltons (Haard *et al.*, 1994). Portions of these compounds are present in the soluble fraction of the sarcoplasm and hence are called sarcoplasmic proteins. The sarcoplasmic proteins normally constitute about 20-25% of total fish muscle proteins and chemically these compounds in fish and shellfish include dilute salt soluble proteins, peptides, amino acids, amines, amine oxides, guanidine compounds, purines and urea (Haard *et al.*, 1994). The physiological roles of these compounds vary from enzyme catalysis, osmoregulation, antifreeze, intermediary metabolism, nitrogen storage and cell structure to transport process.

Myofibrillar proteins, the major soluble protein contributing to over 50% of the total protein content plays a key role in muscle contraction. Besides the major components myosin, actin, tropomyosin and troponin C, I and T, many other components of the myofibrils called scaffold proteins comprising less than 1% of total myofibrillar protein

also contribute to the functional properties of myofibrillar protein (Sikorski, 1994).

Sikorski (1994) defined **myosin** as "*the major component of myofibrillar protein, structurally consists of two heavy chains, which are non-covalently associated with two pairs of light chains. The amino-terminal part of about 800 residues of each heavy chain folds in to a globular domain. Together with one pair of light chains this forms the globular head. The carboxyl-terminal parts of both heavy chains together form a coiled-coil ∝-helical rod. The globular head contains the active site for ATPase activity and binding site for actin, whereas the helical rod participates in the formation of myosin filaments*".

A myosin molecule is formed of approximately 4500 amino acid residues of which 40 are cysteine residues. The native myosin is about 150 nm long with a diameter of 8 nm in the globular region and 1.5-2.0 nm in the rod region (Kielley and Harrington, 1960). There are two types of light chains. The **nonessential** light chain which dissociate from the myosin heavy chain after treatment with DTNB affect the calcium binding ability and the **essential light** (A1 and A2) chains which dissociate at high alkaline pH of 11 have specific sites for calcium binding and are essential for ATPase activity of myosin (Sikorski, 1994). The subunits of fast muscle light chains are of three kinds while those of slow muscle light chains are of two kinds in both warm-blooded vertebrates and fish.

Actin, which comprises of about 20% of the total myofibrillar protein, is the major component of the thin filament of the myofibril (Suzuki, 1981). Actin exists in two forms - globular, G-actin and fibrous, F-actin. G-actin is a monomer of 42 KD molecular weight proteins and polymerises to F-actin depending on the ionic strength of the medium of extraction (Shenouda and Pigott, 1975; Stryer, 1995). The polymerised actin irreversibly binds to phospholipids and neutral lipids. The actin are characterised by the presence of five SH groups in the molecule, three of which are located on the surface. Actin was suggested to increase the thermal stability of myosin probably by the competition of its SH groups with the SH groups of myosin for oxidation (Sikorski, 1994). Fish actin in contrast to that from bovine sources, does not gel on heating in the presence of sodium chloride, a property characteristic of myosin. It forms a curd instead of a gel and thus, does not contribute to the elasticity of fish gels (Sano *et al.*, 1989a). Sano *et. al.*, (1989b) further stated that the increase in elasticity of fish protein gel is proportional to the F-actin/myosin ratio and F-actin adds the viscous element to the natural actomyosin suspension.

Tropomyosin, the third major component of structural proteins (Mannherz and Good, 1976), plays a role in the regulation of calcium dependent interaction of actin and myosin. In skeletal and cardiac muscles it forms an integral part of the thin filament of sarcomere and involves in the calcium regulatory system for contraction and relaxation.

Situated in the two grooves of the double stranded structure of filamentous actin, tropomyosin forms a long filament by aggregation of individual molecules. Throughout its entire length it interacts with seven actin monomers on each of the two strands of F-actin. It also binds one mole of troponin complex. As a result of the binding of Ca2+ to troponin C, tropomyosin may alter its position in the groove of actin filament and permits interaction of myosin heads and actin monomers (Mannherz and Good, 1976).

Troponin (Suzuki, 1981) is a protein highly essential for the action of tropomyosin in muscle contraction. The protein isolated from rabbit has a molecular weight of about 80,000 and is formed of three subunits namely troponin-T which combines with tropomyosin, troponin -I which inhibits the action of ATPase and troponin-C which combines with calcium. The troponin from fish sources is also more or less similar, of course with species difference.

Paramyosin (Sikorski, 1994) is characteristic of invertebrates (3% in scallop, 14% in squid, 19% in oyster and 38% in smooth muscle of oyster) and is present in 0.1 to 10% of myosin. The paramyosin is characterised by the presence of large concentrations of amide, acidic amino acid residues like glutamic acid (20-23%), aspartic acid (12%), and basic amino acid residues like arginine (12%), lysine (9%) and small amounts of proline (Kantha, *et al.*, 1990). The paramyosin rods form the thick core of myofibrils of invertebrate muscle, which is

covered by a layer of myosin and was reported to have a structural function affecting the orientation of the myosin molecule. Functionally paramyosin affects the rheological properties of gels prepared from invertebrate meat by adding elasticity and cohesiveness to the gel than that prepared from fish gels. The action of paramyosin is due to the inhibition of dissociation of myosin from actin (Sano *et. al.*, 1989c).

4. 1. 1. Nature of proteins in food system

In live animal large number of biochemical and physiological changes take place. After death the meat passes through three major states namely, pre-rigor (characterised by soft, pliable tissue and biochemical changes like breakdown of ATP and creatine phosphate and active glycolysis), rigor (stiff and rigid condition) and post rigor (tenderisation of meat) affecting the biochemistry of the meat. Exposure of fish to extreme conditions causes conformational changes in the fish proteins which are accompanied by losses in the functional properties of muscle proteins like solubility, water retention, gelling ability and lipid emulsification characteristics.

The fish muscle myofibrillar proteins are comparatively less stable than those of the mammalian muscle to heat treatment (Sikorski, 1994) and even less stable are those from squid (Tanaka et al, 1982). The stability of myofibrillar proteins depends not only on the species and habitat temperatures but also on the conditions affecting the state of

the proteins like pH and ionic strength. Ca2+ ATPase activity of the protein readily gets altered once a structural alteration takes place with actomyosin molecule. The ATPase from fish habituated at higher temperature has significantly higher thermo stability than those caught from cold waters.

The myosin has a comparatively higher content of sulfhydryl (SH) groups. The fish muscle myosin contains in the range of 29-49 moles SH / $5x10^5$ g protein (Suzuki, 1981). Trout reported to contain 42 moles SH per $5x10^5$ g protein (Buttkus, 1970). The SH groups present in the myosin molecule, especially those present near the globular head and on the light chain determine the characteristics and properties of myosin (Shenouda, 1980).

4. 1. 2. Denaturation of proteins

The chemical, physical and biological changes taking place in protein molecules under the influence of conditions to which they are exposed are called protein denaturation. This actually involves major changes to the original native structure without altering the amino acid sequence (Tanford, 1968). The usual causative agents include exposure to acid, alkali, certain chemicals and physical conditions. As a result of this exposure, unfolding or alteration in the native conformation of the polypeptide chain through changes in secondary bonds like hydrogen and Van der waals occurs. The denaturation is normally reversible but

the irreversible and insoluble nature of protein denaturation often encountered is due to the polymerisation as a result of irreversible disulfide interchange reactions (Tanford, 1968). Therefore, any change in the primary, secondary and tertiary structure of protein molecules taking place as a result of the conditions mentioned above is considered denaturation.

The chemical and physical conditions responsible for denaturation of protein molecules include changes in the temperature, pH, use of denaturing agents like urea, β- mercaptoethanol, dithiothreitol, use of organic solvents like formamides, di and trichloroacetic acid and their salts, use of detergents like sodium dodecyl sulphate (SDS), use of inorganic salts like lithium bromide, sodium iodide and potassium thiocyanate, exposure to high pressure and ultrasonic homogenisation.

The temperature stability of proteins varies widely. Some undergo unfolding at higher temperatures while some others do so at relatively low temperatures. The denaturation takes place as a result of the increase in the entropy associated with the changes in the three dimensional structure of the proteins. As the temperature is increased this entropy also increases and at a particular stage overcomes the energy barrier leading to the denaturation of protein molecule. As a result of this unfolding, the hydrophobic residues buried inside the protein molecule get exposed, leading to intermolecular association

consequently forming aggregates. Thus the formation of hydrogen, hydrophobic (Connell, 1960; 1965) and disulfide bonds (Buttkus, 1971) facilitate the formation of protein aggregates. This essentially makes the denaturation an irreversible process. It is reported that freezing, heating and infusion of salt affected the functional properties of fish proteins resulting in increased or decreased organoleptic acceptability (Poulter *et al*, 1985). However, there are differences in stabilities between the myosin of different fish species (Connell, 1961; Poulter *et.al.*, 1985).

In the case of chemical denaturation, the chemicals break the secondary bonds holding the protein molecule forming strong bond with reactive groups in the protein molecule. Urea or guanidine hydrochloride is used for disrupting the hydrogen bonds and at higher concentrations the proteins undergo high degree of unfolding. In some cases aggregation is made possible by the formation of new disulfide bridges by the sulfhydryl groups exposed to the exterior as a result of unfolding of protein chain. The β - mercaptoethanol and dithiothreitol act by disrupting disulfide bonds while SDS act by disrupting both hydrogen and hydrophobic bonds.

4. 1. 3. Tools for measuring protein denaturation

When protein is heated its physiochemical and biochemical properties change. So the properties, which get altered under the conditions of protein denaturation, can be used as tools for studying the

degree of alteration. Turbidity measurements, light scattering, solubility, electron microscopic studies, circular dichroism, intrinsic and induced fluorescence studies, reactive sulfhydryl groups, estimation of ATPase activity and electrophoretic studies are some of the methods which are normally employed for assessing the degree of denaturation of the protein molecule.

Turbidity and **light scattering** analyses provides a rough estimate of the aggregation of protein molecules because increases in turbidity are caused by discrete particle formation of protein molecules (Deng *et al.*, 1976; Ziegler and Acton, 1984).

Solubility of a protein is the amount of protein or nitrogen constituent soluble in a particular buffer under specific conditions. It is a physicochemical property that is related to other functional properties and is influenced by amino acid composition and sequence, molecular weight and conformation and content of polar and non-polar groups in the proteins (Zayas, 1997). The solubility of different protein components is dependent on temperature, ionic strength and pH.

Estimation of **ATPase activity** has been greatly acknowledged as an important tool in quantifying the extent of changes taking place in actomyosin molecule as a result of heating in both mammalian systems (Hartshorne *et al.*, 1972; Levy and Moy, 1981a & b) and in fish (Arai and Fukuda, 1973; Yamashita *et al.*, 1978; Taguchi *et al.*, 1986) and it was found that the natural actomyosin from fish was comparatively less

stable than that of the mammalian natural actomyosin. Further, the stability of actomyosin from different species is related to its habitat temperatures and the stability increases with increasing habitat temperatures (Arai and Takashi, 1977; Suzuki, 1981).

Although the domains associated with ATPase activity are mainly localised at the head portions of the myosin, some are associated with actin and troponin - tropomyosin complex. Any structural change associated with these sites affect the ATPase activity. Ca^{2+}ATPase of myofibrillar protein is reported to be a good index to evaluate the stability of fish muscle proteins during icing, freezing and subsequent storage (Arai and Takashi, 1977).

The inactivation rate constants of actomyosin and myosin Ca^{2+}ATPase were frequently used for evaluating the thermal stability of fish muscle proteins (Suzuki, 1981). The stability of actomyosin as assessed by inactivation constants of ATPase varied with species and the stability of fish myosin was found to be lower than that of rabbit and whale (Tsai *et al.*, 1989).

Sulfhydryl groups and disulfide bonds are important in maintaining the structure and function of native proteins besides playing important role in the functional properties of proteinaceous foods (Opstvedt *et al.*, 1984). Disulfide cross-linking in proteins may occur from the oxidation of sulfhydryl groups and Sulfhydryl-disulfide interaction (Friedman, 1973). Heating that affected the cysteine / cystine

residues were found to reduce protein utilisation by animals. The disulfide cross links in protein occur as a result of formation of sulfhydryl group and sulfhydryl-disulfide interchange (Cecil and McPhee, 1959) and the same has been demonstrated in fish (Itoh *et al.*, 1979a; Itoh *et al.*, 1979b; Itoh *et al.*, 1980), in frozen fish (Buttkus, 1970) in meat (Hamm and Hofmann, 1965), and in soy protein (Wolf, 1970).

Hydrophobicity of proteins is the sum of side chain hydrophobicity of the constituent amino acids and the hydrophobic interactions are important in proteins for their stability, conformation and function (Tanford, 1970; Kato and Nakai, 1980). The aromatic amino acids are mostly located in the non-helical, head portion of myosin (Molina *et al.,* 1987) and actin (Sano *et al.*, 1994). Since these portions have less ∝-helix content, the intrinsic fluorescence resulted mainly from the aromatic amino acid residues. As far as the biological functions of a protein is concerned the true hydrophobicity of the protein rather than the side chain hydrophobicity was considered to play a role. Some of these hydrophobic residues are exposed to the exterior in the native protein itself, some are buried inside the molecule and some get exposed on denaturation of the protein (Kato and Nakai, 1980). It was reported (Niwa *et al.*, 1986a) that the hydrophobic residues are getting exposed on freezing. This phenomenon is remarkably seen in fish than in other warm blooded animals and is exhibited by actomyosin rather

than by actin. Maximum hydrophobic interactions between amino acid residues occurred between temperatures 30°C -50°C, signifying the emergence of amino acids at elevated temperatures (Sano *et al.*, 1994).

During protein unfolding as a result of the exposure of protein to extreme conditions the hydrophobic aromatic amino acids are exposed to the surface of the molecule. This results in an increase in the intrinsic fluorescence, which in turn cause hydrophobic interaction to reduce free energy. Such changes in the hydrophobic interactions were investigated using Cis-parinaric acid as a probe for inducing fluorescence

Binding of polyene fatty acids, cis-parinaric acid (9,11,13,15-cis.trans.trans.cis-octadecatetraenoic acid) and cis-eleostearic acid (9,11,13-cis trans.trans-octadecatrienoic acid) to protein altered the absorption spectrum, enhanced fluorescence and induced circular dichroism in the polyene chromophore (Sklar *et al.*, 1977). This is considered to be a better tool than the 1-anilinonaphthalene-8-sulphonate-fluorescence quenching method (Sano *et al.*, 1994).

4. 2. Objectives

This chapter is aimed to investigate the functional properties of natural actomyosin (NAM), the major component of the myofibrillar protein responsible for the functional properties of meat products, from

all the three major carps. It also investigates the functionality changes in fish proteins during heat treatment in these fish.

4. 3. Materials and Methods

The fish (rohu, catla and mrigal) were brought from fish farm in iced condition and used for extraction of actomyosin immediately on reaching the laboratory (with in 30 minutes). Small sized fish weighing around 500 g and large sized weighing around 1000 g as shown in Table below, were taken for the study. To evaluate the gonadal maturation stage of each fish the gonadosomatic index, GSI was calculated (Montecchia et al., 1997) as follows:

GSI: (Wet wt gonads / Wet wt (fish – gonads) x 100

Three fish from each group were filleted and mince was separated using Baader - 694 meat picking machine, which was taken for actomyosin extraction.

	Small		**Big**	
	Weight (g)	GSI	Weight (g)	GSI
Rohu	480	0.429	1200	0.335
Catla	470	0.417	1350	0.296
Mrigal	750	0.390	1100	0.352

4. 3. 1. Preparation of actomyosin

The actomyosin (AM) was extracted according to the procedures of Jiang *et. al.*, (1987) as detailed under 2.4.10 of Chapter 2. The actomyosin thus prepared was diluted to a concentration of 5-mg/ ml solution, which in turn was used for the stability studies of AM.

4. 3. 2. Determination of stability of actomyosin

The actomyosin was diluted to a required concentration of 5 mg/ml. 10 ml portions of the actomyosin solution was pipetted out into a series of test tubes and kept in ice. Test tubes in pair were kept in a water bath maintained at different temperatures, namely, 10, 20, 30, 40, 45, 50, 55 and 60° C. The test tubes were removed to the ice bath immediately once the protein solution has attained the respective temperatures. The actomyosin, so exposed to different temperatures, was subjected to different experiments to establish the thermal stability of actomyosin from major carps.

The actomyosin was subjected to ATPase assay, solubility, Sulfhydryl group, surface hydrophobicity and electrophoresis by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to ascertain the changes in actomyosin as a result of thermal treatment.

4. 3. 3. Analytical methods

4. 3. 3. 1. Turbidity

The method described by Sano *et al.*, (1994) was followed. The natural actomyosin as well as the heat-treated samples was diluted to an appropriate concentration of 1 mg per ml with 0.6M NaCl (pH 7.5). Absorbance at 660 nm of the diluted protein solution was expressed as absorbance /mg/ml of protein.

4. 3. 3. 2. Solubility

The heat-exposed samples were centrifuged at 7500 rpm for 15 minutes and the supernatant was taken for protein estimation to find the decrease in solubility as a result of heating. The total salt soluble protein and actomyosin were estimated by biuret method as described earlier (2.4.11.1. of Chapter, 2).

4. 3. 3. 3. Adenosine Tri Phosphatase (Ca^{2+}ATPase-)

The protein solution was diluted to 1:4 with 0.6M NaCl so as to prepare 1 mg/ml actomyosin solution. The ATPase assay was carried out by the method of Jiang et al., (1987b) as described under 2. 4.12 of Chapter, 2. The Ca-ATPase-specific activity was shown as micromoles inorganic phosphate released per milligram actomyosin in 1 min for the reaction at 25°C. The Ca-ATPase-total activity was expressed as micromoles inorganic phosphate released from actomyosin extracted from 10 g of muscle.

4. 3. 3. 3. 1. First order rate constants of Adenosine Tri Phosphatase

The first order rate constant (KD) for inactivation of myofibrillar Ca-ATPase was calculated using the relation described earlier (2. 4. 13 of Chapter 2).

4. 3. 3. 4. Sulfhydryl groups

The total reactive sulfhydryl groups in the protein molecules were estimated as per the method given under Section 2. 4. 14 of Chapter 2.

4. 3. 3. 5. Surface Hydrophobicity

The surface hydrophobicity of the native and heat-treated actomyosin solutions was estimated as per the method given under Section 2. 4. 15 of Chapter 2.

4. 3. 3. 6. Sodium dodecyl sulphate Polyacrylamide gel electrophoresis

The actomyosin and the heat-treated samples were subjected to SDS-PAGE according to the method described under Section 2. 4. 16 of Chapter 2.

4. 3. 4. Statistical methods

The different analytical data were analysed by the window based SPSS statistical software for one-way ANOVA. The variations between different groups were established by Duncan's multiple test analysis.

4. 4. Results and Discussion

4. 4. 1. Characteristics of Natural Actomyosin

4. 4. 1. 1. Natural Actomyosin (NAM) from Rohu

The actomyosin prepared from small and big fish by the precipitation of the total soluble protein in 0.6 M NaCl, were compared (Table 1). The NAM contents were 7.60 and 6.44 g per 100 g fish flesh for the small and big fish respectively. There were no such reports as to the comparison of small and big fish. However, it was reported that mature cod undergoes depletion in certain seasons and further that the pre-spawned fish had a lower content of AM than the post-spawned fish (Montecchia *et al.*, 1997).

Ca^{2+} ATPase activity reflects the integrity and functionality of the myosin molecule in the actomyosin complex. NAM, the important protein fraction responsible for the functional properties had a specific Ca^{2+} adenosine triphosphatase activity of 0.49 for the small fish and in the case of big fish the activity was lower by about 26%. The same trend was noticed in the total activity also. Comparing pre and post-spawned fish it was found that the post-spawned fish had higher Ca^{2+} ATPase activity than the pre spawned fish (Roura *et al.*, 1990; Roura and Crupkin, 1995; Montecchia *et al.*, 1997) The actomyosin from pre-spawned hake was more labile to denaturation, which might have caused

the reduced functionalities of actomyosin in pre-spawned fish (Roura *et al.*, 1990).

The sulfhydryl content of the fish were 52 and 56 micromoles per g protein. In comparison the Harp seal muscle (Synowiecki and Shahidi, 1991), Pacific mackerel and Alaska Pollock (Opstvedt *et al.*, 1984), squid (Synowiecki and Sikorski, 1988), meat (Hamm and Hofmann, 1965) had sulfhydryl contents of 63, 76, 70, 79, and 88 micromoles per g protein respectively. The SH groups occur in the flesh either exposed or masked (Sedlak and Lindsay, 1968) with exposed groups being readily available for reaction with DTNB. It was also reported that in the undenatured protein only 90% of the total SH groups were reactive towards DTNB and the remaining 10% were masked in the actomyosin molecule (Sedlak and Lindsay, 1968; Opstvedt *et al.*, 1984).

Cis-parinaric acid induced fluorescence intensity showed a value of 110 and 70 for the small and the big fish respectively. This shows that the protein of small fish had a higher content of hydrophobic amino acids in comparison to that of the big fish. The fluorescence intensity was relatively lower for the myosin from red muscles of albacore, yellow fin tuna and warm-blooded animals like chicken, pork and whale but higher in white muscle fish (Niwa *et al.*, 1986b).

The statistical analysis showed a significant difference in various parameters studied like actomyosin concentration (0.1% level), ATPase

activity (specific and total, 1% level), SH groups (1% level) and CPA induced fluorescence (0.1% level) between small and big fish (Table 2).

4. 4. 1. 2. Natural Actomyosin (NAM) from Catla

The NAM contents were 5.37 and 5.04 g per 100 g fish flesh for the small and big fish respectively (Table 3). NAM, the important protein fraction responsible for the functional properties has a Ca^{2+} adenosine triphosphatase specific activity of 0.43 u moles Pi/min/mg protein for the small fish and the big fish showed a higher activity of 0.61 u moles Pi/min/mg protein which was about 42% higher than that of the small fish. However, considering the total activity a reverse trend was noticed (Table 3). The sulfhydryl content of the fish were 81 and 98 micromoles per g protein indicating the possibility of the partial unfolding leading to the exposure of SH groups from the interior of the protein molecule.

The Cis-parinaric acid induced fluorescence intensity showed a value of 110 and 122 for the small and the big fish respectively. This shows that the proteins of big fish had a higher complement of hydrophobic amino acids than the small fish.

The statistical analysis using one-way ANOVA showed that the differences in the parameters like ATPase activity (specific (5% level) and total (0.1% level), SH groups (0.1% level) and CPA induced fluorescence (5% level) between small and big fish are highly

significant (Table 4) except in the concentration of NAM, which showed less significance.

4. 4. 1. 3. Natural Actomyosin (NAM) from mrigal

The NAM contents were 5.03 and 4.57 g per 100 g fish flesh for the small and big fish respectively (Table 5). NAM, the important protein fraction responsible for the functional properties had a Ca^{2+} adenosine triphosphatase specific activity of 0.50 u moles Pi/min/mg protein for the small fish and the big fish showed a higher activity of 0.74 u moles Pi/min/mg protein, an increase of about 48%. However, considering the total activity a reverse trend was noticed (Table 5) as seen in the case of catla. The sulfhydryl content of the fish were more or less same in both the fish with 62 and 69 micromoles per g protein.

The Cis-parinaric acid induced fluorescence intensity, used as a measure of protein unfolding showed a value of 60 and 130 for the small and the big fish respectively. This shows that the proteins of big fish are unfolded to a larger extent than that of the big fish.

The statistical analysis showed that the differences in the parameters like actomyosin concentration (1% level), ATPase activity (specific (1%) and total (5%)), and CPA induced fluorescence (0.1% level) between small and big fish are highly significant (Table 6) except in the reactive sulfhydryl groups, which showed less significance.

4. 4. 1. 4. Comparison among Rohu, Catla and Mrigal

4. 4. 1. 4. 1. Small fish

The three species belonging to the major carps behaved almost similarly (Table 7). The extractable natural actomyosin contents were almost same in catla and mrigal, but rohu showed a higher value of 7.6 g per 100 g fish flesh. The Ca^{2+} ATPase activities, both specific and total, were almost comparable but SH contents varied among the fish with catla showing higher content of reactive SH groups (81 micromoles per g protein) followed by mrigal (62 micromoles per g protein) and rohu (52 micromoles per g protein).

The statistical analysis (Table 8), however, showed highly significant differences in NAM concentration (0.1% level), SH groups (0.1% level), CPA fluorescence intensity (0.1% level) and Ca^{2+}ATPase total activities (5% level) while that of the ATPase specific activities did not show significant difference.

4. 4. 1. 4. 2. Big fish

Rohu showed a higher extractable natural actomyosin content followed by catla and mrigal (Table 9). The value for catla and mrigal were 22% and 29% lower than the value for rohu. The Ca^{2+} ATPase activities, both specific and total, showed a reverse trend compared to the NAM content with mrigal showing a higher activity followed by catla and rohu. As far as reactive SH groups were concerned NAM of

catla showed a higher content of SH groups (98 micromoles per g protein) followed by mrigal (69 micromoles per g protein) and rohu (56 micromoles per g protein) signifying the possible unfolding of the catla NAM to a higher degree than the other two. The CPA fluorescence intensity of the mrigal NAM was marginally higher than that of catla and rohu.

The statistical analysis (Table 10), however, showed highly significant differences at 0.1% level for NAM concentration, SH groups, CPA fluorescence intensity and Ca2+ATPase specific activities but the values for ATPase total activities were significant at 5% level.

4. 4. 2. Thermal denaturation of carp actomyosin

The physicochemical properties of protein undergo changes as a result of heating. The protein is expected to undergo alteration in structural conformation or reversible/irreversible denaturation affecting the functional properties of the proteins thereby limiting its possible food applications. Understanding such changes in actomyosin, the chief constituent of proteins in muscle, is of great value in the development of protein-based products. The different parameters taken to elucidate such changes in natural actomyosin (NAM) include solubility, Ca2+ ATPase activity, surface reactive sulfhydryl groups and Cis-parinaric acid induced fluorescence.

4. 4. 2. 1. Turbidity

Turbidity measurements provide rough estimates of aggregation processes of protein molecules on exposure to heating (Acton *et al.*, 1981; Deng *et al.*, 1976). The turbidity values showed some variation between small and big fish but between the groups remained almost comparable. The turbidity in both small and big fish remained more or less constant upto 40°C (Fig 1 and 2) and then increased with increase in temperature except in big mrigal where the values gradually increased from 10°C itself. The intensity of turbidity increased to a noticeable level beyond 40°C. The results of the turbidity measurements revealed the possible aggregation of natural actomyosin at 40°C and more extensive aggregation of molecules occurred at temperature range of 45°C to 60°C. The increase in absorbance of heated fish myosin correlated with the formation of more and larger myosin aggregates. The aggregation of myosin molecules increased with temperature in cod myosin upto 50°C and decreased thereafter presumably due to the disintegration of aggregates as a result of proteolytic action (Yongsawatdigul and Park, 1999). The turbidity values were comparable to those reported in carps (Sano *et al.*, 1994) where the turbidity remained relatively unchanged upto 30°C and then increased upto 80°C.

The one way ANOVA showed that the difference in the turbidity data were highly significant (0.1%level) in both small and big fish (Table 11 and 13). The Duncan's analysis also showed significant difference in the turbidity after 40°C (Tables 12 and 14) in all the three fish namely rohu, catla and mrigal.

4. 4. 2. 2. Solubility

In small fish, solubility almost remained unchanged upto a temperature of 40°C in the case of rohu and decreased slightly in the case of catla and mrigal (Fig 4). The decrease was to the magnitude of 6% and 14% respectively for catla and mrigal. At 45°C the solubility suddenly dropped to 29, 23, and 14 percentages for rohu, catla and mrigal respectively. Heating further to 60°C increased the solubility possibly due to the dissociation of the aggregated molecule associated with sudden cooling that followed.

In the case of big fish the solubility remained unchanged for catla and mrigal but decreased marginally (4%) on exposure to 40°C (Fig 5). The solubility decreased to 36 and 31 percentages in catla and mrigal but only 11% in rohu on increasing the temperature to 45°C. The trend continued in the case of rohu upto 55°C (58% remained soluble) and at 60°C about 64% of the protein remained in solution. The trend for catla and mrigal were similar to that seen in the case of small ones showing a marginal increase at elevated temperatures. Compared to beef

actomyosin, the fish actomyosin exhibited stability at lower temperatures below 40°C (Deng *et al.*, 1976).

Myosin rod is responsible for the salt solubility of myosin, which gets denatured very fast during thermal denaturation (Azuma and Konno, 1998). It is reported that myofibrillar proteins start coagulating on heating at 30-40°C and the coagulation is nearly completed at 55°C (Zayas, 1997). At temperatures of 40-50°C or greater, proteins will denature with decrease in solubility, unfolding of polypeptide chains, and the formation of new electrostatic hydrogen bonds (Zayas, 1997).

If dissociation of myosin and actin molecule takes place as a function of temperature rise, then the composition of soluble and insoluble fractions undergo change upon heating to various temperatures. The SDS-PAGE of the proteins in all the three fish showed (Fig 6, 7 and 8) two intense bands corresponding to myosin heavy chain (MHC) and actin and no insolubilisation had occurred below 40°C. When temperature was increased to 45°C insolubilisation had occurred as shown by the decrease in the intensity of MHC and actin and subsequently at 55°C and 60°C the MHC and actin band thickness increased signifying the possible association of protein molecules. Sano *et al.*, (1994) demonstrated similar results upto 40°C in the case of carp natural actomyosin. The disappearance of some of the

intermediate bands between MHC and actin bands is also of significance in this regard.

Thus, myosin molecules began to dissociate from actin filaments at about 45°C so that the resulting myosin lacking actin molecules would readily aggregate and become insoluble. But at elevated temperatures of 55°C and 60°C, the dissociation of aggregated molecule takes place and this results in the thickening of MHC and actin bands in SDS-PAGE (Fig 3 and 4). The short duration exposure of the protein to the respective temperatures and the immediate cooling to 0°C temporarily dissociated the protein molecules by preventing the formation of disulfide bond thereby facilitating the dissociation of MHC and actin. At elevated temperatures above 40°C the system is complex in which dissociated myosin and myosin lacking natural actomyosin coexisted (Sano *et al.*, 1994).

The one-way ANOVA showed a significant difference at 0.1% level in the solubility values between the three fish (Tables 15 and 17). The Duncan's analysis showed the individual variation in the data above 40°C (Table 16 and 18).

4. 4. 2. 3. Adenosine Triphosphatase activity

The Ca^{2+}ATPase activity is one of the characteristic properties of natural actomyosin. The ATPase activity of the natural actomyosin from

fish source is less stable when compared to that of the mammalian muscle (Arai *et al.*, 1973; Arai and Fukuda, 1973; Taguchi *et al.*, 1986).

The Ca^{2+} ATPase activity decreased with increasing temperatures (Fig 9). In the case of small fish the Ca^{2+} ATPase activity more or less remained same upto 40°C followed by a rapid loss of activity from 40°C to 50°C. However, in catla there was a loss of ATPase activity (around 27%) just above 20°C itself and this was followed by a major drop above 40°C. Heat treatment above 50°C resulted in almost complete loss of activity.

In the case of big fish (Fig 10), the overall trend remained the same as in the case of small fish. However, in the case of catla the ATPase decreased twice – one above 20°C resulting in about 7% loss and the other major drop above 40°C. The ATPase total activities also followed the same trend (Fig 11 and 12).

The differences in the stability of ATPase among natural actomyosins of various fish species result from the difference in the habitat temperatures (Hashimoto *et al.*, 1982; Sano *et al.*, 1994). A systematic study on the thermo stabilities of about 40 species of fish led to the conclusion that the differences were due to the physiological adaptation of the enzyme to the environment at the molecular level (Hashimoto *et al.*, 1982). There are reports available comparing post spawned hake with pre spawned fish (Roura and Crupkin, 1995)

indicating high ATPase activities in post spawned fish than pre-spawned fish and the higher proteolytic activity of the pre-spawned fish contribute to this effect.

The one-way ANOVA showed a significant difference (0.1% level) in the ATPase activity, in the three fish (Table 19 and 21). The result of Duncan's analysis showed the individual variation in the data with increasing temperature (Table 20 and 22).

The inactivation rate constants of actomyosin Ca^{2+}ATPase were frequently used for evaluating the thermal stability of fish muscle proteins (Suzuki, 1981; Tsai *et al.*, 1989). Considering both small and big fish, when stabilities of actomyosin at 40°C and 45°C were compared (Table 23), catla (small) was the most unstable and then catla (big), mrigal (big), mrigal (small), rohu (big) and rohu (small). But catla small was most unstable at 30°C itself. The inactivation constants of all the fish increased with increasing temperature. Considering the K_D values of all the fish upto 45°C rohu actomyosin was found to be the most stable. However, at higher temperatures the K_D values of mrigal was found to be higher and was of less significance considering the fact that most of the myosin had already undergone conformational changes leading to the complete loss of Ca^{2+} ATPase activity. The K_D values of rohu, catla and mrigal at 40°C were higher than that reported for milk fish, tilapia, carp, tuna, sea bream and rainbow trout which had K_D

values of 41.3, 37.5, 58.7, 15.3, 33.8, 46.1 x 10^{-5} respectively (Tsai *et al.*, 1989).

The results indicate that considerable conformational changes in the myosin head portion in the case of rohu, catla and mrigal take place above 40°C except in the case of catla, which seems to be more labile to conformational changes at much lower temperatures from 20°C to 30°C. The unfolding of protein molecules increased the hydrophobic amino acid residues and the SH groups at the surface. The formation of hydrophobic and disulfide bonds among the exposed amino acid residues and the SH groups would affect the structure around the sites responsible for ATPase activity (Sano *et al.*, 1994). The thermal denaturation studies on carp natural actomyosin demonstrated a rapid loss of activity from 40°C to 50°C and complete loss of activity on heat treatment above 50°C (Sano *et al.*, 1994).

4. 4. 2. 4. Sulfhydryl groups

In small fish (Fig 13), the SH contents in rohu increased upto 5% of the original on heating to a temperature of 40°C and then dropped suddenly by 57 % on heating to 45°C. This is followed by a marginal increase by 4%, 23% and 37% respectively in the SH contents on heating to temperatures of 50°C, 55°C, and 60°C. In the case of small catla a 6% increase was noticed on heating to 40°C, which then dropped by 23% at 45°C followed by a small increase of 2 and 4% on further

heating to 55°C and 60°C respectively. In mrigal the changes in SH contents behaved in a similar fashion to rohu with an initial increase upto 40°C (6%) followed by a major drop at 45°C (43%). The value then marginally increased by 15%, 23% and 29 % on further heating to higher temperatures of 50°C, 55°C and 60°C.

Among big fishes (Fig 14), rohu showed an increase of 10% of the original value on heating to 40°C and then dropped by 18% on heating to 45°C. This was followed by an increase of 4%, 9% and 8% in the SH contents on heating to temperatures of 50°C, 55°C, and 60° C respectively. In catla, there was no initial increase but decreased gradually from 10°C onwards and the loss was 17% SH at 40°C. At 45°C the SH contents dropped by 35%. This was followed by a small increase of 8 and 13% on further heating upto 60°C. In mrigal only marginal initial increase was noticed upto 40°C but dropped by 63 % at 45°C. The SH contents increased as in the above cases by 7% and 17 % on heating to higher temperatures.

The surface reactive sulfhydryls increased marginally during heat treatment from 20°C to 40°C. The content decreased sharply from 40° to 45°C. The increase noticed in sulfhydryls on heating the actomyosin from 20° to 40°C was due to the emergence of SH groups to the surface as a result of the unfolding of actomyosin at higher temperatures. The

decrease noticed above 50° C was due to the oxidation of the exposed sulfhydryls preferably to disulfide bonds.

Heating Pollock and mackerel meat to different temperatures showed (Opstvedt *et al.*, 1984) no change upto 50°C but increased on heating upto 60° C and then decreased. Alongside this drop in sulfhydryls there was increase in disulfide bonds. These changes were noticed upon 20 minutes of heating. The temperatures above 50°C facilitated the formation of disulfide bonds through oxidative transformation of SH groups to disulfide bonds in fish meat (Opstvedt *et al.*, 1984). Similar findings were reported while studying the heat induced changes in the SH groups in Harp seal muscle proteins (Synowiecki and Shahidi, 1991). On the other hand the exposure of sulfhydryls and the formation of disulfide bond took place at lower temperatures in extracted actomyosins (Itoh *et al.*, 1979a; Itoh *et al.*, 1980; Sano *et al.*, 1994).

One-way ANOVA (Table 24 and 26) showed that the values of SH contents were significantly different (0.1 % level) between both small and big fish. The Duncan's analysis showed that among the values of SH contents for different fish, the values for mrigal had significant variation at all temperatures compared to rohu and catla (Table 25 and 27).

4. 4. 2. 5. CPA induced fluorescence

Among the small fishes, the CPA fluorescence intensity (Fig 15), in rohu, increased upto 58% of the original on heating to a temperature of 40°C and then increased substantially by about three folds on heating to 45°C. This fluorescence further increased with increase in temperature. In the case of catla, the increase was rather quick and the fluorescence increased by 77% on heating to 40°C, which increased further and reached more than five times on heating to 60°C. In the case of small mrigal, the increase in fluorescence intensity upto 133 % was noticed on heating to 40°C and reached three-fold on heating to 60°C.

Among the big fishes (Fig 16), the increase in fluorescence intensity in rohu was double that of small fish. The fluorescence intensity increased by one hundred percent on heating to 40°C and on further increasing the temperature to 60°C by about four folds. In catla, in contrast to the small fish, only 37% increase in fluorescence intensity was noticed on heating to 40°C; the value increased 7 times on heating to 60°C. The Increase in fluorescence intensity was much slower in the case of mrigal, and only about 40 % increase was noticed on heating to 40°C. On subsequent increase in temperature upto 60°C, the value increased by about three and half times only.

This showed that the CPA fluorescence intensity increased slowly in all the three fishes upto 40°C then increased with increasing temperature. The increase was substantial above 40°C. These results indicated that hydrophobic interactions among the actomyosin molecules occurred above 40°C and most extensively at higher temperatures. This suggested the emergence of hydrophobic aromatic amino acid residues to the surface of the actomyosin molecule leading to hydrophobic interaction.

In the case of carp natural actomyosin (Sano *et al.*, 1994), the ANS fluorescence intensity remained relatively unchanged upto 30°C and then increased with increasing temperature indicating that in carp the emergence of hydrophobic amino acids to the surface of the molecule occurred at 30°C. Similar increases in the ANS fluorescence intensity and hence hydrophobic amino acids to the surface was noticed on freezing of actomyosin from different fish as well (Niwa *et al.*, 1986a; Niwa *et al.*, 1986b). So, these types of changes in hydrophobic interactions are associated with changes in actomyosin irrespective of the treatment.

The difference in the fluorescence intensity for rohu, catla and mrigal were highly significant (0.1% level) for both small and big fish (Table 28 and 30). The fluorescence intensity data of rohu big and

mrigal small showed variations at all temperatures on Duncan's multiple analyses (Table 29 and 31).

Functional and textural characteristics of meats depend on myofibrillar proteins (Goll *et al.*, 1977). This is of significance in fish muscle than for mammalian muscle because of its lower collagen content.

Changes in the actomyosin composition of mature Argentine hake (*merlucius hubbsi marini*) are influenced by the metabolic state of the fish and is related to its reproductive cycle (Crupkin *et al.*, 1988). Crupkin *et al.*, (1988) reported that the actomyosin from pre spawning fish had relatively lower percentage of myosin. Actomyosin from fish caught in post spawning condition had higher ATPase activity, reduced viscosity and better gelling properties than those of pre spawning stages (Beas *et al.*, 1988; Roura *et al.*, 1990). The loss of filamentous structure in actomyosin from pre-spawned hake related to a decrease in the affinity between myosin and actin (Roura *et al.*, 1992).

Studies on the storage life of iced southern blue whiting (*Micromesistius australis*) demonstrated the biological conditions of the fish influencing the rheological properties of myofibrillar protein extracts (Barassi *et al.*, 1981; Beas *et al.*, 1988). It has been demonstrated in hake that in gonadal resting periods, the natural actomyosin was characterised by the significantly lower content of myosin heavy chains and more actin as components of actomyosin

system compared to pre and post spawned hake (Crupkin *et al.*, 1988; Roura *et al.*, 1990) and was reported to be related to the sensitivity of actomyosin to proteolysis in vivo. (Crupkin et *al.*, 1988).

The deteriorative changes taking place in seafood on frozen storage result from the deteriorative biochemical reactions occurring in lipids and proteins (Suzuki, 1981). Aggregation of proteins frequently occurred after prolonged frozen storage, which consequently caused a decrease in gelation during the preparation of minced fish products (Kurokawa, 1979). The stability of the proteins varied with species and storage conditions (Tsai *et al.*, 1989). However, changes as a result of long time storage are associated with protein denaturation or aggregation especially in the myosin – actomyosin system (Jiang *et al.*, 1987a). Thus, the properties of myofibrillar proteins are highly related to the quality of minced fish products. The content and the composition of free amino nitrogen are related to the stability of protein (Jiang and Lee, 1985; Jiang *et al.*, 1987b). Studies revealed that myosin in solutions or frozen condition tends to aggregate forming dimers, trimers and high molecular weight polymers (Buttkus, 1970; Jiang *et al.*, 1987a).

The decrease in the sulfhydryl group of fish and rabbit actomyosin during the storage at 0°C (Buttkus, 1971) at 4°C (Hamada *et al.*, 1977) and at –20°C (Jiang *et al.*, 1986b) clearly indicated the oxidation of SH groups at the active sites for ATPase activity in the

myosin head. However, subsequent studies (Sompongse *et al.*, 1996a; Sompongse *et al.*, 1996b; Sompongse *et al.*, 1996c; Sompongse *et al.*, 1996d) did not indicate the involvement of myosin head in the oxidation process but the sulfhydryl groups in the tail portion of the myosin molecule is assumed to play a role in the oxidation of actomyosin.

Thus, there were marginal differences in the NAM properties between small and big sized fish within the group. When the natural actomyosin from rohu, catla and mrigal were heated to 30°C relatively little change was noticed in any of the analysis. The natural actomyosin molecules began to unfold around 40°C and substantial unfolding occurred between 40°C and 60°C. As unfolding proceeded, the numbers of hydrophobic amino acid residues and number of SH groups increased at the surface of the molecule. Considering the actomyosin from these three fishes, the AM from rohu was more stable followed by mrigal and catla.

The interaction between the hydrophobic amino acids and the SH groups lead to the aggregation of molecule which began slowly at 40°C and extensively between 45° to 60°C. As the time of exposure of actomyosin to different temperatures is limited, there is possibility of formation of other weak bonds like hydrogen bond etc. between the actomyosin molecules. As a result of this, the protein molecules dissociate to some extent on subsequent cooling.

Table 1. Comparison of actomyosin characteristics from small and big rohu*

	Small fish	Big fish
NAM content (g/100g fish flesh)	7.60	6.44
Ca2+ ATPase, specific (μ moles Pi/min/mg P)	0.49	0.36
Ca2+ ATPase, total (μ moles Pi/min/mg P)	37.25	23
Reactive SH groups (μ moles/g P)	52	56
CPA fluorescence index	110	70

* mean of triplicate analysis

Table 2. Oneway ANOVA of the NAM characteristics from rohu

		Sum of Squares	df	Mean Square	F	Sig.
NAM concentration	Between Groups	2.018	1	2.018	91.954	.001
	Within Groups	8.780E-02	4	2.195E-02		
	Total	2.106	5			
ATPase activity, Specific	Between Groups	2.535E-02	1	2.535E-02	46.091	.002
	Within Groups	2.200E-03	4	5.500E-04		
	Total	2.755E-02	5			
ATPase activity, Total	Between Groups	294.000	1	294.000	73.500	.001
	Within Groups	16.000	4	4.000		
	Total	310.000	5			
CPA fluorescence	Between Groups	2400.000	1	2400.000	150.000	.000
	Within Groups	64.000	4	16.000		
	Total	2464.000	5			
SH groups	Between Groups	24.000	1	24.000	24.000	.008
	Within Groups	4.000	4	1.000		
	Total	28.000	5			

Table 3. Comparison of actomyosin characteristics from small and big catla*

	Small fish	Big fish
NAM content (g/100g fish flesh)	5.37	5.04
Ca2+ ATPase, specific (μ moles Pi/min/mg P)	0.43	0.61
Ca2+ ATPase, total (μ moles Pi/min/mg P)	37.25	31
Reactive SH groups (μ moles/g P)	81	98
CPA fluorescence index	110	122

* Mean of triplicate analysis

Table 4. Oneway ANOVA of NAM characteristics from catla

		Sum of Squares	df	Mean Square	F	Sig.
Atpase activity, Specific	Between Groups	4.860E-02	1	4.860E-02	16.475	.015
	Within Groups	1.180E-02	4	2.950E-03		
	Total	6.040E-02	5			
ATPase activity, Total	Between Groups	96.000	1	96.000	17.455	.014
	Within Groups	22.000	4	5.500		
	Total	118.000	5			
NAN Concentration	Between Groups	.660	1	.660	3.521	.134
	Within Groups	.750	4	.187		
	Total	1.410	5			
CPA Fluorescence intensity	Between Groups	216.000	1	216.000	7.714	.050
	Within Groups	112.000	4	28.000		
	Total	328.000	5			
SH groups	Between Groups	433.500	1	433.500	216.750	.000
	Within Groups	8.000	4	2.000		
	Total	441.500	5			

Table 5. Comparison of actomyosin characteristics from small and big mrigal*

	Small fish	Big fish
NAM content (g/100g fish flesh)	5.03	4.57
Ca2+ ATPase, specific (μ moles Pi/min/mg P)	0.5	0.74
Ca2+ ATPase, total (μ moles Pi/min/mg P)	25	34
Reactive SH groups (μ moles/g P)	62	69
CPA fluorescence index	60	130

* Mean of triplicate analysis

Table 6.Oneway ANOVA of NAM characteristics from mrigal

		Sum of Squares	df	Mean Square	F	Sig.
ATPase activity, Specific	Between Groups	8.640E-02	1	8.640E-02	27.000	.007
	Within Groups	1.280E-02	4	3.200E-03		
	Total	9.920E-02	5			
ATPase activity, Total	Between Groups	121.500	1	121.500	9.346	.038
	Within Groups	52.000	4	13.000		
	Total	173.500	5			
CPA Fluorescence intensity	Between Groups	7350.000	1	7350.000	350.000	.000
	Within Groups	84.000	4	21.000		
	Total	7434.000	5			
NAM Concentration	Between Groups	.317	1	.317	26.672	.007
	Within Groups	4.760E-02	4	1.190E-02		
	Total	.365	5			
SH groups	Between Groups	73.500	1	73.500	3.868	.121
	Within Groups	76.000	4	19.000		
	Total	149.500	5			

Table 7. Comparison of actomyosin characteristics from small rohu, catla and mrigal*

	Rohu	catla	Mrigal
NAM content (g/100g fish flesh)	7.60	5.37	5.03
Ca2+ ATPase, specific (μ moles Pi/min/mg P)	0.49	0.43	0.5
Ca2+ ATPase, total (μ moles Pi/min/mg P)	37	37	25
Reactive SH groups (μ moles/g P)	52	81	62
CPA fluorescence intensity	110	110	60

* Mean of triplicate analysis

Table 8. oneway ANOVA of NAM from small rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
ATPase activity, Specific	Between Groups	8.600E-03	2	4.300E-03	1.402	.316
	Within Groups	1.840E-02	6	3.067E-03		
	Total	2.700E-02	8			
ATPase activity, Total	Between Groups	344.000	2	172.000	21.500	.002
	Within Groups	48.000	6	8.000		
	Total	392.000	8			
CPA Fluorescence intensity	Between Groups	5000.000	2	2500.000	120.968	.000
	Within Groups	124.000	6	20.667		
	Total	5124.000	8			
NAM Concentration	Between Groups	10.656	2	5.328	37.953	.000
	Within Groups	.842	6	.140		
	Total	11.498	8			
SH groups	Between Groups	1302.000	2	651.000	177.545	.000
	Within Groups	22.000	6	3.667		
	Total	1324.000	8			

Table 9. Comparison of actomyosin characteristics from big rohu, catla and mrigal*

	Rohu	Catla	Mrigal
NAM content (g/100g fish flesh)	6.44	5.04	4.57
Ca2+ ATPase, specific (μ moles Pi/min/mg P)	0.36	0.61	0.74
Ca2+ ATPase, total (μ moles Pi/min/mg P)	23	31	34
Reactive SH groups (μ moles/g P)	56	98	69
CPA fluorescence intensity	70	122	130

* Mean of triplicate analysis

Table 10. Oneway ANOVA of NAM characteristics from big rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
ATPase activity, Specific	Between Groups	.224	2	.112	79.929	.000
	Within Groups	8.400E-03	6	1.400E-03		
	Total	.232	8			
ATPase activity, Total	Between Groups	194.000	2	97.000	13.857	.006
	Within Groups	42.000	6	7.000		
	Total	236.000	8			
CPA Fluorescence intensity	Between Groups	6368.000	2	3184.000	140.471	.000
	Within Groups	136.000	6	22.667		
	Total	6504.000	8			
NAM Concentration	Between Groups	5.678	2	2.839	396.126	.000
	Within Groups	4.300E-02	6	7.167E-03		
	Total	5.721	8			
SH groups	Between Groups	2774.000	2	1387.000	126.091	.000
	Within Groups	66.000	6	11.000		
	Total	2840.000	8			

Table 11. Oneway ANOVA of turbidity data from small rohu,catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
catla young	Between Groups	6.385E-02	7	9.122E-03	68.846	.000
	Within Groups	2.120E-03	16	1.325E-04		
	Total	6.597E-02	23			
mrigal young	Between Groups	.165	7	2.352E-02	119.007	.000
	Within Groups	3.162E-03	16	1.976E-04		
	Total	.168	23			
Rohu young	Between Groups	.136	7	1.944E-02	27.873	.000
	Within Groups	1.116E-02	16	6.975E-04		
	Total	.147	23			

Table 12. Duncan's analysis of turbidity data from small rohu, catla and mrigal

mrigal small

Duncan[a]

TEMP	N	Subset for alpha = .05			
		1	2	3	4
10.00	3	.2870			
20.00	3	.2950			
30.00	3	.2960			
40.00	3	.3120			
45.00	3		.3820		
50.00	3			.4250	
55.00	3				.4840
60.00	3				.5020
Sig.		.061	1.000	1.000	.136

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Catla small

Duncan[a]

TEMP	N	Subset for alpha = .05				
		1	2	3	4	5
10.00	3	.3460				
20.00	3		.3850			
30.00	3		.3980			
40.00	3		.3990			
45.00	3			.4250		
50.00	3				.4580	
55.00	3					.4970
60.00	3					.5010
Sig.		1.000	.177	1.000	1.000	.676

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Rohu small

Duncan[a]

TEMP	N	Subset for alpha = .05			
		1	2	3	4
10.00	3	.2260			
20.00	3	.2320			
30.00	3	.2370			
40.00	3	.2500			
45.00	3		.3410		
50.00	3		.3580	.3580	
55.00	3			.3980	.3980
60.00	3				.4210
Sig.		.321	.442	.082	.302

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 13. Oneway ANOVA of turbidity data from big rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
Catla old	Between Groups	7.818E-02	7	1.117E-02	88.379	.000
	Within Groups	2.022E-03	16	1.264E-04		
	Total	8.020E-02	23			
Mrigal old	Between Groups	.247	7	3.526E-02	216.323	.000
	Within Groups	2.608E-03	16	1.630E-04		
	Total	.249	23			
Rohu old	Between Groups	.114	7	1.631E-02	119.679	.000
	Within Groups	2.180E-03	16	1.363E-04		
	Total	.116	23			

Table 14. Duncan's analysis of turbidity data from big rohu, catla and mrigal

Catla old

Duncan[a]

TEMP	N	Subset for alpha = .05			
		1	2	3	4
10.00	3	.3520			
20.00	3	.3540			
30.00	3	.3590			
40.00	3	.3600			
45.00	3		.3850		
50.00	3			.4260	
55.00	3				.4890
60.00	3				.4980
Sig.		.435	1.000	1.000	.341

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Mrigal old

Duncan[a]

TEMP	N	Subset for alpha = .05				
		1	2	3	4	5
10.00	3	.2360				
30.00	3	.2360				
20.00	3	.2570				
40.00	3		.2980			
45.00	3			.3620		
50.00	3				.4380	
55.00	3					.4810
60.00	3					.4910
Sig.		.073	1.000	1.000	1.000	.352

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Rohu old

Duncan[a]

TEMP	N	Subset for alpha = .05					
		1	2	3	4	5	6
20.00	3	.2540					
10.00	3	.2580					
30.00	3	.2680					
40.00	3		.2980				
45.00	3			.3210			
50.00	3				.3680		
55.00	3					.3960	
60.00	3						.4580
Sig.		.182	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 15. Oneway ANOVA of solubility data from small rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
Catla young	Between Groups	82.555	7	11.794	583.479	.000
	Within Groups	.323	16	2.021E-02		
	Total	82.878	23			
Mrigal young	Between Groups	93.203	7	13.315	633.282	.000
	Within Groups	.336	16	2.102E-02		
	Total	93.540	23			
Rohu young	Between Groups	45.175	7	6.454	83.867	.000
	Within Groups	1.231	16	7.695E-02		
	Total	46.406	23			

Table 16. Duncan's analysis solubility data from small rohu,catla and mrigal

Catla young

Duncan[a]

TEMP	N	Subset for alpha = .05		
		1	2	3
50.00	3	1.0500		
45.00	3	1.1300		
55.00	3	1.1800		
60.00	3	1.2000		
40.00	3		4.7100	
30.00	3		4.7600	4.7600
20.00	3		4.9300	4.9300
10.00	3			4.9800
Sig.		.251	.090	.090

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Mrigal young

Duncan[a]

TEMP	N	Subset for alpha = .05			
		1	2	3	4
50.00	3	.7600			
45.00	3	.9600	.9600		
55.00	3	1.0100	1.0100		
60.00	3		1.0800		
30.00	3			4.5200	
40.00	3			4.5600	
10.00	3				5.0900
20.00	3				5.2800
Sig.		.061	.351	.740	.128

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Rohu young

Duncan[a]

TEMP	N	Subset for alpha = .05		
		1	2	3
45.00	3	1.4800		
50.00	3		2.5600	
55.00	3		2.8400	
60.00	3		2.9600	
30.00	3			4.9700
10.00	3			5.0900
40.00	3			5.0900
20.00	3			5.1500
Sig.		1.000	.113	.476

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 17. Oneway ANOVA of solubility data from big rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
Catla old	Between Groups	56.874	7	8.125	606.332	.000
	Within Groups	.214	16	1.340E-02		
	Total	57.088	23			
Mrigal old	Between Groups	47.162	7	6.737	254.123	.000
	Within Groups	.424	16	2.651E-02		
	Total	47.586	23			
Rohu old	Between Groups	18.145	7	2.592	60.742	.000
	Within Groups	.683	16	4.267E-02		
	Total	18.828	23			

Table 18. Duncan's analysis of slubility data from big rohu, catla and mrigal

Catla old

Duncan[a]

TEMP	N	Subset for alpha = .05		
		1	2	3
45.00	3	1.8400		
50.00	3	1.8600		
55.00	3	1.9800	1.9800	
60.00	3		2.1600	
40.00	3			4.9400
20.00	3			5.0400
10.00	3			5.0600
30.00	3			5.0900
Sig.		.179	.075	.163

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Mrigal old

Duncan[a]

TEMP	N	Subset for alpha = .05		
		1	2	3
45.00	3	1.5000		
50.00	3	1.7500		
55.00	3		2.3400	
60.00	3		2.5900	
40.00	3			4.6600
20.00	3			4.7900
30.00	3			4.8300
10.00	3			4.8300
Sig.		.078	.078	.256

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Rohu old

Duncan[a]

TEMP	N	Subset for alpha = .05				
		1	2	3	4	5
55.00	3	3.3200				
60.00	3	3.6400				
50.00	3		5.0400			
45.00	3		5.1000	5.1000		
30.00	3		5.3800	5.3800	5.3800	
40.00	3			5.4600	5.4600	5.4600
10.00	3				5.6800	5.6800
20.00	3					5.7700
Sig.		.076	.073	.059	.110	.100

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 19. Oneway ANOVA of ATPase activity data from small rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
Catla young	Between Groups	.569	7	8.134E-02	82.367	.000
	Within Groups	1.580E-02	16	9.875E-04		
	Total	.585	23			
Mrigal young	Between Groups	.864	7	.123	224.113	.000
	Within Groups	8.816E-03	16	5.510E-04		
	Total	.873	23			
Rohu young	Between Groups	1.259	7	.180	125.158	.000
	Within Groups	2.300E-02	16	1.437E-03		
	Total	1.282	23			

Table 20. Duncan's analysis of ATPase data from small rohu, catla and mrigal

Catla young

Duncan[a]

TEMP	N	Subset for alpha = .05			
		1	2	3	4
55.00	3	5.000E-02			
60.00	3	5.000E-02			
50.00	3	7.000E-02			
45.00	3		.2000		
40.00	3			.3100	
30.00	3			.3200	
10.00	3				.4300
20.00	3				.4400
Sig.		.471	1.000	.702	.702

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Mrigal young

Duncan[a]

TEMP	N	Subset for alpha = .05		
		1	2	3
55.00	3	.0000		
60.00	3	.0000		
50.00	3	1.000E-02		
45.00	3		.3200	
30.00	3			.4000
40.00	3			.4000
10.00	3			.4140
20.00	3			.4200
Sig.		.628	1.000	.351

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Rohu young

Duncan[a]

TEMP	N	Subset for alpha = .05		
		1	2	3
55.00	3	.0000		
60.00	3	.0000		
50.00	3	1.000E-02		
45.00	3		.1800	
40.00	3			.4800
10.00	3			.4900
20.00	3			.5000
30.00	3			.5000
Sig.		.764	1.000	.561

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 21. Oneway ANOVA of ATPase activity data from big rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
Catla old	Between Groups	1.362	7	.195	108.095	.000
	Within Groups	2.880E-02	16	1.800E-03		
	Total	1.391	23			
Mrigal old	Between Groups	1.306	7	.187	112.189	.000
	Within Groups	2.660E-02	16	1.662E-03		
	Total	1.332	23			
Rohu old	Between Groups	.396	7	5.657E-02	113.132	.000
	Within Groups	8.000E-03	16	5.000E-04		
	Total	.404	23			

Table 22. Duncan's analysis of ATPase data from big rohu, catla and mrigal

Catla old

Duncan[a]

TEMP	N	Subset for alpha = .05			
		1	2	3	4
55.00	3	7.000E-02			
60.00	3	7.000E-02			
50.00	3	8.000E-02			
45.00	3		.1900		
40.00	3			.4800	
30.00	3				.5700
10.00	3				.6100
20.00	3				.6100
Sig.		.788	1.000	1.000	.290

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Mrigal old

Duncan[a]

TEMP	N	Subset for alpha = .05	
		1	2
55.00	3	1.000E-02	
60.00	3	1.000E-02	
50.00	3	2.000E-02	
45.00	3	7.000E-02	
40.00	3		.4800
30.00	3		.4900
10.00	3		.5000
20.00	3		.5000
Sig.		.116	.589

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Rohu old

Duncan[a]

TEMP	N	Subset for alpha = .05			
		1	2	3	4
55.00	3	5.000E-02			
60.00	3	5.000E-02			
50.00	3	7.000E-02			
45.00	3		.2100		
40.00	3			.3100	
20.00	3			.3300	.3300
30.00	3			.3300	.3300
10.00	3				.3600
Sig.		.315	1.000	.315	.138

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 23. Effect of the incubation temperature on the rate constants for inactivation[a] of actomyosin Ca^{2+} ATPase of rohu, catla and mrigal

Temp ° C	K_D x10^{-3},s^{-1}					
	A	B	C	D	E	F
10	0	0	0			0
20	-0.084	0.704	0.284	0.167	-0.210	0.183
30	-0.168	0.665	3.381	0.764	0.198	1.119
40	0.161	1.222	3.865	2.628	0.433	0.781
45	7.072	4.159	6.169	9.000	13.5	5.5
50	30.2	11.8	13.9	14.9	20.7	28.5
55	25.9	15.8	16.1	13.7	28.5	24.6
60	28.2	13.8	15.7	7.3	35.7	31.8

[a] Kd = (ln C_0-ln C_t)/t where C_o= Ca-ATPase specific activity before incubation, C_t= Ca-ATPase
specific activity after t-s incubation, and t= incubation time (s). Concentration of actomyosin
1 mg/ml; pH 7.0. Means of three determinations from each sample were used to calculate KD values. A, rohu small; B, rohu big; C- catla small; D, catla big; E, mrigal small; F, catla big

Table 24. Oneway ANOVA of SH group data from small rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
Catla young	Between Groups	3192.000	7	456.000	34.743	.000
	Within Groups	210.000	16	13.125		
	Total	3402.000	23			
Mrigal young	Between Groups	9966.000	7	1423.714	142.371	.000
	Within Groups	160.000	16	10.000		
	Total	10126.000	23			
Rohu young	Between Groups	3722.625	7	531.804	54.544	.000
	Within Groups	156.000	16	9.750		
	Total	3878.625	23			

Table 25. Duncan's analysis of SH group data from small rohu, catla and mrigal

Catla young

Duncan[a]

TEMP	N	Subset for alpha = .05	
		1	2
60.00	3	59.0000	
55.00	3	60.0000	
50.00	3	62.0000	
10.00	3		81.0000
45.00	3		81.0000
20.00	3		85.0000
30.00	3		86.0000
40.00	3		86.0000
Sig.		.351	.145

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Mrigal young

Duncan[a]

TEMP	N	Subset for alpha = .05			
		1	2	3	4
60.00	3	17.0000			
55.00	3	21.0000	21.0000		
50.00	3		26.0000		
45.00	3			35.0000	
10.00	3				62.0000
20.00	3				62.0000
40.00	3				66.0000
30.00	3				67.0000
Sig.		.141	.071	1.000	.092

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Rohu young

Duncan[a]

TEMP	N	Subset for alpha = .05		
		1	2	3
45.00	3	22.0000		
50.00	3	24.0000		
55.00	3		34.0000	
60.00	3		39.0000	
20.00	3			51.0000
10.00	3			52.0000
30.00	3			52.0000
40.00	3			55.0000
Sig.		.444	.068	.167

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 26. Oneway ANOVA of SH group data from big rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
Catla old	Between Groups	8842.500	7	1263.214	32.918	.000
	Within Groups	614.000	16	38.375		
	Total	9456.500	23			
Mrigal old	Between Groups	9989.625	7	1427.089	83.334	.000
	Within Groups	274.000	16	17.125		
	Total	10263.625	23			
Rohu old	Between Groups	1916.625	7	273.804	19.384	.000
	Within Groups	226.000	16	14.125		
	Total	2142.625	23			

Table 27. Duncan's analysis SH group data from big rohu, catla and mrigal

Catla old

Duncan[a]

TEMP	N	Subset for alpha = .05			
		1	2	3	4
55.00	3	51.0000			
60.00	3	51.0000			
50.00	3	56.0000	56.0000		
45.00	3		64.0000		
40.00	3			81.0000	
30.00	3				94.0000
20.00	3				95.0000
10.00	3				98.0000
Sig.		.363	.133	1.000	.465

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Mrigal old

Duncan[a]

TEMP	N	Subset for alpha = .05		
		1	2	3
45.00	3	25.0000		
50.00	3	26.0000		
55.00	3	30.0000	30.0000	
60.00	3		37.0000	
10.00	3			69.0000
20.00	3			70.0000
30.00	3			70.0000
40.00	3			70.0000
Sig.		.179	.055	.789

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Rohu old

Duncan[a]

TEMP	N	Subset for alpha = .05	
		1	2
55.00	3	41.0000	
60.00	3	42.0000	
50.00	3	44.0000	
45.00	3	46.0000	
10.00	3		56.0000
20.00	3		61.0000
40.00	3		62.0000
30.00	3		63.0000
Sig.		.152	.051

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 28. Oneway ANOVA of CPA fluorescence data from small rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
Catla young	Between Groups	312412.5	7	44630.357	458.335	.000
	Within Groups	1558.000	16	97.375		
	Total	313970.5	23			
Mrigal young	Between Groups	281690.6	7	40241.518	576.939	.000
	Within Groups	1116.000	16	69.750		
	Total	282806.6	23			
Rohu young	Between Groups	268558.5	7	38365.500	369.788	.000
	Within Groups	1660.000	16	103.750		
	Total	270218.5	23			

Table 29. Duncan's analysis of CPA fluorescence data from small rohu, catla and mrigal

Catla young

Duncan[a]

TEMP	N	Subset for alpha = .05				
		1	2	3	4	5
10.00	3	110.0000				
20.00	3		180.0000			
30.00	3		180.0000			
40.00	3		195.0000			
45.00	3			350.0000		
50.00	3				370.0000	
55.00	3					410.0000
60.00	3					415.0000
Sig.		1.000	.096	1.000	1.000	.544

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Mrigal young

Duncan[a]

TEMP	N	Subset for alpha = .05					
		1	2	3	4	5	6
10.00	3	60.0000					
20.00	3	60.0000					
30.00	3		120.0000				
40.00	3			190.0000			
45.00	3				210.0000		
50.00	3					240.0000	
55.00	3						350.0000
60.00	3						355.0000
Sig.		1.000	1.000	1.000	1.000	1.000	.474

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Rohu young

Duncan[a]

TEMP	N	Subset for alpha = .05				
		1	2	3	4	5
10.00	3	110.0000				
20.00	3		158.0000			
30.00	3		170.0000			
40.00	3		174.0000			
45.00	3			320.0000		
50.00	3				340.0000	
55.00	3					380.0000
60.00	3					390.0000
Sig.		1.000	.086	1.000	1.000	.247

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 30. Oneway ANOVA of CPA fluorescence data from big rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
Catla old	Between Groups	270329.6	7	38618.518	589.596	.000
	Within Groups	1048.000	16	65.500		
	Total	271377.6	23			
Mrigal old	Between Groups	74682.292	7	10668.899	293.977	.000
	Within Groups	580.667	16	36.292		
	Total	75262.958	23			
Rohu old	Between Groups	347044.5	7	49577.786	856.636	.000
	Within Groups	926.000	16	57.875		
	Total	347970.5	23			

Table 31. Duncan's analysis CPA fluorescence data from big rohu, catla and mrigal

Catla old

uncan[a]

TEMP	N	Subset for alpha = .05					
		1	2	3	4	5	6
10.00	3	122.0000					
20.00	3	134.0000					
30.00	3		159.0000				
40.00	3		168.0000				
45.00	3			254.0000			
50.00	3				289.0000		
55.00	3					385.0000	
60.00	3						412.0000
Sig.		.088	.192	1.000	1.000	1.000	1.000

eans for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Mrigal old

Duncan[a]

TEMP	N	Subset for alpha = .05			
		1	2	3	4
10.00	3	95.0000			
20.00	3		110.0000		
30.00	3		110.0000		
40.00	3			135.0000	
45.00	3				220.0000
50.00	3				220.0000
55.00	3				220.0000
60.00	3				228.3333
Sig.		1.000	1.000	1.000	.138

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Rohu old

Duncan[a]

TEMP	N	Subset for alpha = .05							
		1	2	3	4	5	6	7	8
10.00	3	70.0000							
20.00	3		120.0000						
30.00	3			140.0000					
40.00	3				165.0000				
45.00	3					230.0000			
50.00	3						290.0000		
55.00	3							390.0000	
60.00	3								421.0000
Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

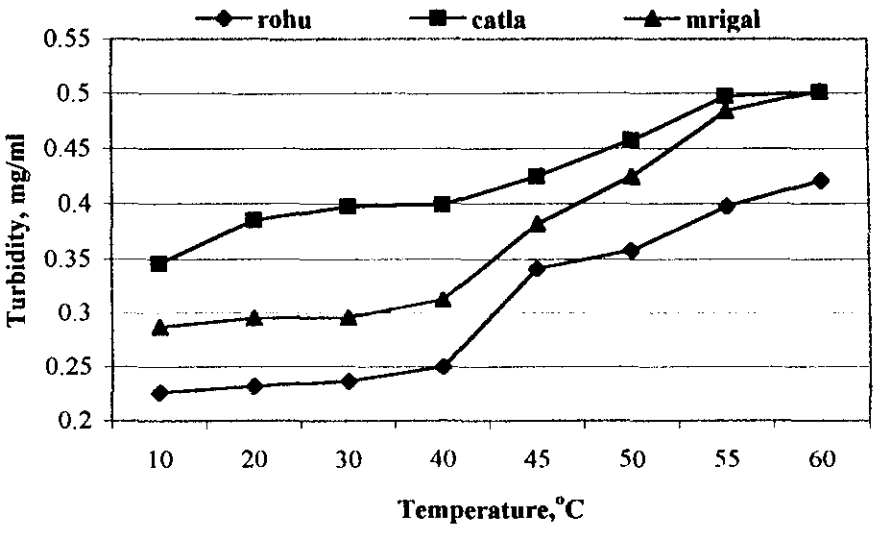


Fig 1. Changes in turbidity of natural actomyosin after heat treatment in small fish

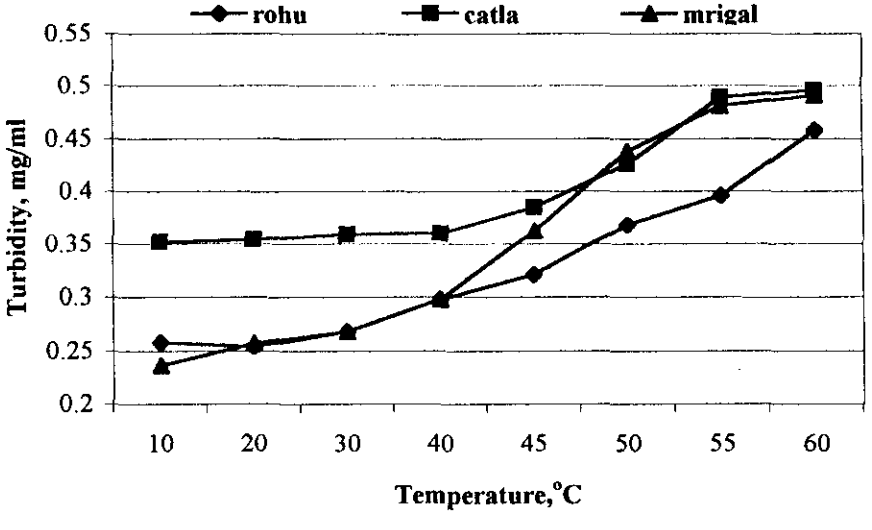


Fig 2. Changes in turbidity of natural actomyosin after heat treatment in big fish

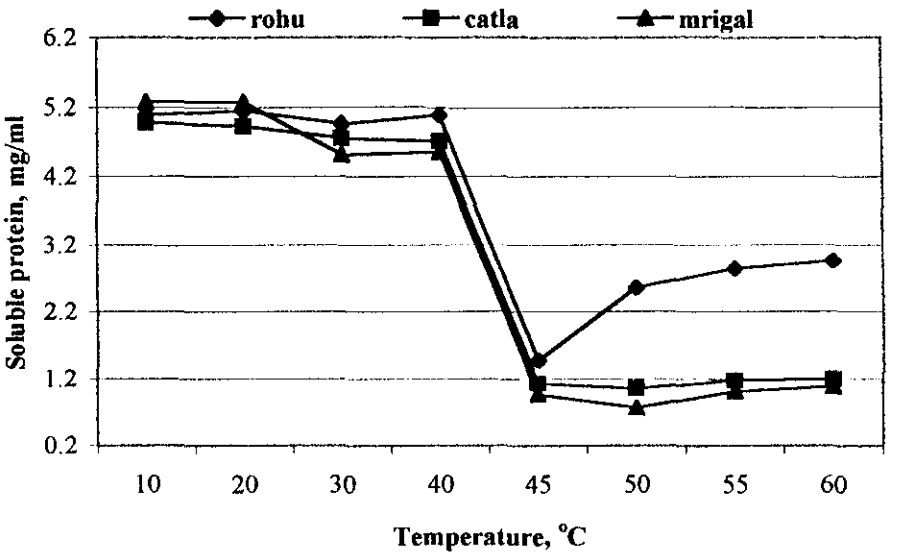


Fig 3. Changes in solubility of natural actomyosin after heat treatment in small fish

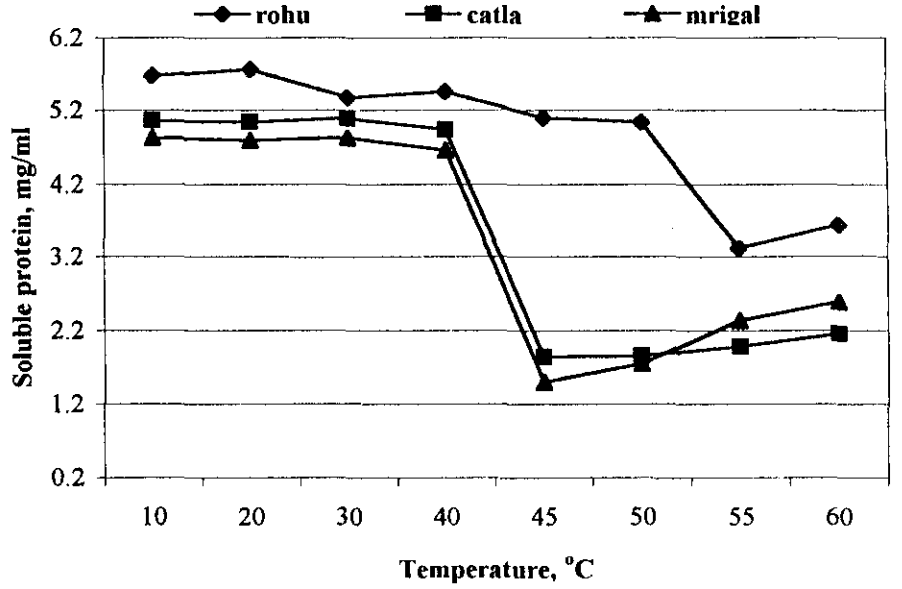


Fig 4. Changes in solubility of natural actomyosin after heat treatment in big fish

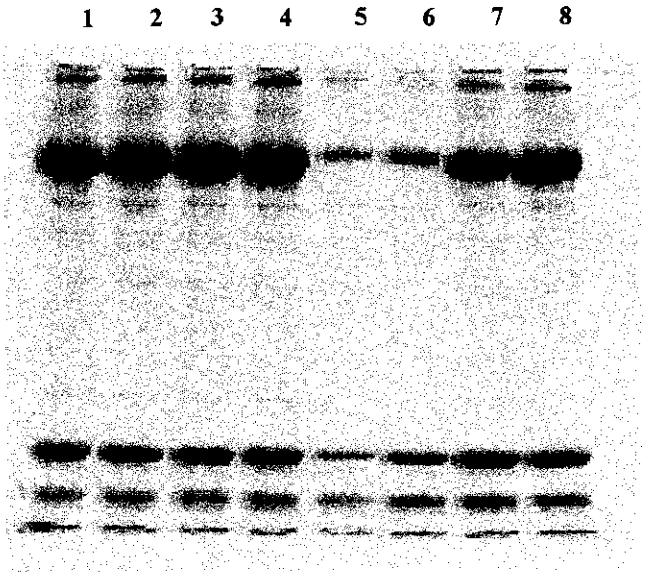


Fig. 5a. Electrophoretic separation of rohu (small) actomyosin heated to different temperatures. Lanes 1-8 treatments at 10, 20, 30, 40, 45, 50, 55 and 60°C.

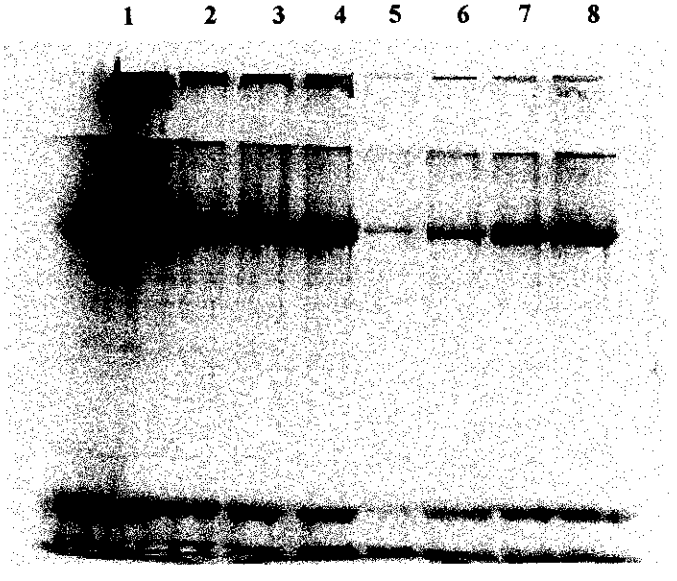


Fig. 5b. Electrophoretic separation of rohu (big) actomyosin heated to different temperatures. Lanes 1-8 treatments at 10, 20, 30, 40, 45, 50, 55 and 60°C.

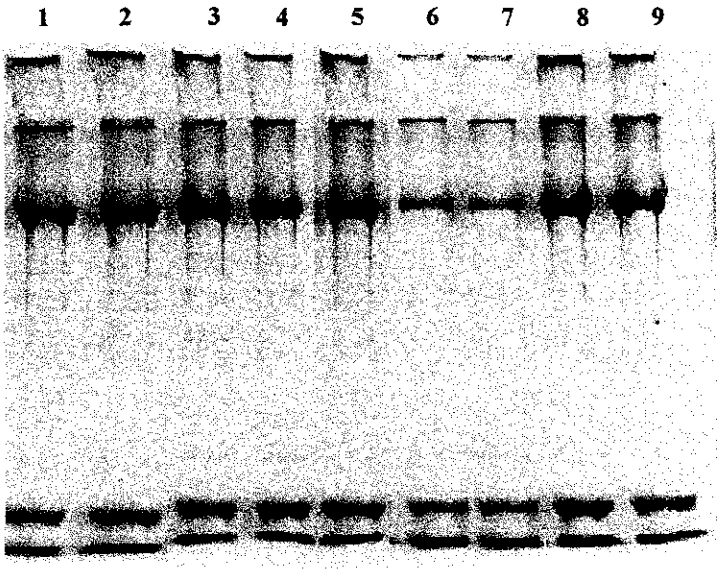


Fig. 6a. Electrophoretic separation of catla (small) actomyosin heated to different temperatures. 1- control; 2-8 indicate treatments at 10, 20, 30, 40, 45, 50, 55 and 60°C.

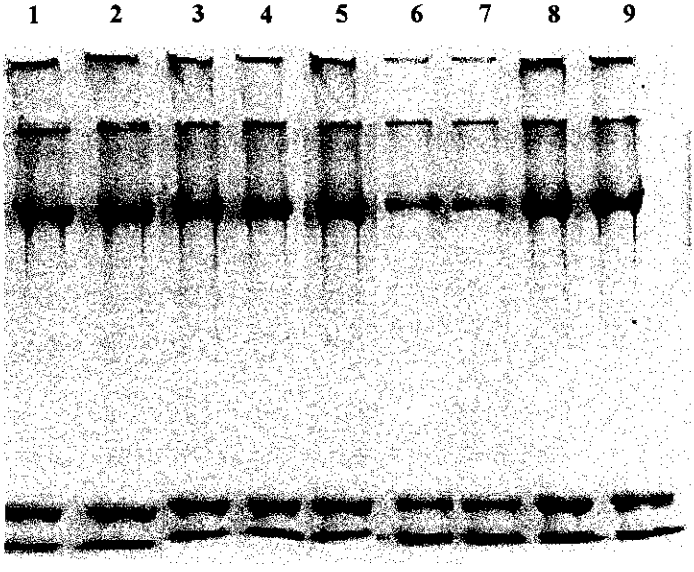


Fig. 6b. Electrophoretic separation of catla (big) actomyosin heated to different temperatures. Lane 1- control; Lanes 2-8 treatments at 10, 20, 30, 40, 45, 50, 55 and 60°C.

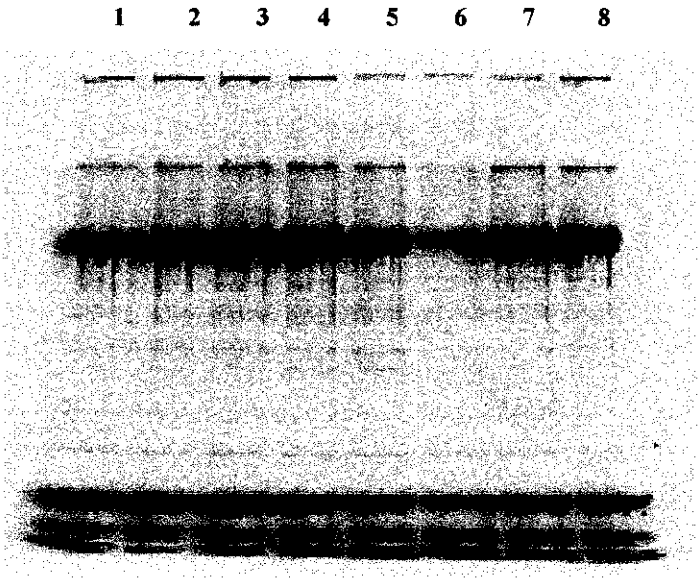


Fig. 7a. Electrophoretic separation of mrigal (small) actomyosin heated to different temperatures. Lanes1-8 treatments at 10, 20, 30, 40, 45, 50, 55 and 60°C.

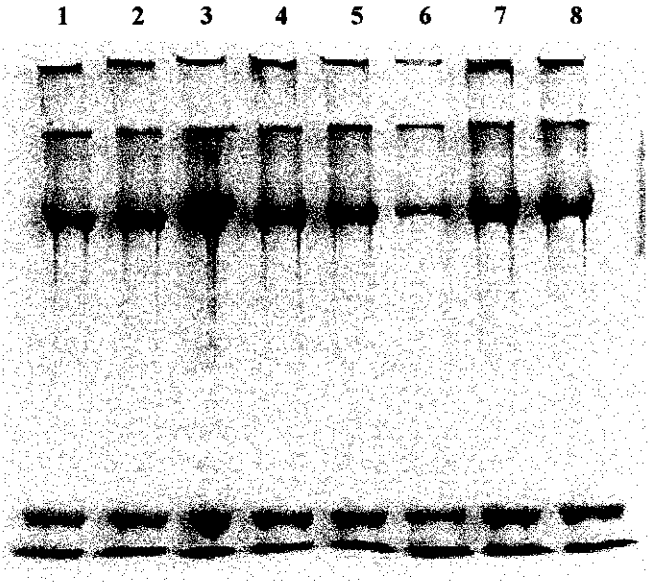


Fig. 7b. Electrophoretic separation of mrigal (big) actomyosin heated to different temperatures. Lanes 1-8 treatments at 10, 20, 30, 40, 45, 50, 55 and 60°C.

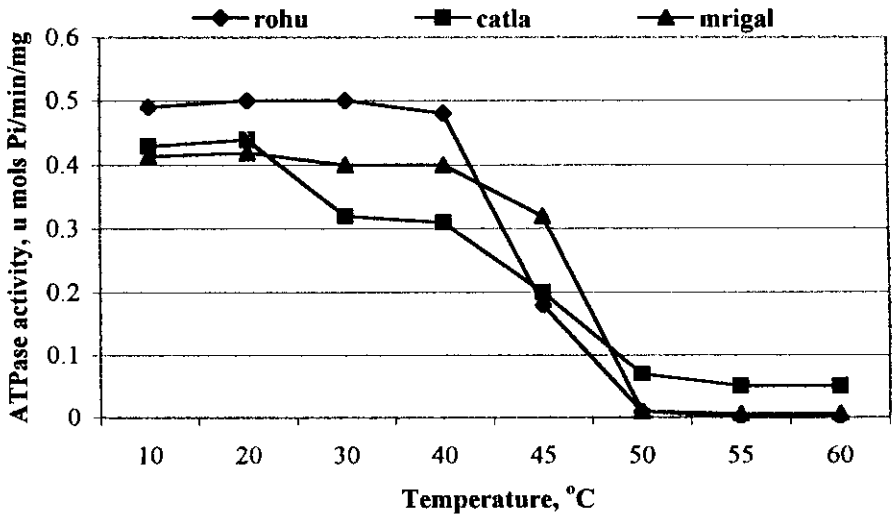


Fig 8. Changes in the Ca^{2+} ATPase specific activity of natural actomyosin after heat treatment in small fish

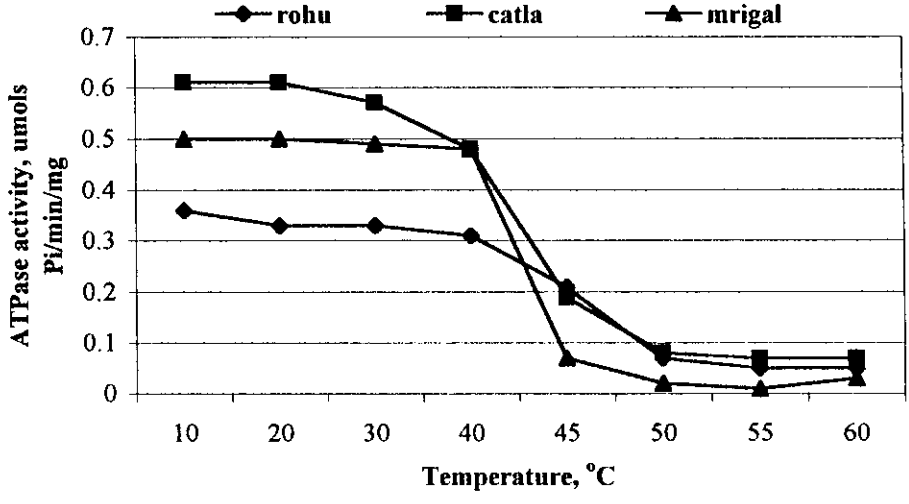


Fig 9. Changes in the Ca^{2+} ATPase specific activity of natural actomyosin after heat treatment in big fish

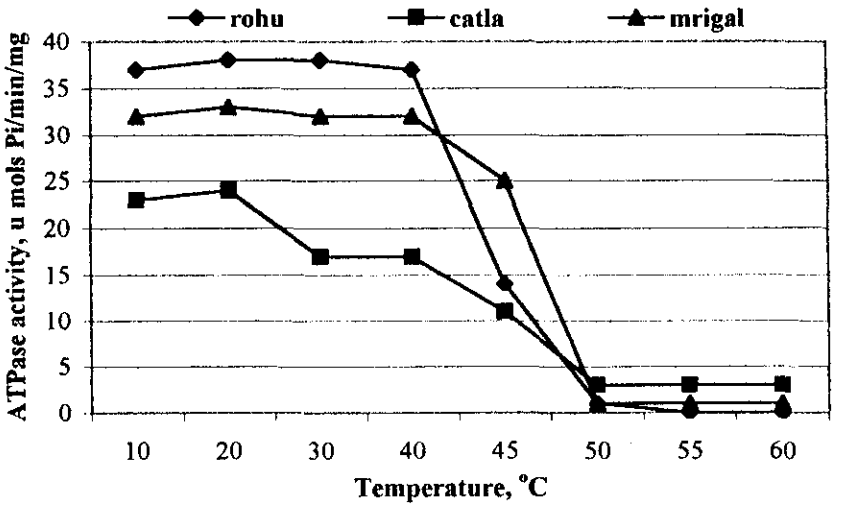


Fig 10. Changes in the Ca^{2+} ATPase Total activity of natural actomyosin after heat treatment in small fish

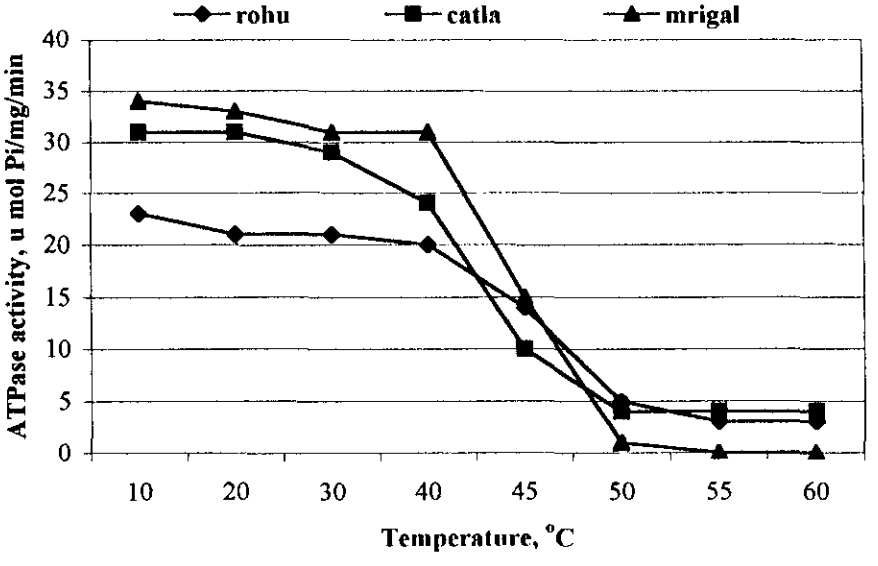


Fig 11. Changes in the Ca^{2+} ATPase Total activity of natural actomyosin after heat treatment in big fish

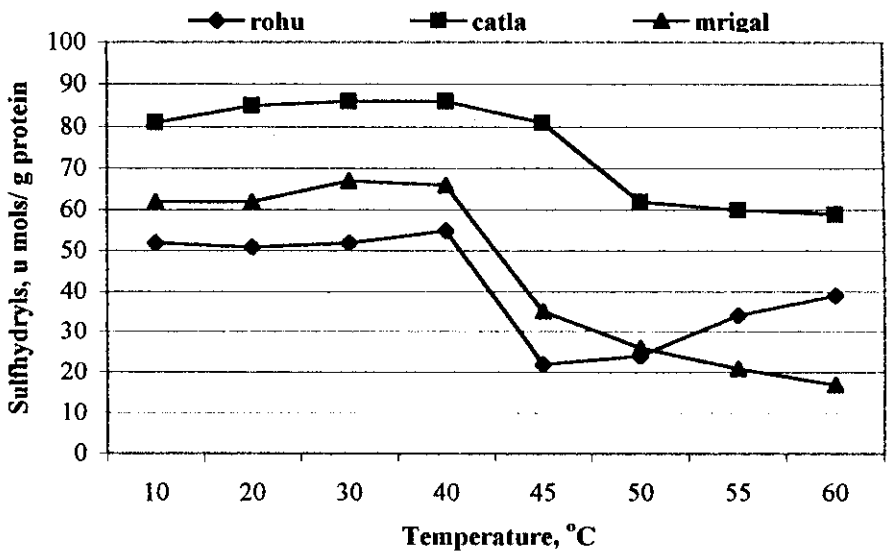


Fig 12. Changes in the surface sulfhydryls (SH) of natural actomyosin after heat treatment in small fish

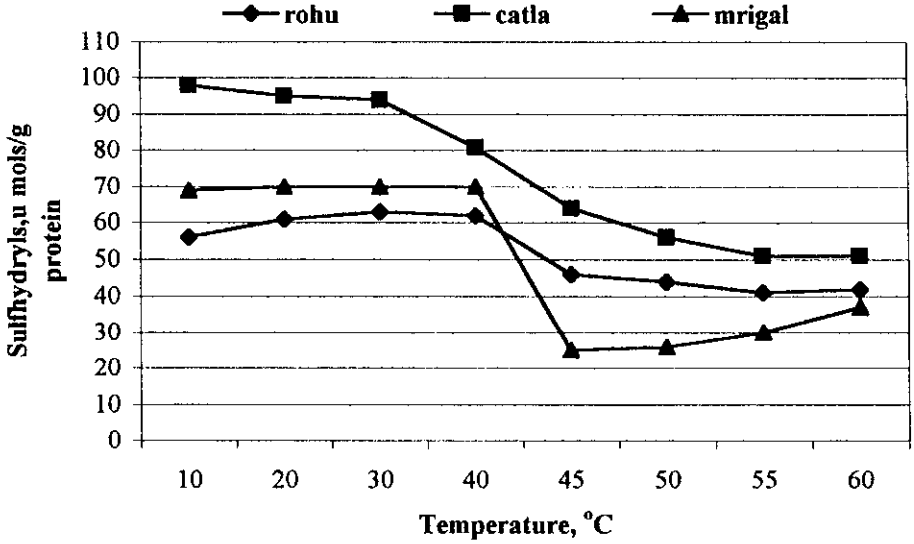


Fig 13. Changes in the surface sulfhydryls (SH) of natural actomyosin after heat treatment in big fish

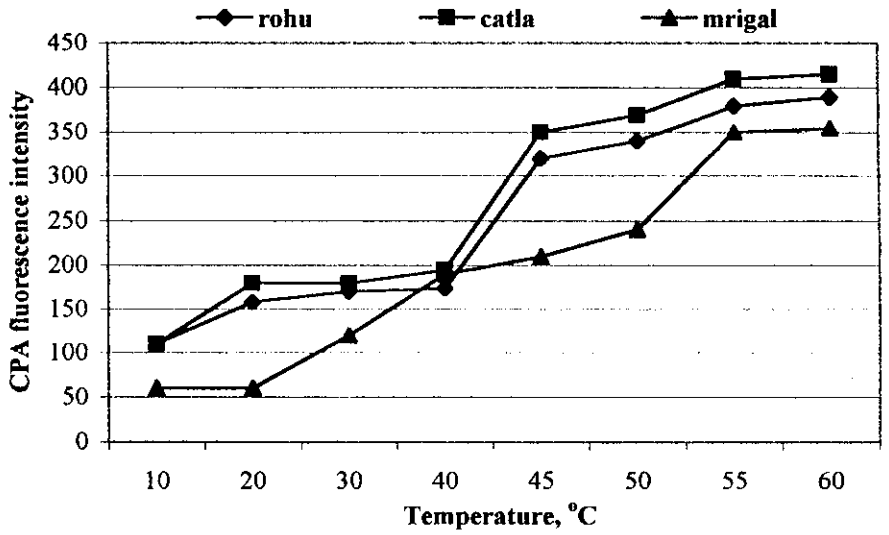


Fig 14. Changes in CPA fluorescence of natural actomyosin after heat treatment in small fish

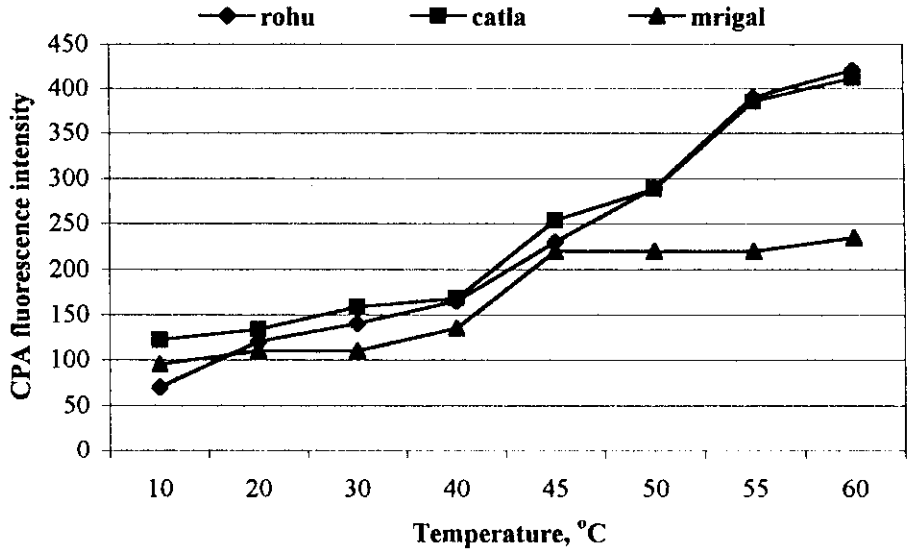


Fig 15. Changes in CPA fluorescence of natural actomyosin after heat treatment in big fish

Chapter – 5

Preparation of myofibrillar protein concentrate (Surimi)

Section - 1

Introduction

Myofibrillar protein concentrate or surimi is washed, refined fish mince, stabilised by cryoprotectants for a good frozen shelf life. It is a highly concentrated myofibrillar protein having great potential as a protein ingredient with specific properties, which make them suitable for the preparation of a number of fabricated food products.

Surimi is usually prepared from low fatty white meat with good gel forming ability to have an elastic texture and desirable characteristics like bland flavour and functional properties like emulsification properties, gel strength and elasticity. Though, generally most of the fishes are suitable for the preparation of surimi, fish with low fat content is often preferred because of its storage characteristics. Besides this, actomyosin, the principal component responsible for the above said functional properties, decides the suitability of a particular species of fish for preparation of myofibrillar protein concentrate. An elaborate processing schedule is necessary to get the desired quality washed mince and the washing process depends on the quality of mince from a particular fish. The gel forming ability of surimi decreases with an increase in water content (Lee, 1984). High water content is associated with lower myofibrillar protein content and decreases in the cross-link density. Since surimi preparation process is species specific, establishing the exact correlation between separating methods and test data becomes the task of each experiment.

5. 1. 1. Review of Literature

5. 1. 1. 1. Raw material

The selection of raw material is one of the important steps in surimi processing. The freshness of the fish is extremely important. Besides freshness the size and seasonality also affect the quality of the surimi (Suzuki, 1981; Lee, 1986; Lin & Morrissey, 1995). Freshness is a time dependent factor and the quality of the surimi is again dependent on the storage time and conditions of storage. The gel strength of kamaboko made from three-day ice stored lizardfish is only 50% of that from fresh fish (Kurukawa, 1979). A reduction in the gel strength of 10 - 20% for each day of storage in ice is reported in Pacific whiting (Morrissey *et al.*, 1992). Sardines were usable for surimi making only for 24 hours after catch while Pacific mackerel and white hake could be used for upto two days in iced condition (Hashimoto, 1987).

The season of the fish catch affect the quality of the mince as well as the surimi prepared from it. The fish caught during feeding season produce surimi of high quality because of the low moisture content and high nitrogen content of the fish meat (Lee, 1986). The fish from spawning season on the other hand have high pH resulting in the increased water retention; hence poor quality of the surimi. Mince from fatty fishes are highly unstable on storage. The higher content of highly unstable lipids, being more susceptible to oxidation and hydrolysis,

contribute to the protein denaturation and loss of gelling properties (Lanier, 1994). Deep skinning or high pressure water spray, water floatation or centrifugal separation of fat and a novel vacuum leaching to accelerate floatation of lipids and high fat dark meat are some of the methods developed to remove fat from the fatty fish (Nishioka *et al.*, 1990). Mince from very lean fish species and surimi from very fresh fish are almost comparable in terms of the gelling properties (McDonald *et al.*, 1990). However, on storage the gelling property of the whole mince is lost due to the presence of different enzyme systems and other water-soluble denaturants (Suzuki, 1981).

5. 1. 1. 2. Processing of mince to surimi

The method of mincing and the changes in the minced fish have been already discussed under Chapter 1. Introduction. Washing and mechanical dehydration of minced fish is carried out to produce a white, colourless and bland material. The minced fish essentially contains all the enzymes and other water-soluble substances, which are reported to interfere with the quality of the mince and hence need to be removed at the earliest. However, the process also removes a considerable amount of solubles decreasing the yield (Lee, 1984). Excessive washing, improves the quality of surimi but decreases the yield. Surimi yields of 21.4% on Pacific whiting (Lee *et al,* 1990) 23 - 32% for different fishes from Indian waters (Muraleedharan *et al.*, 1996a; Muraleedharan *et al.*,

1996b; Muraleedharan *et al.*, 1997) and 17% in northern squawfish (Lin & Morrisey, 1995) have been reported.

Washing the mince to remove the unwanted materials has been an accepted process for improving the quality of mince (Regenstein, 1986). Washing not only removes fat and other undesirable materials such as blood, pigments and other water soluble constituents of the mince like enzymes responsible for degradation of myosin (Lanier *et al.*, 1981; Lanier, 1994) but more importantly concentrates the myofibrillar protein (Lee, 1984; Babbit, 1986;). Water-soluble sarcoplasmic proteins, which include water-soluble nitrogen, different enzymes, substrates etc., retard gel network formation by interfering with the actomyosin cross-linking (Okada, 1964). As mincing leads to tissue disruption, exposing all the enzymes responsible for the different biochemical reactions in the live fish, the composition of the meat is lost if proper care is not taken at the earliest. Continuous washing in cold water of temperature less than 10°C with slow agitation gives good result. Excessive washing, however, does not completely remove different enzymes but only reduces their levels (Lin *et al.*, 1980). The remaining alkaline protease is responsible for the degradation of texture during cooking of the surimi products in several species of fish (Hamann *et al.*, 1990; Saeki et *al.*, 1995).

The efficiency of washing depends on the quality of water, ratio of meat to water and the number of washing cycles. Temperature, degree

of hardness, mineral content, pH, salinity and level of chlorination are some of the parameters of water affecting the washing efficiency and hence the final product. Higher content of calcium and magnesium is reported to affect the texture and that of iron and manganese affect the colour of the product. Hence, a soft water of minimum level of Ca/Mg and Fe/Mn is recommended (Lee, 1986). Keeping the meat in water for a long time, however, hydrates the meats affecting its functional properties. Extensive data is available on the leaching out of fish meat and the effect of different ratio of water to meat used to remove the water-soluble fractions (Lee, 1984). The use of 0.1-0.3% NaCl in the final washing to facilitate the effective removal of water from the mince was also suggested. The number of washing cycles depends on the quantity of materials to be removed. A maximum of two washings were suggested if a stirring time of 10 minutes is used (Lee, 1986). It was also reported that effective washing was also achieved, by using 1:3 meat to water (of pH 4.9 to 6.7) ratio (Pacheo-Agulier *et al.*, 1989).

The washed mince is strained to remove the foreign bodies like bones, scales, connective tissues etc. to give high quality surimi. As the heat sensitive myofibrillar protein fraction is treated through this process, proper care must be taken to prevent rise in temperature, which otherwise is likely to interfere with the quality of the product.

5. 1. 1. 3. Stabilising the surimi

As the idea behind the preparation of surimi is to utilise the fish protein as an ingredient in the preparation of value added products, it is mandatory to have the material in exceptionally good form with high stability. Fish protein being relatively low heat stable, denaturation and aggregation of myofibrillar protein play a dominant role in changing the functional property of frozen fish meat (Shenouda, 1980). The retention of gel forming ability and water holding capacity of actomyosin are essential in the manufacture of surimi-based products (Lee, 1984). The stabilisation hence requires the removal or inactivation of denaturants and protection of protein from the remaining denaturant action. Water washing of fish meat accomplishes the first part and addition of cryoprotectants play a role in stabilising the washed meat. Low molecular sugars, sugar alcohol, phosphates etc. have been used for the purpose.

The leached mince was mixed with a suitable cryoprotective agent in a silent cutter before packing. The most commonly used cryoprotectant is a mixture of sucrose (4%), sorbitol (4%) and tripolyphospahate (TPP- 0.2%) (Suzuki, 1981). Incorporating sucrose and sorbitol mixture effectively inhibited denaturation although excessive sweetness posed problems (Lee, 1984). A number of chemicals such as sodium glutamate (Naguchi and Matsumoto, 1970)

amino acid and related compounds (Noguchi and Matsumoto, 1970; 1975a), carboxylic acids (Noguchi and Matsumoto, 1975b; Rao, 1984), palatinit, lactitol and polydextrose (Sych *et al.*, 1990b) etc. have been used to overcome the problem of sweetness. Improvements in the quality of surimi were noted upon addition of a mixture of sucrose (5%, sorbitol (5%), 0.05% salt of carboxylic acids (calcium lactate and calcium citrate), 0.1% sodium bicarbonate and 0.3% sodium polyphosphate (Yamamoto *et al.*, 1992).

The free sulfhydryl groups in the protein molecule are responsible for its functional properties in the intact proteins. Upon denaturation, intra- molecular bonding affecting the functionality of the protein saturates these groups. Additions of reducing agents like, 0.1% sodium bisulphite, 0.15% L-cysteine, 8 μ. mol /g hydroquinone, 7 μ mol /g ascorbic acid into the mince increased the gel forming ability by recovering the reactive –SH groups (Lan *et al.*, 1987). Further addition of oxidising agents like hydrogen peroxide and potassium bromate into the gel containing the reducing agents increased the gel strength. During ice storage of actomyosin from carp, *Cyprinus carpio*, a decrease in the sulfhydryl group and Ca^{2+} ATPase activity were noticed along with dimerisation of myosin heavy chains. In the presence of sucrose, sorbitol or sodium glutamate the Ca^{2+} ATPase activity got stabilised but oxidation of –SH and dimerisation reactions proceeded indicating that

these reactions are independent of the presence of cryoprotectants (Sompongse *et al.*, 1996a).

The polyphosphates in association with the sugar and/or sorbitol stabilised the product by minimising the pH shift and maintaining a neutral pH and reduced the freeze-induced aggregation (Noguchi and Matsumoto, 1971; Park and Lanier, 1987; Park *et al.*, 1988). Addition of phosphates to surimi increased both solubility and the water holding capacity of the proteins (Yu *et al.*, 1994). Among the phosphates sodium tripolyphosphate offered good protection.

Sucrose, glucose syrup, sorbitol and sucrose/sorbitol mixture (1:1 w/w) offered best cryoprotection (Sych *et al.*, 1990a). Similar effects of sucrose and sorbitol were noticed in studies with *Nemipterus tolu* surimi (Yu *et al.*, 1994). Sugars and polyols stabilised proteins in solutions by stabilising the intra-molecular hydrophobic interactions (Black *et al.*, 1979) besides selectively hydrating the protein molecule.

Freeze-induced aggregation was effectively reduced by combination of phosphates and sugars. Poly dextrose (8%) and sucrose / sorbitol produced a better effect than that the cryoprotectants individually (Park and Lanier, 1987). In studies involving freeze-dried carp myofibrillar protein, the protection by sugars was related to the number and configuration of hydroxyl group in the sugar molecule (Matsumoto *et al.*, 1992).

Lactitol (D-galactosyl-β (1→4)-D-glucitol) produced by catalytic hydrogenation of lactose and palatinit produced from sucrose, containing equimolar mixture of (D-glucosyl-α (1→1)-D-glucitol) and (D- glucosyl-α (1→1)-D-mannitol) have reduced calorific value and sweetness in comparison to sucrose (Sych *et al.*, 1990b). Another sugar polymer, malto-dextrose, though had a similar effect on the solubility of salt soluble protein to that of sucrose/sorbitol mixture, it adversely affected the gel forming ability (Park *et al.*, 1988). On the other hand, polydextrose, a non-toxic highly branched glucose polymer of glucose produced by the thermal polymerization of glucose was patented as a effective substitute for sucrose/sorbitol as cryoprotective agent for surimi (Park *et al.*, 1988). At 8% level palatinit, lactitol and polydextrose produced similar cryoprotective effect as exhibited by sucrose/ sorbitol mixture (Sych *et al.*, 1990b). The formation of hydrogen bonds between these agents and the protein, hydrates the protein molecules and thereby reducing the chances of aggregation.

5. 1. 2. Aim and Objectives

This chapter aims to

- Optimize surimi processing methodology,
- Investigate the feasibility of recovering mince from fresh water major carps using a mechanical deboner,

- Evaluate the gel-forming behaviour of the surimi from fresh water fishes.
- Identify the differences if any between different sized fishes with respect to gel forming ability.

Results of the study are discussed in the following four sections, namely,

1. Changes in the nature of carp meat during washing
2. Pattern of yield during the processing of surimi from major carps
3. Gel forming ability of surimi from major carps, and
4. Comparison of rheological properties of surimi from different sizes of major carps.

Chapter – 5

Section - 2

Changes in the nature of carp meat during washing

5. 2. 1. Introduction

The main objective of surimi preparation is to remove the sarcoplasmic proteins (SPP) and to concentrate the myofibrillar proteins (MFP) to improve the gel forming ability of the proteins. The mince thus produced is white, odourless and bland flavoured, which can act as a vehicle for the production of value added products. But the removal of SPP is usually associated with considerable loss of myofibrillar proteins and this could be explained by the solubility of the MFP in water (Hennigar *et al.*, 1988; Lin *et al.*, 1995; Lin and Park, 1996). The solubility of MFP in water and low ionic strength solutions has been reported earlier (Stefansson and Hultin, 1994; Wu *et al.*, 1991).

Fish from salt water contain different salt concentrations in the muscle equivalent to 0.145 ionic strength (Wu *et al.*, 1991), which might inhibit the solubilisation of proteins. However, during repeated washing the salt from the meat is usually eliminated in the initial washings and this in turn affect the solubility of the proteins, especially the myofibrillar proteins (Wu *et al.*, 1991). Matsumoto (1959) reported that when muscle extracted by salt solutions are repeatedly extracted by water, further proteins become soluble. Washing cycles (Lee, 1986) and washing time (Pacheco-Aguilar, 1989), which have a direct bearing on the ionic strength of the meat are the main contributors affecting the solubility of MFP, besides pH (Foegeding, 1987; Pacheco-Aguilar,

1989), temperature (Lin and Chen, 1989), particle size (Acton, 1972), state of fish, like rigor or not (Suzuki, 1981; Park et al, 1990) and proteolytic activity of the meat (Chang-Lee *et al.*, 1990; Xiong and Brekke, 1989). When fish meat from white croaker and walleye pollock were washed with a solution containing $CaCl_2$, a controlled swelling associated with increase in the MFP was noticed but it also caused denaturation of MFP (Saeki and Hirata, 1994). The lower the thermal stability of MFP the more labile it became to $CaCl_2$ treatment (Saeki, 1995).

The effect of washing on the meat of major carps was studied under the following heads:

1. Effect of salt content and washing cycles in the extraction of soluble proteins from rohu, catla and mrigal during the washing process.
2. Changes in the composition of carp meat during washing procedures.

5. 2. 2. Materials and Methods

5. 2. 2. 1. Efficiency of water and brine in washing fish mince

The major carp (rohu, catla and mrigal) meat collected from a 5 mm perforation of the Baader 696 meat-picking machine from post rigor stage was subjected to washing. The washing schedule (Lin and Park,

1996) was carried out at (0 to 1°C) in a cold room. 40g fish meat was stirred with 160 ml (1:4 meat to wash solution ratio) wash solution (water or 0.2% NaCl) for 10 minutes and filtered using two layer nylon mesh (mesh 26). This completed one cycle and the residue and wash solution were taken for analysis. The residue from cycle one was subjected to further two cycles of washing. The filtrate from each washing step was considered to be soluble protein and the final residue was taken as insoluble proteins.

5. 2. 2. 1. 1. Protein studies

The protein extraction was carried out as described in Section 2. 4. 9 under Materials and Methods (Chapter 2). The protein content in the supernatant solutions were determined by the method of Lowry *et. al.* (Section 2. 4. 11. 2 of Chapter 2) using bovine serum albumin as standard. The amount of recovered and soluble proteins was expressed as percentage of total protein in the unwashed mince.

Electrophoresis of the samples was carried out as per Section 2. 4. 16 of Chapter 2 without equalising protein concentration. Water retention of both mince and washed mince were carried out by the method of Kocher and Foegeding (1993) as described under Section 2. 4. 17 of Chapter 2.

5. 2. 2. 2. Changes in composition during washing of fish mince

The washing schedule followed is shown in Fig 1. Washed mince from each washing was subjected to the analysis mentioned below.

Different analyses carried out include proximate composition (2. 4. 1), total soluble protein (2. 4. 6), water-soluble sarcplasmic protein - SPP(2. 4. 7), myofibrillar protein – MFP (2. 4. 8), Ca2+ ATPase activity (2. 4. 12) and rheological characteristics namely folding test (2. 4. 17. 2), gel strength and compressibility (2. 4. 17. 3).

5. 2. 3. Results and Discussion

5. 2. 3. 1. Efficiency of water and brine in washing fish mince

The protein retained in the meat, as measured by, total SDS soluble protein in the washed mince increased after washing with both water and 0.2% salt solution (Fig. 1, 4 and 7). In the case of rohu, the SDS soluble protein content increased from 59% to 68%, on dry weight basis, after the first wash and this value increased to 80 % after three washing cycles in the water to meat ratio of 4:1 and a stirring time of 10 minutes (Fig 1). In the case of mrigal the initial SDS soluble protein showed a decrease after the first wash but subsequently increased and a protein content of 87% was noticed at the end of three washes (Fig 7). A different observation was seen in the case of catla (Fig 4) where the washing decreased the SDS soluble proteins and a reduction of about

6% protein was noticed during the three washing cycles. The higher protein content, seen in unwashed mince did not appreciably increase as a result of washing indicating the possibility of MFP loss. On the other hand washing with salt solution did not appreciably increase the protein content in the meat. The increases after three washings in salt solutions were 3, 6, and 7 percentages respectively for rohu, catla and mrigal. This supports the finding (Wu *et al.*, 1991) that the presence of salt in the fish muscle might inhibit the solubilisation of proteins.

Considering the protein lost in the wash solution (water or brine) in the case of rohu first washing accounted for 18% proteins. The subsequent washings removed additional 6% and 4% respectively for the second and third washings (Fig. 2). In catla, the initial washing removed about 22% proteins in the wash solution followed by 9.5% and 4.5% for the subsequent washings (Fig. 5). This is related to the higher SPP content associated with catla meat (Chapter 2). However, for mrigal the values were 17%, 8% and 2% respectively for the three washings (Fig. 8). Lin and Park (1996) reported similar results during the washing of Pacific whiting meat with water washing accounting for higher loss of proteins.

Washing with salt solutions (three washings together) accounted for slight increase in the protein loss of, upto 30% in rohu (Fig 3). This could be the effect of salt on the solubility of MFP. In the case of catla

(Fig 6) almost similar loss was noticed but in the case of mrigal loss was 25% (Fig 9). The electrophoretic analysis also revealed maximum loss of proteins in the first washing (Fig 10 to 15). Washing with water (0 % NaCl) resulted in the removal of more proteins. The electrophorogram showed the intensity of high molecular weight protein (actomyosin) decreasing in the meat during the first, second and third washings. The electrophoresis of the wash water showed the presence of high molecular weight components having comparable molecular weights to myosin heavy chain and actin and majority of protein loss is seen in the first wash solution. This finding is similar in all the three fishes studied. Uses of higher water to meat ratio resulted in the higher loss of myofibrillar proteins (Lin and Park, 1996) and hence optimisation of water to meat ratio is of utmost importance in the washing processes. In the case of freshwater carps, which contains more sarcoplasmic proteins and almost no or negligible content of TMAO, water washing with 4:1 water to meat ratio and a stirring time of 10 min was found to be suitable to remove the sarcoplasmic proteins almost completely.

5. 2. 3. 1. 1. Moisture content and water retention of washed mince

The initial moisture contents of the unwashed meats were 78.79%, 80.6% and 79.03% respectively for rohu, catla and mrigal. After washing with large amount of water, the moisture content of the

washed mince increased substantially in all the three fishes. In rohu meat, the moisture increased by 5%, 9% and 12 % respectively for first, second and third washing cycles (Fig 16). The water uptake was almost similar for mrigal (Fig 18), namely, 3%, 8%, and 12% for the three washing cycles but in the case of catla (Fig 17), the increase was comparatively low (3, 5 and 10%) and is related to higher initial water content of catla meat.

There was increase in moisture content as a result of washing in salt solutions also. Adding salt (0.2% sodium chloride) to wash solutions facilitated the removal of water during the dehydration step and hence reduced moisture content of the washed minces when compared to the minces washed with water alone. In rohu, the increases in water contents as a result of washing with salt solution were 1, 5 and 6 % respectively for first, second and third washing cycles (Fig 16, 17 and 18). The values were 2, 4 and 6 for mrigal and 2, 5, and 9% for catla for the three washing cycles. These results compared well with the results of similar studies in Pacific whiting (Lin and Park, 1996). However, higher contents of salt in the wash solutions increased the water hydration properties of the washed mince (Lin and Park, 1996).

Eliminating water and water-soluble protein from the minced meat increased the MFP content in the washed meat. Removal of water-soluble proteins affects the natural conformation of the actomyosin

component of the MFP leading to an increase in the hydrophilicity of the myofibrillar proteins (Lin and Park, 1996). In this case, the catla meat exhibited higher water retention capacity in both type of washings and was followed by rohu and mrigal (Fig 19, 20 and 21). As a result of this the washed mince tend to hydrate and swell making removal of water from the washed mince a difficult process. Under these conditions small quantities (0.1 to 0.3%) of sodium chloride was usually added to facilitate the removal of water (Lee, 1984) and 0.25% sodium chloride was found to be sufficient for Pacific whiting (Lin and Park, 1996). The sodium chloride reduced the water holding capacity of the myofibrils by the interaction of sodium ions and thereby effecting the concentration of proteins. $CaCl_2$ was also reported to behave in a similar fashion, regardless of its concentration, preventing the washed meat from swelling and concentrating the myofibrillar proteins in white croaker and walleye pollock (Saeki and Hirata, 1994). Lin and Park (1996) further stated that at higher concentrations of salt (>2%) the hydrophillicity of the washed mince increased due to the strong affinity of the chloride ions than the sodium ions for myofibrillar proteins. As a result, the myofibrillar proteins repel each other leading to swelling.

5. 2. 3. 2. Changes in composition of meat during washing procedure

The components of the fish meat undergo changes during washing, an important step in the preparation of surimi. The meat

absorbed water during the process in all the three fishes with alterations in the composition of the meat.

5. 2. 3. 2. 1. Moisture

In the case of rohu, in the initial washing only a marginal increase (0.8%) in the moisture was noticed (Table 1) and it could be due to the higher moisture in the unwashed meat as a result of icing of the fish. About 3-3.5% water uptake was noticed in the subsequent washings. The total protein content, calculating on dry weight basis, decreased by 3.6 % in the first washing itself and second and third washings removed additional 1.2% and 0.5% proteins only. As far as fat is concerned, about 48% of the total fat is removed in three washings; of which more than 41% is removed in the first washing itself. The ash content increased by 85% in the first wash cycle itself and in the subsequent washing cycles increased the ash by additional 12 to 20%. This could be due to the fact that the final washing step in each washing cycle was carried out using 0.2% brine solution to facilitate the easy removal of water from the washed mince. The increased ash content in second and third washing cycles may be related to the increased loss of proteins associated with the increased absorption of NaCl by the meat.

The moisture content of surimi decreased by 6% to 9% as a result of addition of cryoprotectants to the washed minces. The final composition of surimi as a result of repeated washing is given in Table 2.

The Duncan's multiple test analysis (Table 3) showed that there was significant difference between the unwashed mince and two and three times washed minces as far as moisture content and protein contents were concerned. But in the case of fat, washing showed a significant difference from the unwashed mince.

There was about 1% increase in moisture in the case of catla after the first wash (Table 4). The second and third washings increased the same by about 3 %. The total protein (on dry weight basis) loss was about 4% in the first washing itself and second resulted in a loss of further 2.5% loss and the third washing did not remove any protein further. The initial fat was low, compared to rohu, and the first washing removed about 17% of the total fat, which increased to 29% in the second washing; the total loss at the end of three washings was 38%. The ash content as in the case of rohu increased by about 100% in the first wash itself and the subsequent washings did not increase the ash content further. Composition of surimi (Table 5) was slightly altered as a result of the addition of cryoprotectants.

The Duncan's multiple test analysis (Table 6) showed that the moisture and protein content did not differ significantly between the unwashed and the once washed meat but the fat and the ash differed significantly between unwashed and washed meat.

There was 1.6 % increase in moisture in the case of mrigal after the first wash (Table 7). The second washing increased the same by

about 5% but decreased in the third wash marginally due to the effective removal of water. The total protein loss on dry weight basis was about 4 to 5% after three washing cycles. About 45% decrease in the fat content was noticed in the first washing itself, which did not decrease any further due to further washing. As in the case of rohu and catla, the ash content in mrigal also increased during the washing cycles. Composition of surimi (Table 8) was slightly altered as a result of addition of cryoprotectants.

The Duncan's multiple test analysis (Table 9) showed significant difference in the fat and ash contents between washed and unwashed meat but the moisture and protein content showed significant differences between the unwashed, twice washed and thrice washed mrigal meat.

Aqueous washing of mechanically separated fish meat has been successfully used to produce surimi with excellent functional properties (Lanier, 1986). Loss of crude protein during washing has been reported during the preparation of surimi from Northern Squawfish (Lin and Morrissey, 1995). In all the three fishes, 38 to 48 % of the lipids were removed in the first washing itself and the subsequent washings removed the fat only marginally. However, this is of less significance considering the low fat content of major carps. Washing the meat to remove the fat content is of significance in preparing surimi from fatty fishes like sardine where, 60% (in the case of ordinary meat) to 80% (in

the case of whole or dark meat) fat is removed by washing step (Suzuki, 1981). However, washing with 0.5% solution of sodium bicarbonate was found to be very effective in the removal of fat from the meat of fatty fish and to enhance the *ashi* forming ability of the fish meat (Suzuki, 1981). Washing procedure incorporating lipid and water-soluble anti-oxidants to prevent the lipid oxidation and rancidity development has been tried during the preparation of beef heart surimi and its products (Wang *et al.*, 1997; Xiong and Brekke, 1993). The changes in the proximate composition of the washed meat showed significant difference when comparing with that of unwashed mince except for the moisture content in the first washing. However, between the washing cycles there was no significant difference in proximate composition of the mince. These findings are in line with the earlier reports with marine fishes (Chang-Lee *et al.*, 1990; Pacheco-Augilar *et. al.*, 1989; Lin & Morrissey, 1995). The higher values for the ash noticed in the washed meat were due to the incorporation of 0.1% NaCl in the wash water, which facilitate the removal of water from the hydrated meat (Lee, 1984).

5. 2. 3. 2. 2. Proteins

MFP, in fish, constitutes about 66-77% of total proteins and the water-soluble sarcoplasmic proteins to about 20-25% (Suzuki, 1981) and the range in content reflect the variations due to species, age of fish and processing method. Patashnik *et al.* (1982) noted that the MFP content

was 11% lower and SPP content was 15% higher in parasitised whiting than in uninfected fish meat. The total soluble proteins in rohu and mrigal meat accounted for 72% and 76% on dry weight basis while for catla the value was slightly higher (85%) (Tables 10, 12and 14). The water-soluble SPP contributed to 39% of this total soluble protein in rohu while for mrigal and catla the values were 35% and 33% respectively. The MFP contributed to 65% of total soluble protein in the case of fresh mrigal while for catla and rohu the values were 61% and 67% respectively.

Generally removing the lipids and water-soluble constituents from the meat concentrates the MFP content. The first washing cycle, in rohu (Table 11), effected the removal of 29% of total salt soluble proteins from the meat. 20% of this loss was from water-soluble SPP and 9% was from MFP. Further washings removed 7 to 8% SPP only and concentrated the MFP. The three washings accounted for the removal of 29% (%of the soluble protein) of the SPP, which was actually the removal of 74% of the total water-soluble SPP. The removal of SPP resulted in the concentration of MFP upto 15%.

In catla, the first washing itself (Table 13), removed 29% of total soluble proteins from the meat. 20% of this was water-soluble SPP and the remaining 9% MFP and the catla meat behaved almost similar to that of rohu. Further washings removed only about 3% SPP. The three

washings together accounted for the removal of about 23% of SPP (% total soluble proteins), which was infact about 68% of the total water-soluble SPP. The removal of SPP resulted in the concentration of MFP (5 to 8%).

The soluble proteins were reduced by 35% in the first washing itself in mrigal and the loss was almost equally shared between SPP and MFP (Table 15). The subsequent washings removed only the SPP (2-3%) and concentrated the MFP. The three washings accounted for the removal of about 21% SPP (% total soluble proteins), which was actually the removal of about 59% of the total water-soluble sarcoplasmic proteins. The removal of sarcoplasmic proteins resulted in the concentration of MFP upto 5%.

Loss of water-soluble proteins upto 50% in white croaker and 60% in Walleye Pollock during surimi production has been reported (Saeki and Hirata, 1994). Loss of MFP had taken place in all the three fishes but the proportion was higher in rohu where loss of MFP to the tune of 18% of total soluble protein. The loss of MFP in the other two fishes was almost comparable.

The retention of salt soluble proteins at the end of the washing depends on the initial content of soluble proteins, and the washing process. In the case of rohu (Fig 22, 23 and 24), the MFP concentration increased from 61% (of total soluble proteins) in the unwashed mince to

87% at the end of three washings. In catla, the increase was from 67% to 87% of total soluble proteins and in the case of mrigal 65 % to 80%. The MFP, contrary to the reports (Suzuki, 1981; Saeki and Hirata, 1994) decreased after the first washing by about 9%, 9% and 17% respectively for rohu, catla and mrigal. This could be attributed to the loss of myofibrillar proteins during the processing cycles or partly due to the proteolysis of the meat during processing (Suzuki, 1981; Patashnik, 1982; Chang-Lee *et al.*, 1990). The loss of MFP during the washing processes have been reported in Pacific Whiting (Chang-Lee *et al.*, 1990), Walleye Pollock (Saeki & Hirata, 1994), and freshwater Catla (Sankar and Ramachandran, 1998). Studies on the recovered proteins from wash water also revealed considerable loss of myofibrillar proteins during surimi processing (Lin *et.al.*, 1995).

5. 2. 3. 2. 3. Adenosine Triphosphatase activity

The myofibrillar ATPase is located in the myosin head region. The ATP hydrolysing activity of myosin is used to explain the conformational changes taking place in the myosin molecule even though it does not necessarily imply the unfolding of protein chain (Chan *et al.*, 1995). The ATPase activity of the actomyosin (Table 16) showed alteration in all the three fishes as a consequence of washing. The residual ATPase activity in the fish meat after washing showed a decrease in the case of mrigal but increased in the case of rohu and catla.

The first washing itself resulted in the loss of about 5% activity in mrigal and in the subsequent washings decreased by 18% and 22% respectively. About 16% increase in activity was noticed in the first washing in the case of catla while in rohu it was only 18%. In both fishes subsequent washings reduced the activity marginally. Maximum increase in activity in the case of rohu and catla is related to the concentration of MFP after washing. This probably explains the conformational changes taking place in fish myosin as a result of washing. Takashiki *et al.* (1962) found that the helical content of myosin molecule decreased by only a few percent even after ATPase activity completely disappeared. However, Kawashima et al (1973) found a close correlation between the gel strength and amount of actomyosin by means of Ca^{2+} ATPase activity. In this case, even though, a marginal loss in ATPase activity was noticed, the washed meat still retained its gelling properties.

5. 2. 3. 2. 4. Gelling characteristics

The unwashed mince itself was found to have good elastic characteristic and gelling properties. The rheological properties such as gel strength and compressibility of the washed meat, however, increased as a result of washing. Besides this the colour of the mince also improved as a result of washing. The unwashed fish meat from rohu,

catla and mrigal itself showed better gel strength and compressibility besides having good folding test characteristics.

Washing the meat once increased gel strength marginally in rohu (Table 17), which decreased on further washing due to increase in the water content in the washed meat. The compressibility of the cooked gel also increased about 47% in once washed meat, which decreased on further washing but still was higher than the control. The statistical analysis of the gel strength (Table 18) data did not differ significantly (sig. 0.065) between the unwashed and washed meat while the compressibility data showed high significance between the unwashed, once washed and twice washed meat but not with thrice washed meat.

In the case of catla, washing the meat increased the gel strength and compressibility by more than 100% (Table 19). But subsequent washings decreased the rheological characteristics as in the case of rohu. There was significant difference in the gel strength data between unwashed, once washed and twice washed meat but not between the twice washed and three times washed meat (Table 20). The compressibility data were significantly different from each other.

Washed mrigal meat behaved almost similar to that of washed catla meat with gel strength data showing double the values compared to unwashed fish meat (Table 21). The compressibility data almost increased three times on washing the fish meat once, which decreased marginally on subsequent washings. The statistical analysis of the gel

strength data showed significant difference between the unwashed, once washed and twice washed meat but not with the thrice-washed meat as in the case of catla (Table 22). The compressibility data showed statistical significance between unwashed, once washed and three times washed samples.

Different characteristics including the water content of the meat and the degree of proteolysis in the meat affect the rheological properties of the gel formed. Increased water content dilutes the protein content and limits the protein availability for gel formation. Increased myosin content has got a direct effect on the gel strength, measured as tensile strength and shear stress (Takagi and Simidu, 1972; Hamada, 1992; Hamman and MacDonald, 1992; Reppond and Babbit, 1997). Ofstad *et al.*, (1993) reported improvements in the shear stress and shear force in cod muscles washed with a solution containing Ca and Mg ions. The gel characteristics are also related to the water content of the meat and an inverse relationship was noticed between the gel characteristics and moisture content of surimi from different fishes (Reppond and Babbit, 1997; Chen and Lee, 1997).

Thus, there is considerable loss in salt extractable protein, with a marginal loss of MFP in the first washing itself. The elasticity of the gel, the gel strength and the compressibility of the fish meat, however, increased as a result of washing. Mrigal had comparatively higher gel

strength followed by catla and rohu. Washing resulted in the conformational changes in fish actomyosin to some extent, as shown by the decrease in the Ca^{2+} ATPase activity. There are no interfering substances like TMA in the meat of freshwater fish and the quality improvement as seen by colour, flavour and acceptability of mince and kamaboko produced, is noticed in one wash itself. Hence, a single washing with mince to water (containing 0.2% NaCl) ratio of 1:4 could be recommended for the processing of rohu, catla and mrigal mince.

Table 1. Mean proximate composition* (% wet weight) of rohu meat during surimi preparation

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Unwashed flesh	83.09 (0.14)	15.45 (0.29)	1.05 (0.09)	1.02 (0.15)
Once washed	83.83 (0.78)	14.24 (1.48)	0.56 (008)	1.80 (0.69)
Twice washed	85.58 (0.67)	12.54 (0.33)	0.45 (0.18)	1.71 (021)
Thrice washed	86.04 (0.49)	12.68 (0.51)	0.43 (0.03)	1.72 (0.03)

* Values are mean of three determinations with standard deviation

Table 2. Mean proximate composition* (% wet weight) of rohu surimi[a]

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Once washed	77.30 (0.60)	15.13 (0.24)	0.57 (0.03)	2.57 (0.22)
Twice washed	78.57 (0.63)	13.10 (0.13)	0.51 (0.03)	3.12 (0.26)
Thrice washed	78.31 (0.41)	12.7 (0.25)	0.45 (0.04)	4.01 (0.62)

* Values are mean of three determinations with standard deviation

[a] Surimi is composed of washed mince mixed with 4.0% sucrose,40% sorbitol and 0.2% tripolyphosphate.

Table 3. Duncan analysis of composition in rohu

Moisture

		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	unwashed	3	83.090	
	Washing cycle 1	3	83.827	
	Washing cycle 2	3		86.047
	Washing cycle 3	3		86.583
	Sig.		0.360	0.453

Protein

		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	Washing cycle 3	3	12.680		
	Washing cycle 2	3	12.540		
	Washing cycle 1	3		14.200	
	Unwashed	3			15.457
	Sig.		0.723	1.000	1.000

Fat

		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	Washing cycle 3	3	0.430	
	Washing cycle 2	3	0.450	
	Washing cycle 1	3	0.560	
	Unwashed	3		1.005
	Sig.		0.086	1.000

Ash

		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	unwashed	3	1.023	
	Washing cycle 1	3		1.710
	Washing cycle 2	3		1.720
	Washing cycle 3	3		1.803
	Sig.		1.000	0.781

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000

Table 4. Mean proximate composition* (% wet weight) of catla meat during surimi preparation

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Unwashed flesh	83.30 (0.95)	16.54 (052)	0.45 (0.08)	0.60 (005)
Once washed	84.34 (0.25)	14.94 (1.29)	0.35 (0.07)	1.16 (0.10)
Twice washed	86.44 (0.66)	12.55 (0.51)	0.26 (0.05)	1.19 (0.05)
Thrice washed	86.14 (0.36)	13.01 (1.10)	0.23 (0.04)	1.21 (0.45)

* Values are mean of three determinations with standard deviation

Table 5. Mean proximate composition* (% wet weight) of catla surimi[a]

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Once washed	77.52 (0.43)	14.59 (0.22)	0.31 (0.03)	1.70 (0.35)
Twice washed	78.69 (0.75)	11.89 (0.21)	0.21 (0.02)	1.75 (0.28)
Thrice washed	77.33 (0.79)	12.64 (0.31)	0.23 (0.05)	1.96 (0.45)

* Values are mean of three determinations with standard deviation

[a] Surimi is composed of washed mince mixed with 4.0% sucrose,40% sorbitol and 0.2% tripolyphosphate.

Table 6. Duncan analysis of composition in catla

Moisture

		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	unwashed	3	83.300	
	Washing cycle 1	3	84.343	
	Washing cycle 3	3		86.143
	Washing cycle 2	3		86.437
	Sig.		0.071	0.575

Protein

		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	Washing cycle 2	3	12.550	
	Washing cycle 3	3	13.013	
	Washing cycle 1	3		14.943
	Unwashed	3		16.543
	Sig.		0.555	0.066

Fat

		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	Washing cycle 3	3	0.230	
	Washing cycle 2	3	0.260	
	Washing cycle 1	3	0.347	0.347
	Unwashed	3		0.450
	Sig.		0.065	0.084

Ash

		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	Unwashed	3	0.603	
	Washing cycle 1	3		1.157
	Washing cycle 2	3		1.193
	Washing cycle 3	3		1.213
	Sig.		1.000	0.364

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000

Table 7. Mean proximate composition* (% wet weight) of mrigal meat during surimi preparation

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Unwashed flesh	82.3 (1.14)	16.18 (0.38)	1.06 (0.06)	1.22 (0.24)
Once washed	83.60 (0.87)	15.05 (1.96)	0.54 (0.13)	2.11 (0.53)
Twice washed	86.17 (0.36)	12.05 (1.07)	0.51(0.26)	2.03 (0.22)
Thrice washed	85.65 (1.46)	12.78 (0.97)	0.48 (0.05)	2.28 (0.18)

* Values are mean of three determinations with standard deviation

Table 8. Mean proximate composition* (% wet weight) of mrigal surimi[a]

Samples	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Once washed	76.80 (0.95)	14.57 (0.19)	0.41 (0.07)	1.85 (0.29)
Twice washed	77.40 (1.71)	11.84 (0.19)	0.45 (0.04)	1.94 (0.08)
Thrice washed	77.24 (0.83)	11.22 (0.24)	0.40 (0.02)	3.36 (0.67)

* Values are mean of three determinations with standard deviation

[a] Surimi is composed of washed mince mixed with 4.0% sucrose,40% sorbitol and 0.2% tripolyphosphate.

Table 9. Duncan analysis of composition in mrigal

Moisture

		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	unwashed	3	82.300		
	Washing cycle 1	3	86.600	83.600	
	Washing cycle 3	3		85.150	85.150
	Washing cycle 2	3			86.170
	Sig.		0.163	0.104	0.263

Protein

		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	unwashed	3	12.050		
	Washing cycle 1	3	12.783	12.783	
	Washing cycle 3	3		15.050	15.050
	Washing cycle 2	3			16.180
	Sig.		0.537	0.081	0.349

Fat

		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	Washing cycle 3	3	0.480	
	Washing cycle 2	3	0.510	
	Washing cycle 1	3	0.540	
	Unwashed	3		1.060
	Sig.		0.656	1.000

Ash

		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	unwashed	3	1.220	
	Washing cycle 2	3		2.010
	Washing cycle 1	3		2.170
	Washing cycle 3	3		2.280
	Sig.		1.000	0.127

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 3.000

Table 10. Effect of washing on the protein compositions in rohu meat

	Sarcoplasmic protein* (g/100g flesh)	Total soluble Protein * (g/100g flesh)	Myofibrillar protein * (g/100g flesh)
Unwashed	28.27 ± 0.31	72.32 ± 2.28	44.06 ± 0.41
Washing cycle 1	13.67 ± 0.53	51.21± 0.65	37.54 ± 0.65
Washing cycle 2	8.11 ± 0.25	56.93 ± 0.59	48.82 ± 0.73
Washing cycle 3	7.31 ± 0.44	58.35 ± 1.00	51.04 ± 0.83

Values are mean of three determinations with standard deviation
*on dry weight basis

Table 11. Effect of washing on the loss of protein in rohu meat

	Sarcoplasmic protein		Myofibrillar protein	
	Loss (%)	Loss, % of total soluble protein	Loss (%)	Loss, % of total soluble protein
Washing cycle 1	51.65	20.19	14.79	9.02
Washing cycle 2	71.31	27.88	-10.81	-6.58
Washing cycle 3	74.14	28.98	-15.85	-9.65

Values are mean of three determinations with standard deviation

Table 12. Effect of washing on the protein compositions in catla meat

Fish flesh	Sarcoplasmic protein* (g/100g flesh)	Salt soluble Protein * (g/100g flesh)	Myofibrillar protein * (g/100g flesh)
Unwashed	28.26 ± 1.36	85.27 ± 2.72	57.01 ± 1.40
Washing cycle 1	11.37 ± 1.03	60.47 ± 0.83	49.11 ± 2.58
Washing cycle 2	9.15 ± 0.38	72.57 ± 1.09	63.42 ± 1.01
Washing cycle 3	9.09 ± 1.66	70.85 ±1.08	61.76 ± 2.38

Values are mean of three determinations with standard deviation
* on dry weight basis

Table 13. Effect of washing on the loss of protein in catla meat

Fish flesh	Sarcoplasmic protein		Myofibrillar protein	
	Loss (%)	Loss, % of total soluble protein	Loss (%)	Loss, % of total soluble protein
Washing cycle 1	59.78	19.82	13.86	9.25
Washing cycle 2	67.65	22.43	-11.24	-7.52
Washing cycle 3	67.84	22.49	-8.33	-5.57

Values are mean of three determinations with standard deviation

Table 14. Effect of washing on the protein compositions in mrigal meat

Fish flesh	Water soluble protein* (g/100g flesh)	Salt soluble Protein * (g/100g flesh)	Myofibrillar protein * (g/100g flesh)
Unwashed	27.23 ± 2.69	76.44 ± 15.9	49.21± 13.35
Washing cycle 1	14.15 ± 1.41	49.57 ± 7.69	35.43 ± 8.02
Washing cycle 2	12.11 ± 2.74	57.89 ± 3.44	45.78 ± 1.04
Washing cycle 3	11.29 ± 1.17	56.37 ± 0.80	45.08 ± 1.60

Values are mean of three determinations with standard deviation
* on dry weight basis

Table 15. Effect of washing on the loss of protein in mrigal

Fish flesh	Sarcoplasmic protein		Myofibrillar protein	
	Loss (%)	Loss, % of total soluble protein	Loss (%)	Loss, % of total soluble protein
Washing cycle 1	48.05	17.12	28.01	18.03
Washing cycle 2	55.52	19.78	6.96	4.48
Washing cycle 3	58.54	20.86	8.38	5.39

Values are mean of three determinations with standard deviation

Table 16. Effect of washing on Ca^{2+}ATPase activity of rohu, catla and mrigal meat

	ATPase activity, umols Pi/mg/min		
Fish flesh	**Rohu**	**Catla**	**Mrigal**
Unwashed	0.57± 0.02	0.37± 0.06	0.41± 0.18
Washing cycle 1	0.67± 005	0.46± 0.03	0.39 ± 0.05
Washing cycle 2	0.64± 0.02	0.42± 0.04	0.34 ± 0.05
Washing cycle 3	0.65± 0.01	0.41± 0.01	0.32 ± 0.07

Values are mean of three determinations with standard deviation.

Table 17. Effect of washing on the rheological properties of rohu meat

	Washing cycles			
	Unwashed	**I**	**II**	**III**
Folding test[a]	AA	AA	AA	A
Gel strength, (g x cm)	190 ± 10	211 ± 6.0	209 ± 15.0	195 ± 9.0
Compressibility, (g)	159 ± 5.0	232 ± 22.0	205 ± 14.0	153 ± 10.0

value are mean of three determinations with standard deviation.

[a] folding test grading - AA-no crack after folding twice; A – cracks on second folding; B- cracks on folding once, C- cracks immediately after folding once, D- breakable by finger touch without folding

Fig 18. Duncan analysis of Gel strength and compressibility in rohu

Gel Strength

		N	Subset for alpha = 0.05
			1
Duncan[a]	unwashed	3	190.33
	Washing cycle 3	3	194.00
	Washing cycle 2	3	209.33
	Washing cycle 1	3	210.67
	Sig.		0.065

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 3.000

Compressibility

		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	Washing cycle 3	3	152.67		
	unwashed	3	158.67		
	Washing cycle 2	3		204.67	
	Washing cycle 1	3			232.33
	Sig.		0.623	1.000	1.000

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 3.000

Table 19. Effect of washing on the rheological properties of catla meat

	Washing cycles			
	Unwashed	**I**	**II**	**III**
Folding test[a]	AA	AA	AA	A
Gel strength, (g x cm)	211 ± 14.5	422 ± 14.7	402 ± 17.6	371 ± 26.1
Compressibility, (g)	100 ± 10.5	242 ± 14.2	207 ± 12.1	136 ± 16.0

value are mean of three determinations with standard deviation.

[a] folding test grading - AA-no crack after folding twice; A – cracks on second folding; B- cracks on folding once, C- cracks immediately after folding once, D- breakable by finger touch without folding

Fig 20. Duncan analysis of Gel strength and compressibility in catla

Gel Strength

		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	unwashed	3	211.00		
	Washing cycle 3	3		371.33	
	Washing cycle 2	3		402.00	402.00
	Washing cycle 1	3			422.33
	Sig.		1.000	0.081	0.222

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 3.000

Compressibility

		N	Subset for alpha = 0.05			
			1	2	3	
Duncan[a]	unwashed	3	100.00			
	Washing cycle 3	3		136.33		
	Washing cycle 2	3			207.00	
	Washing cycle 1	3				241.66
	Sig.		1.00	1.00	1.00	1.00

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 3.000

Table 21. Effect of washing on the rheological properties of mrigal meat

	Washing cycles			
	Unwashed	**I**	**II**	**III**
Folding test[a]	AA	AA	AA	A
Gel strength, (g x cm)	246 ± 5.0	522 ± 12.0	513 ± 7.0	498 ± 9.0
Compressibility, (g)	85 ± 3.0	256 ± 9.0	252 ± 4.0	213 ± 9.0

value are mean of three determinations with standard deviation.

[a] folding test grading - [a] folding test grading - AA-no crack after folding twice; A – cracks on second folding; B- cracks on folding once, C- cracks immediately after folding once, D- breakable by finger touch without folding

Fig 22. Duncan analysis of Gel strength and compressibility in mrigal

Gel Strength

		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	unwashed	3	246.33		
	Washing cycle 3	3		498.00	
	Washing cycle 2	3		513.33	513.33
	Washing cycle 1	3			522.00
	Sig.		0.163	0.104	0.263

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 3.000

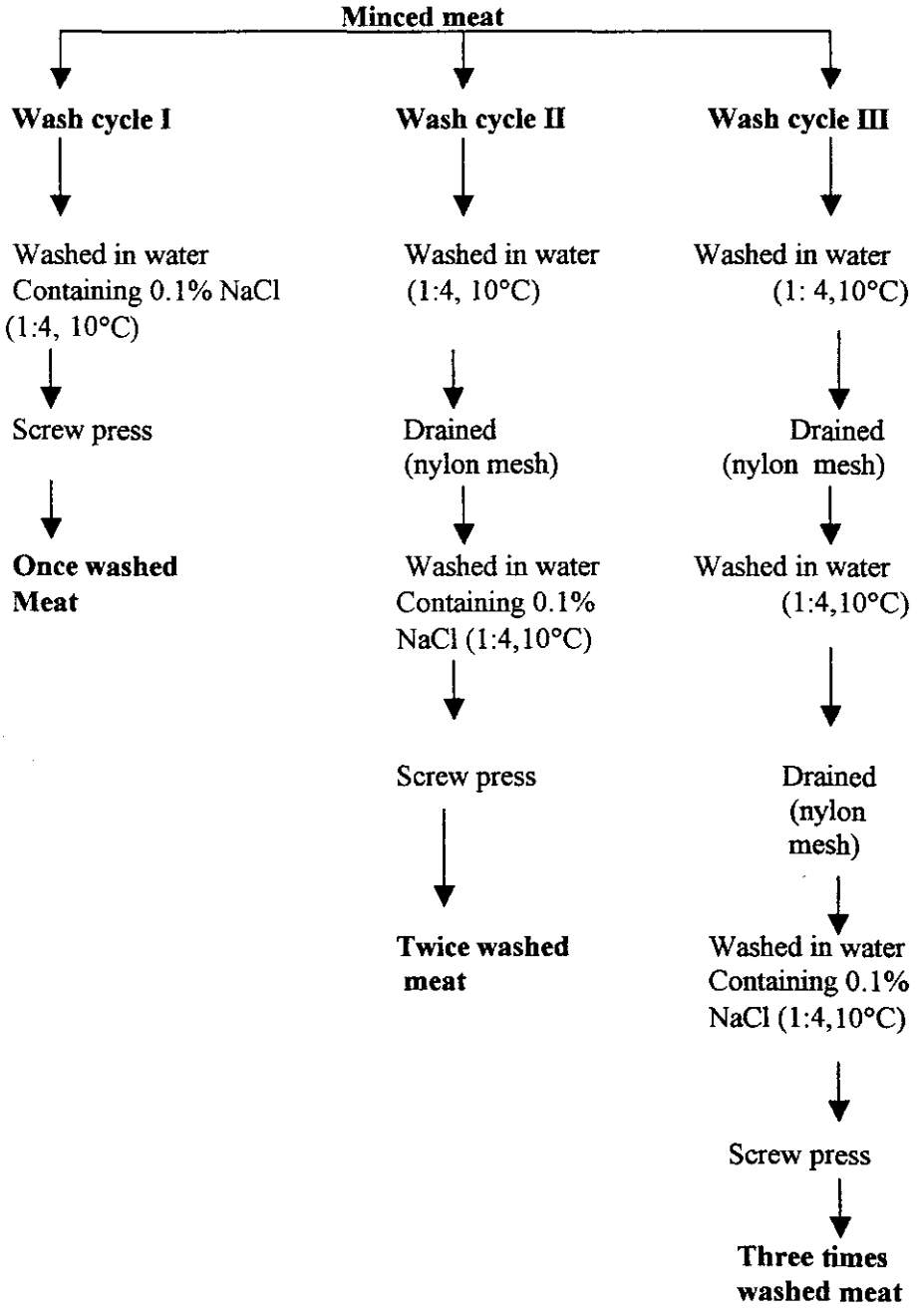
Compressibility

		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	unwashed	3	85.667		
	Washing cycle 3	3		213.00	
	Washing cycle 2	3			254.33
	Washing cycle 1	3			256.00
	Sig.		1.000	1.000	0.781

Means for groups in homogeneous subsets are displayed

a. Uses Harmonic Mean Sample Size = 3.000

Fig 1. Washing schedule for fish meat



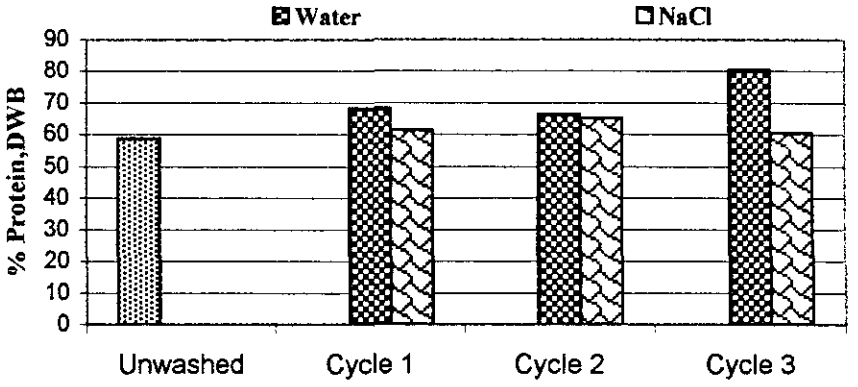


Fig 2. Protein retention in rohu flesh after various washings

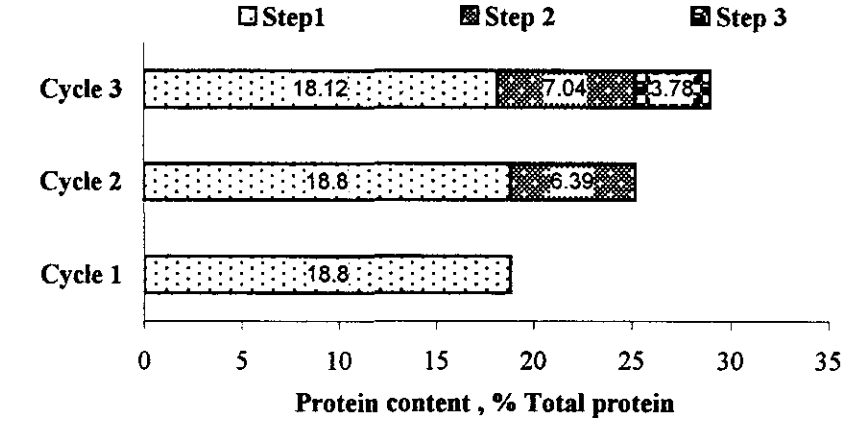


Fig 3. Removal of proteins in wash solution during water washing in different washing cycles in rohu

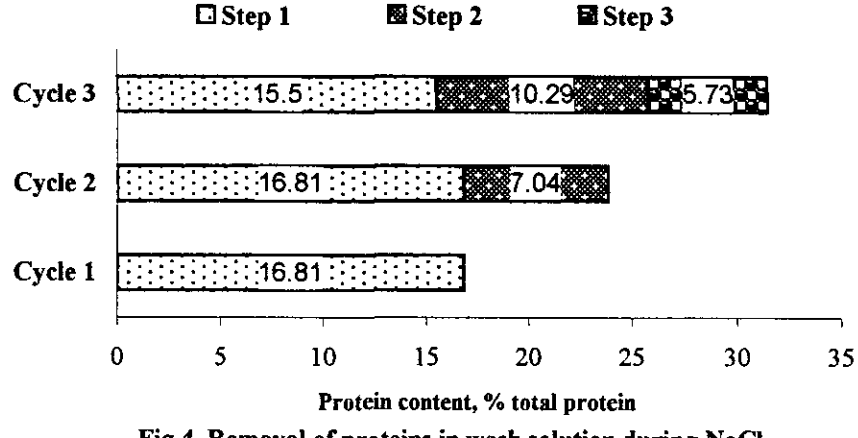


Fig 4. Removal of proteins in wash solution during NaCl washing in different washing cycles in rohu

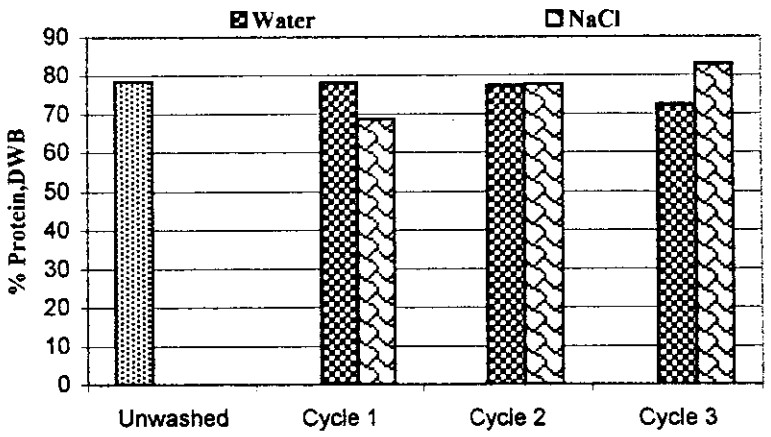


Fig 5. Protein retention in catla flesh after various washings

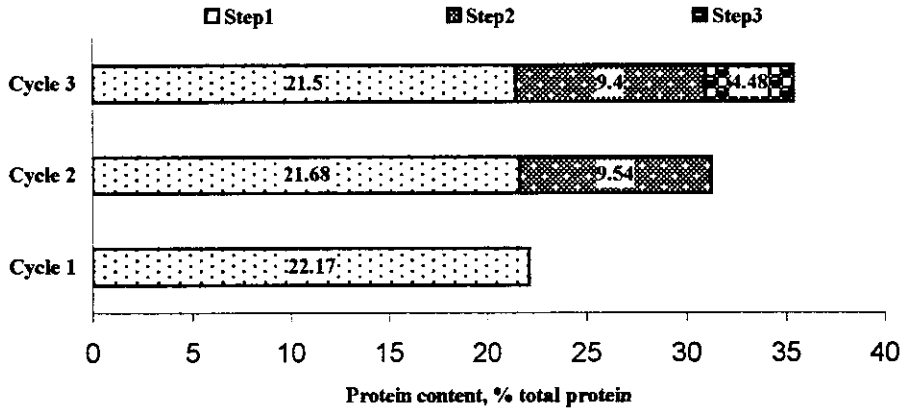


Fig 6. Removal of proteins in wash solution during water washing in different washing cycles in catla

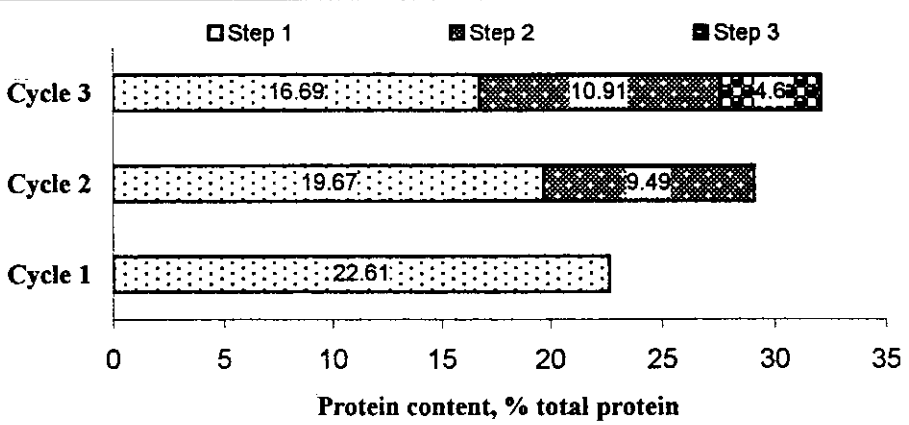


Fig 7. Removal of proteins in wash solution during Nacl (0.2%) washing in different washing cycles in catla

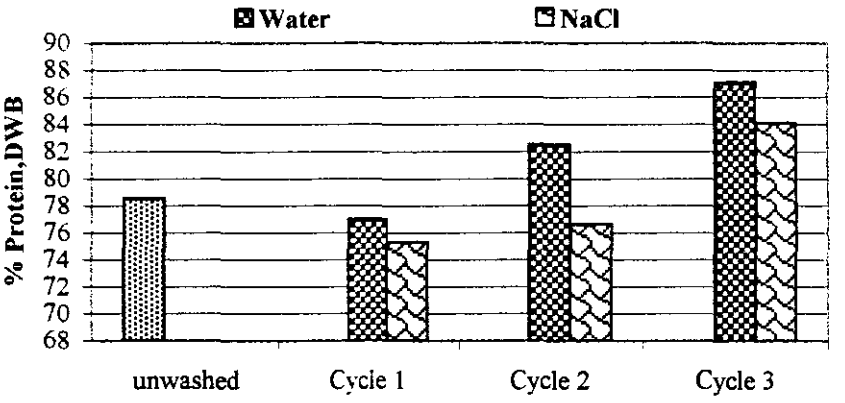


Fig 8. Protein retention in mrigal flesh after various washings

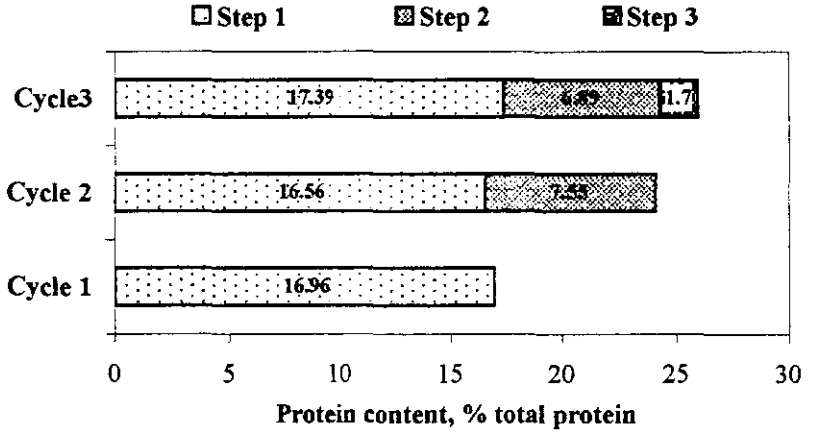


Fig 9. Removal of proteins in wash solution during water washing in different washing cycles in mrigal

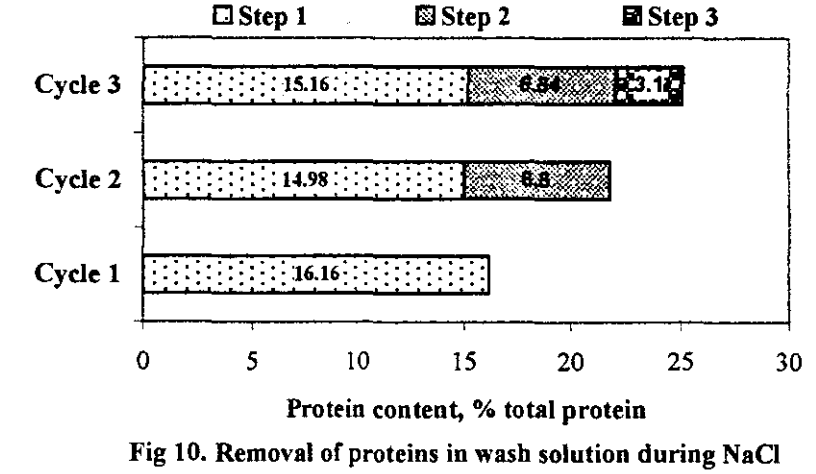


Fig 10. Removal of proteins in wash solution during NaCl (0.2%) washing in different washing cycles in mrigal

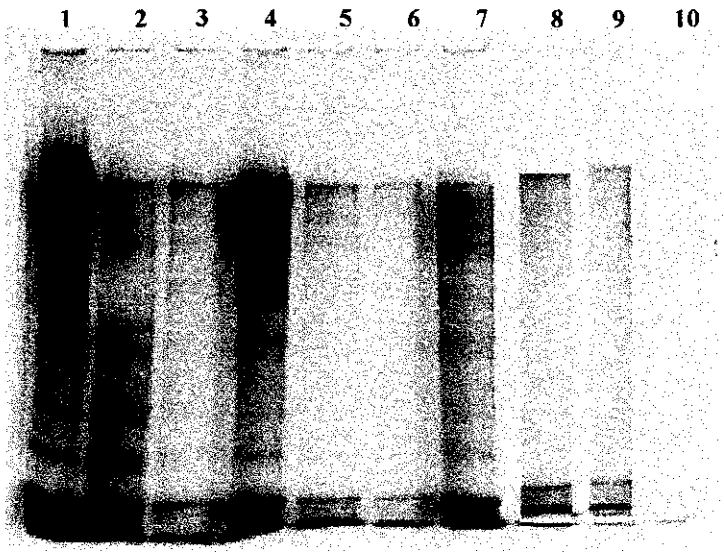


Fig 11. SDS-PAGE profiles of soluble proteins from rohu meat in 0% NaCl; Lane 1-control; Lanes 2,4, & 7 washed meat from 1, 2and 3 washing cycles; Lanes 3,5 &8 wash solution from first washing step; Lanes 6&9 wash solution from 2nd washing step; Lane 10 wash solution from 3rd washing step.

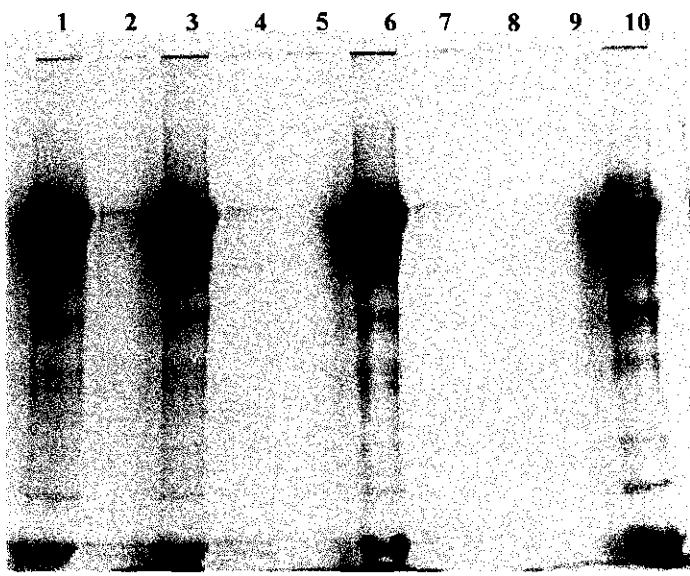


Fig 12. SDS-PAGE profiles of soluble proteins from rohu meat in 0.2% NaCl; Lanes 1,3,& 6 washed meat from 1,2and 3 washing cycles; Lanes 2,4&7 wash solution from first washing step; Lanes 5&8 wash solution from 2nd washing step; Lane 9 wash solution from 3rd washing step; Lane 10-control

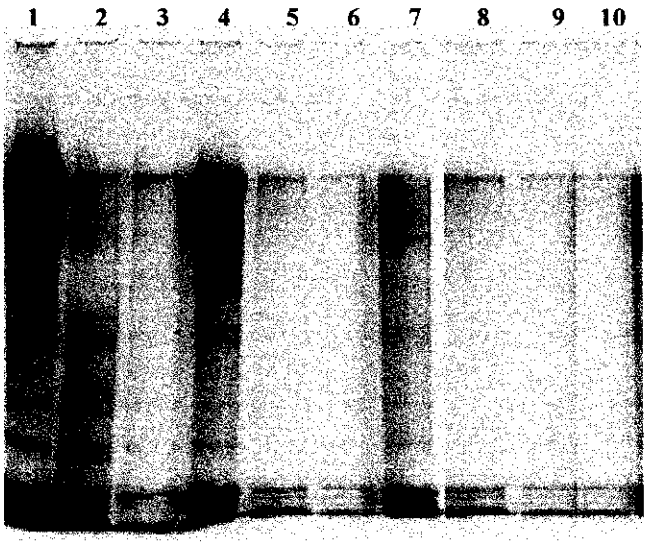


Fig13. SDS-PAGE profiles of soluble proteins from catla meat in 0% NaCl; Lane 1-control; Lanes 2,4, & 7 washed meat from 1,2and 3 washing cycles; Lanes 3,5 & 8 wash solution from first washing step; Lanes 6&9 wash solution from 2nd washing step; Lane 10 wash solution from 3rd washing step.

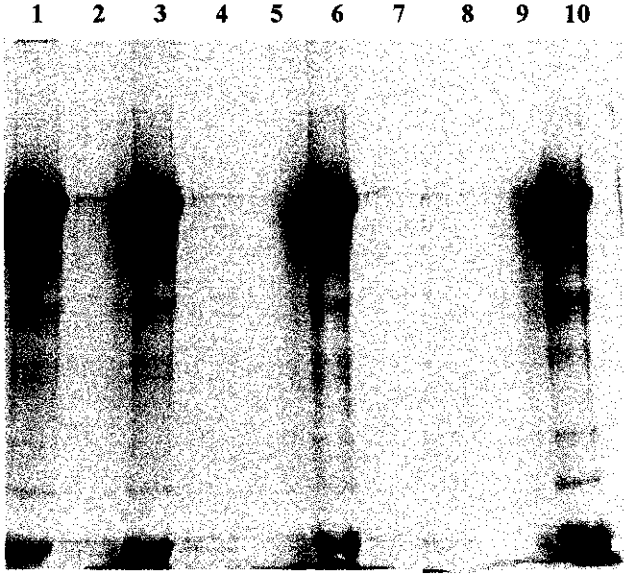


Fig 14. SDS-PAGE profiles of soluble proteins from catla meat in 0.2% NaCl; Lanes 1,3, & 6-washed meat from 1,2and 3 washing cycles; Lanes 2,4 and 7 wash solution from first washing step; Lanes5&8 wash solution from 2nd washing step; Lane 9 wash solution from 3rd washing step; Lane 10 control.

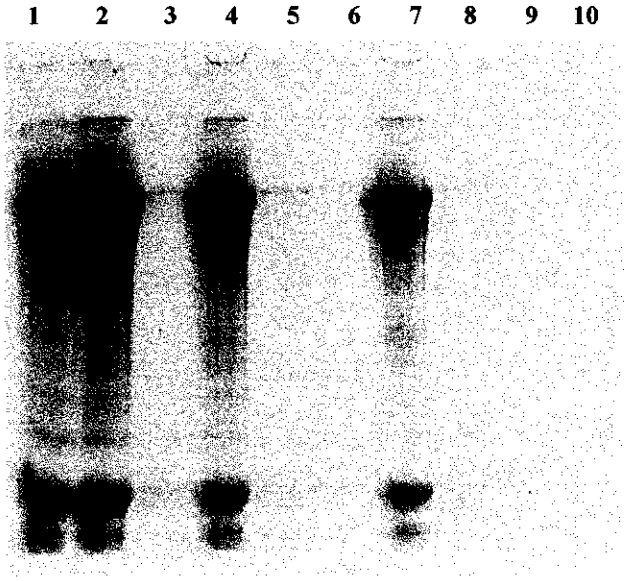


Fig 15. SDS-PAGE profiles of soluble proteins from mrigal meat in 0% NaCl; Lane 1-control; Lanes 2,4, and 7 washed meat from 1,2 and 3 washing cycles; Lanes 3, 5 & 8 wash solution from first washing step; Lanes 6 and 9 wash solution from 2nd washing step; Lane 10 wash solution from 3rd washing step.

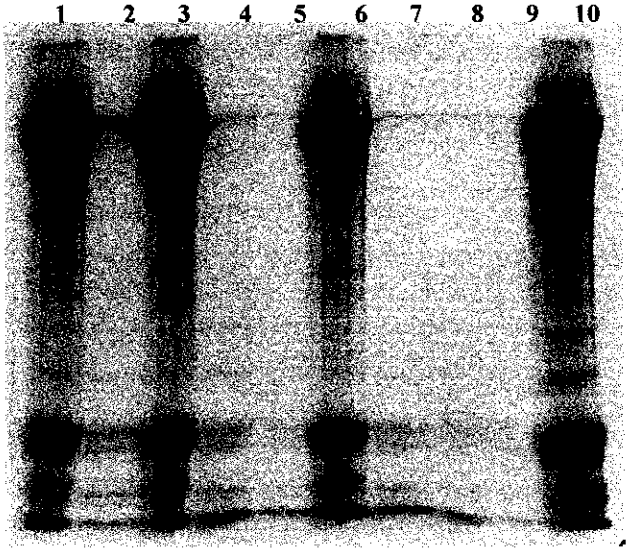


Fig 16. SDS-PAGE profiles of soluble proteins from mrigal meat in 0.2 % NaCl; Lane 1-control; Lanes 2,4,& 7 washed meat from 1, 2and 3 washing cycles; Lanes 3,5 &8 wash solution from first washing step; Lanes 6 and 9 wash solution from 2nd washing step; Lane 10 wash solution from 3rd washing step.

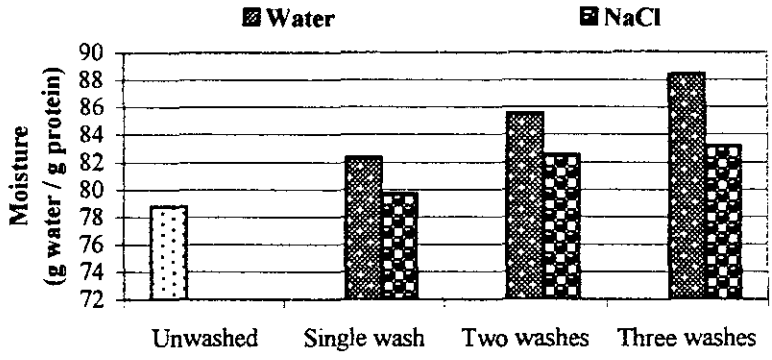


Fig 17. Effect of water and NaCl on the moisture content of rohu meat during different washing cycles

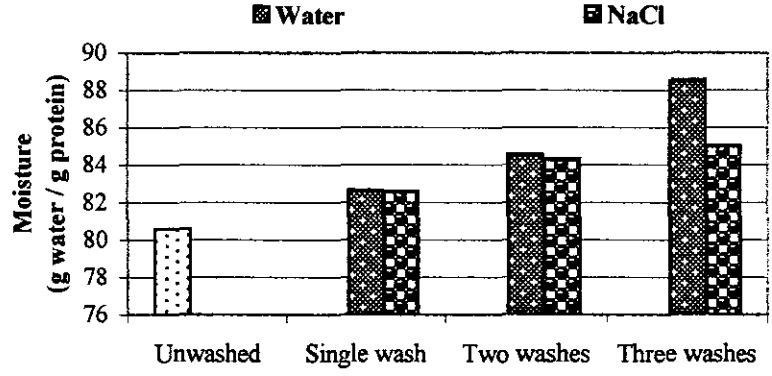


Fig 18. Effect of water and NaCl on the moisture content of catla meat during different washing cycles

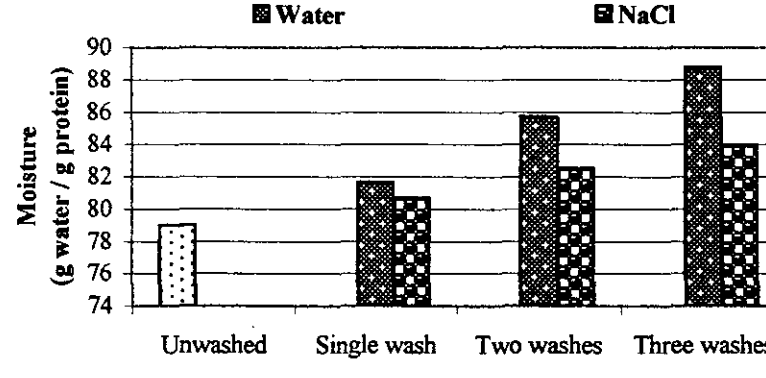


Fig 19. Effect of water and NaCl on the moisture content of mrigal meat during different washing cycles

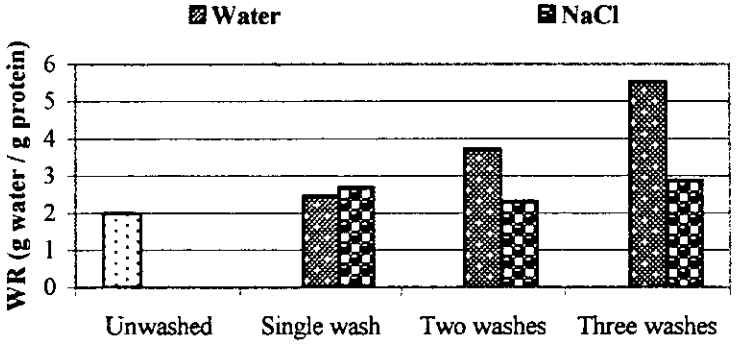


Fig 20. Effect of water and NaCl on the water retention properties of rohu meat during different washing cycles

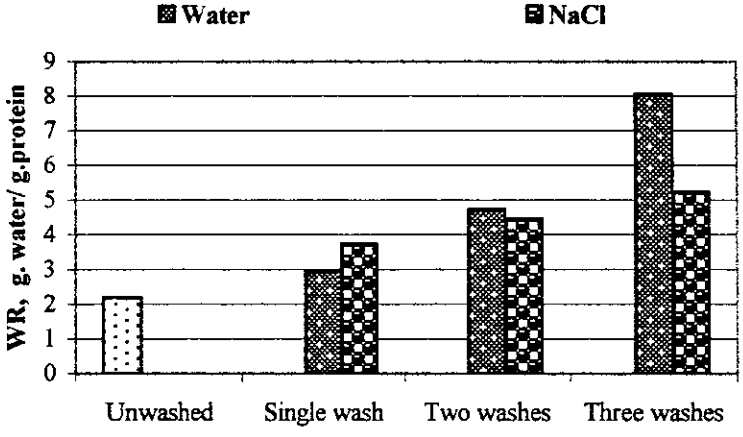


Fig 21. Effect of water and NaCl on the water retention properties of catla meat during different washing cycles

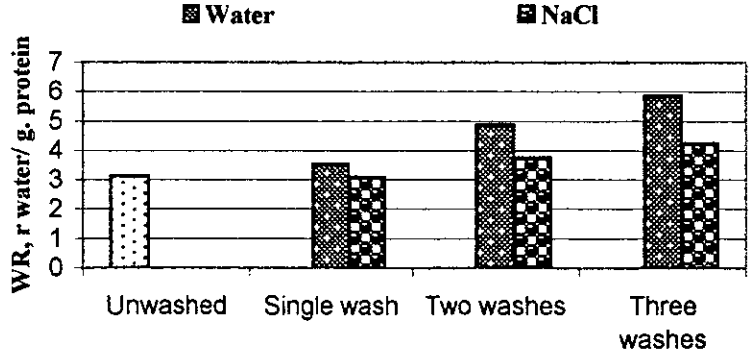


Fig 22. Effect of water and NaCl on the water retention properties of mrigal meat during different washing cycles

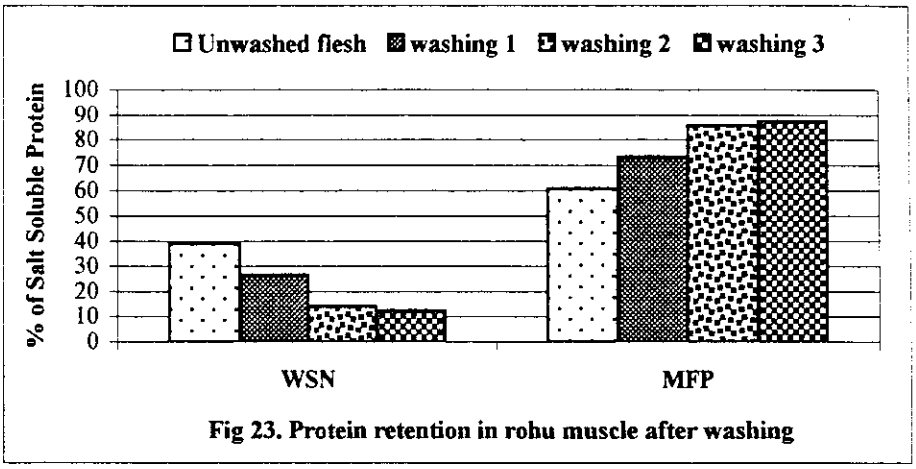


Fig 23. Protein retention in rohu muscle after washing

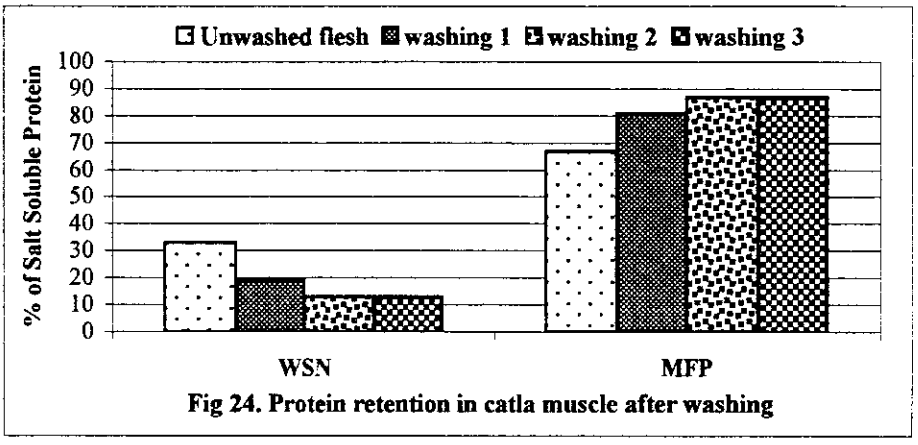


Fig 24. Protein retention in catla muscle after washing

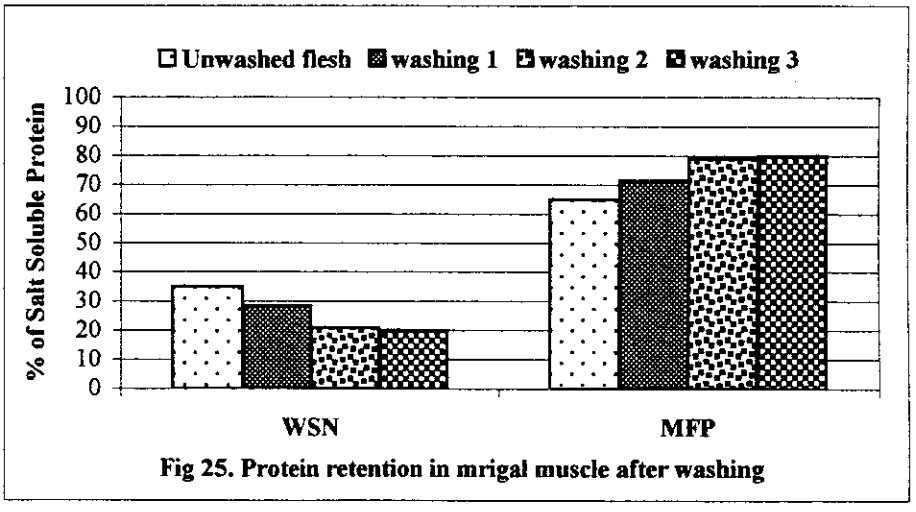


Fig 25. Protein retention in mrigal muscle after washing

Chapter – 5

Section -3

Pattern of yield during the processing of major carps for surimi

5. 3. 1. Introduction

The yield of any processed material depends on the quality, size and the nature of the raw materials besides the processing method. The preparation of a product in a fish processing unit involves different steps to which the fish /fish meat is subjected to before it is ultimately processed into the desired product. The yield of mince and hence surimi from various fish varies from species to species. For different fishes the yield varied from 37% to 60% based on round weight (Miyauchi and Steinberg, 1970) and is generally affected by the processing techniques.

5. 3. 2. Materials and Methods

The post-rigor rohu, catla and mrigal were used for the preparation of surimi. The headed, gutted and cleaned fish was taken as processed fish. The processed fish was split and fed into a deboning machine to collect the bone (Chapter 2) free fish mince, which was subjected to further procedures of surimi preparation. The yield of the in process material was calculated after gutting and cleaning, deboning and washing.

5. 3. 3. Results and Discussion

Rohu (*Labeo rohita*) yielded 66% processed fish or planks (Table 1), which produced about 35% machine-separated minced flesh based on the round weight. Catla (*Catla catla*) yielded 62% and 30 %

respectively and mrigal (*Cirrhinus mrigala*) recorded 65% and 34% for the processed fish and mince respectively (Table 2 and 3). The yield was comparable in the case of rohu and mrigal but the yield in the case of catla was low and is related to the large head. The average yield of mince from major carps is thus 30 to 35 %, which is considered to be lower when compared to that from marine fishes (Perigreen et al., 1979). Varying yields of mince, have been reported (Muraleedharan *et al.*, 1996) for different fishes from Indian waters, namely, *Tachysurus spp.*, (27%), *Megalopsis cordyla* (31%), *Uppinus vittatus* (46%), *Johnius* sp.(34%), *Saurida tumbil* (56%) and *Trichurus savala* (53%). Among the other fish 41 to 45% yield of deboned meat for silver carps (Gleman and Benjamin, 1989), 40% for Pacific hake, 45% for cod (Crawford et al, 1972) and 44-46% for threadfin bream (Joseph and Perigreen, 1983) have been reported which were slightly higher than the yield for major carps. In major carps, head constitutes a major portion of the body comprising almost 20 to 27% of the total body weight, the maximum weight being for catla, which also contribute to the reduced mince yield.

The yield of mince depends on the processing method and the machinery used for picking meat (Crawford *et al.*, 1972; Lee *et al.*, 1990). About 43% yield of planks and 36% of mince in the case of Pacific whiting when Yanagiya flesh separator was used (Lee *et al.*, 1990). Pacheo-Aguilar *et al.* (1986) reported 43 % plank and 32% mince

when Ikeuchi flesh separator was used for the same species. Lin and Morrissey (1995) reported 39% and 27% respectively for planks and mince in the case of Squawfish. Yield values ranging from 32 to 54 % have been reported for different tropical marine fishes processed by Baader 694 deboning machine (Gopakumar *et al.*, 1992).

The 1:4 meat to water, single wash exchange yielded 24%, 23% and 24% washed mince for rohu, catla and mrigal respectively. The yield slightly increased upon addition of cryoprotectants (4% sucrose, 4% sorbitol and 0.2% tripolyphosphate) to 25.5%, 24% and 25.6% for rohu, catla and mrigal respectively (Table 4). A surimi yield of 21.4% for Pacific whiting (Lee et al, 1990), 23-34% for different marine fishes from Indian waters (Muraleedharan *et al.*, 1996b; Muraleedharan *et al.*, 1997) and 17% in northern squawfish (Lin & Morrisey, 1995) have been reported. The yield of surimi observed was comparable to an economically feasible yield of 22% reported by Lee *et.al.* (1990).

Extraction or washing efficiency is a function of agitation time and it has been reported that an agitation time of nine minutes is sufficient to remove total sarcoplasmic proteins (Lee, 1986). The yield of surimi is related to the washing cycles, the ratio of flesh to water taken for leaching the mince. In the case of major carps, the yield of surimi decreases with the number of washing cycles (Table 4). More over, extensive washing of flesh is necessary to remove the TMAO and

its break down products TMA and formaldehyde completely so as to improve the quality of surimi formed. As such these chemicals are reported to be absent in freshwater fishes and hence single washing with 1:4 meat to water ratio was sufficient in this case as it improves the yield as well.

Table 1. Yield of meat from surimi processing of rohu [a]

	Percent of round weight			
Sample	Lot 1	Lot 2	Lot 3	Mean ± S.D.
Round fish	100.00	100.00	100.00	100.00
Processed fish	65.81	66.12	64.85	65.6 ± 0.67
Mince	36.38	34.52	35.36	35.4 ± 0.93
Leached meat	24.77	24.35	23.94	24.53 ± 0.41

[a] each value is an average of three experiments

Table 2. Yield of meat from surimi processing of catla [a]

	Percent of round weight			
Sample	Lot 1	Lot 2	Lot 3	Mean ± S.D.
Round fish	100.00	100.00	100.00	100.00
Processed fish	62.93	62.48	60.23	61.88 ± 1.5
Mince	30.74	31.26	30.12	30.71 ± 0.57
Leached meat	25.48	24.00	21.14	23.54 ± 2.2

[a] each value is an average of three experiments

Table 3. Yield of meat from surimi processing of mrigal [a]

	Percent of round weight			
Sample	Lot 1	Lot 2	Lot 3	Mean ± S.D.
Round fish	100.0	100.0	100.0	100.0
Processed fish	65.85	66.12	63.27	65.08 ± 1.57
Mince	34.41	35.13	33.55	34.36 ± 0.79
Leached meat	25.68	24.12	22.56	24.12 ± 1.56

[a] each value is an average of three experiments

Table 4. Yield of surimi in different washing processes in rohu, catla and mrigal[a, b]

	Mean ± S.D.		
Washing cycles	Rohu	Catla	Mrigal
1	25.53 ± 0.49	24.09 ± 4.8	25.66 ± 1.56
2	20.16 ± 0.57	20.99 ± 0.41	23.18 ± 0.98
3	19.23 ± 0.47	17.24 ± 0.35	22.35 ± 0.93

[a] each value is an average of three experiments
[b] surimi preparation- stirring in1:4 mince to water ratio for 10 min.

Chapter – 5

Section - 4

Gel forming ability of surimi from major carps

5. 4 .1. Introduction

The gelling capacity of food proteins is an important functional attribute in the manufacture of food products. In the production of food products like milk-based products, comminuted fish or meat based products, fruit jellies, baked products etc. gelation of proteins with other constituents like pectin, starches and gums is the basic mechanism leading to the formation of strong gels. The heat induced gelation properties of myofibrillar proteins during the thermal processing are important in the stability and textural aspects of comminuted and reformed meat products.

5. 4. 1. 1. Mechanism of gelation

Gelation is defined as a hydration, structural, textural and rheological property of proteins (Kinsella, 1976). Schmidt (1981) defined gelation as a protein aggregation phenomenon, in which the polymer-polymer and polymer-solvent interactions are so balanced leading to the formation of a tertiary network of gel matrix while Foegeding (1989) described gels as consisting of interconnected unit structures with liquid phase through out the three dimensional matrix.

The specific properties of gels are due to the three-dimensional network of proteins (Zayas, 1997). The gels are formed when partially unfolded proteins develop uncoiled polypeptide segments that interact at specific points forming a three dimensional cross linked network. The

partial unfolding of the native protein with slight changes in the secondary structure of protein is required for gelling. This is usually accomplished by the application of heat or otherwise by treatment with mild denaturants like alkali, acid, urea etc. The formation of three-dimensional network during the gelling is due to protein-protein and protein-solvent interactions. In a gel, the liquid prevents three-dimensional matrix from collapsing into a compact mass and the matrix prevents the liquid from flowing away (Zayas, 1997).

High molecular weight proteins and a high percentage of hydrophobic amino acids tend to establish a strong network of gel system. A strong correlation is reported between the hydrophobicity and the strength of the heat induced gel system (Nakai *et al.*, 1986). The increase in the number of hydrophobic residues facilitated denaturation during heating and the increase in the number of –SH and –S-S- groups during denaturation strengthen the gel structure. The breakage of –S-S-bonds and release of reactive –SH groups formed as a result of protein unfolding form new intermolecular disulfide bonds creating a network. Gel formation is a result of hydrogen bonding, ionic and hydrophobic interactions, Van der Waals forces and covalent disulfide bonding.

5. 4. 1. 2. Heat induced gelation

The heat-induced gelation is suggested to be a two-stage process.

In the first step, partial denaturation of native protein takes place, which is followed by protein-protein and protein – solvent interaction resulting in a three-dimensional network. Ferry (1948) suggested the following mechanism for the gelation process.

♣ heat, heat and /or cooling xP_N ----------> xP_D ----------> $(P_D)x$ ♣

where, x is the number of protein molecule, P_N is the number of native protein, and P_D is the denatured protein. The final state is the aggregate of partially denatured protein.

The texture profile of the heat-induced gels varies with the washing and subsequent cooking schedule, besides the conditioning schedule of the salt-ground meat (Katoh *et al.*, 1984). The formation of heat induced gels is governed by the balance between two forces (Zayas, 1997), the attractive forces induced by the various functional groups exposed by thermal unfolding of proteins and the repulsive forces created by the surface charge. In globular proteins, denaturation precedes aggregation and is the basic reason for the protein-protein interaction.

The interaction between protein and water plays an important role in gel formation especially during conversion of sol to gel (Zayas, 1997). Gel properties are determined by protein water interaction and the water may be bonded by hydrogen bonding to polar groups, by

dipolar groups to ionic groups or retained as structural water. Comparatively strong gels are produced with 98% water and 2% gelling agent.

5. 4. 1. 3. Factors affecting the gel properties

The actual protein concentration, amino acid composition, molecular weight and hydrophobicity of the protein have a direct influence on the gel formation (Mulvihill and Kinsella, 1987). Besides this, the development of three-dimensional network during gel formation is influenced by the method of protein preparation, processing conditions including the length and rate of heating and cooling, and environmental factors like pH and ionic strength.

The strength of the gel increased with protein concentration and the concentration of protein required depends on the properties of protein (Zayas, 1997). For example, gelatin is required at a very low concentration while globular proteins are required at a relatively higher concentration. At low protein concentration, the interaction between proteins tends to occur within the molecule rather than between the molecules and the gel network is not formed.

Protein denaturation, protein-protein and protein-water interactions are affected by pH and ionic strength and hence the gel strength. The gel formed at isoelectric pH (pI) of the protein is less hydrated and less firm because of the lack of repulsive forces. The type

and the stability of gel formation are influenced by the net charge on the protein molecule (Hermansson and Lucisano, 1982). Above and below pI, charge and hence the properties including the ionisation behaviour of the amino acids within the protein changes leading to the formation of firm gels. Increasing ionic strength by addition of salts increases the moisture loss and therefore the gels produced at low ionic strength environment showed fine microstructure while those prepared at high ionic strength showed coarsely aggregated microstructure (Hermansson *et al.*, 1986).

5. 4. 1. 4. Fish proteins

The gelling capacity of fish proteins in comminuted fish products is one of their important functional properties. The gelling properties of surimi, the washed and stabilised fish flesh have already been utilised in the commercial preparation of imitation products. The myofibrillar proteins of fish are known for their gel forming ability on heating and cooling while the sarcoplasmic proteins coagulate. The salt solubilisation of myofibrillar protein with adequate amount of water results in the formation of sol, which subsequently turns into an elastic gel upon heating (Lee, 1992). The gelling properties of fish myofibrillar proteins are reported to be higher than that from other meat sources. The gel forming capacity of fish protein is significantly affected by temperature.

Myosin is the essential component contributing to the elasticity of the fish gels and the conformational state of myosin molecule is the most important factor in the development of gel net work (Chan *et. al.*, 1995). The gelling capacity of fish myosin varies from species to species and is attributed to the differences in the cross-linking of myosin heavy chains, to the differences in the surface hydrophobicity of the unfolded domain of the myosin heavy chain and to the heating temperature (Chan *et al.*, 1992).

The heat-induced gelation in fish protein is reported to take place in two stages. According to Lanier *et al.*, (1982), setting of fish proteins at below 40°C takes place when the partially denatured proteins interacted noncovalently and formed an elastic network. A setting at below 40°C or as low as 5 – 10° C prior to cooking at 90°C resulted in a stronger and elastic gel than just cooking alone (Okada 1959a; Okada 1959b; Kimura *et al.*, 1991; Stone and Stanley, 1992). Setting increased the viscoelasticity of heat set gels with respect to dispersion of the network structure (Niwa, 1992). Gelation at 30°C to 40°C takes place as a result of interactions among the tail portions of the myosin molecule while at higher temperatures the interaction among the hydrophobic amino acids exposed as a result of protein unfolding, at the myosin head portion contributed stability to the gels formed (An *et al.*, 1996). Certain disulfide and covalent bonds through the action of muscle transglutaminase action have also been reported to play a role in the gel

network formation (Saeki *et al.*, 1990). While at cooking temperatures i.e., above 80°C mainly intermolecular disulfide bonds and more extensive intermolecular hydrophobic interactions are involved (Niwa, 1992). One step heating at higher temperatures gave good gel strength in chum salmon (Saeki *et al.*, 1995) while low temperatures setting followed by cooking at high temperature provided good gel strength in herring (Reppond *et al.*, 1995), red hake and Alaska pollock surimi (Schwarz and Lee, 1988).

This formation of translucent gels from the salt solubilised myofibrillar protein or sol at around 40°C is called "*suwari*" setting. The phenomenon is reported in most of the marine fishes and is related to the covalent cross-link formation of myosin heavy chain (Nakamura *et al.*, 1985; 1990; Saeki *et al.*, 1992). At the same time, a marked decrease in gel elasticity of the heat-induced gels occurred when salted surimi paste was cooked at 50°C to 60°C. This type of gel degradation at higher temperatures in the case of induced gels is called "*modori*" and this condition impairs the texture of the surimi-based products (Okamura, 1961; Shimizu *et al.*, 1981). In the case of lizardfish it was seen that the increased gel degradation was noticed in washed mince rather than in unwashed mince in all seasons (Itoh, *et al.*, 1995; Itoh *et al.*, 1997). The proteolytic degradation in tilapia surimi was initiated above 40°C and reached maximum activity at 65°C (Yongsawatdigul *et al.*, 2000).

The aim of this work is to study the rheological characteristics of gels from major carp surimi as a function of setting temperature, setting duration, cooking temperature and duration of cooking with a view to understand the *suwari* and *modori* phenomena in major carp gels.

5. 4. 2. Materials and Methods

The fish caught from freshwater pond in prime condition were brought to the laboratory partially iced and kept overnight to resolve rigor. The post rigor fish was washed thoroughly in ice-cold water and surimi was prepared (Fig 1). Sucrose (4 %), sorbitol (4%) and tripolyphosphate (0.2%) were added as cryoprotectants. The packed surimi was frozen at -40°C and frozen stored at –20°C for analysis.

About 100 g portions of the surimi was ground with 3 g sodium chloride in frozen condition in a kitchen model mixer-grinder for three minutes. Care was taken to prevent the development of heat by cooling the mixer- grinder by keeping inside a freezer after every one minute of operation. The thoroughly mixed surimi or sol was packed, manually in the polypropylene tubing of diameter 5 cm and subjected to setting experiments.

The packed gels were divided into two sets. Both were subjected to setting at different temperatures, namely, 25°C, 35°C, 40°C, 50°C and 60°C for 30/60 minutes. One set was subjected to cooking at 90°C for 30/60 minutes after setting. Both uncooked (suwari) and cooked gels

(kamaboko) were cooled in ice and stored at 5°C overnight before analysis.

Folding test, gel strength and compressibility were the rheological parameters analysed as described under Materials and Methods (Chapter 2).

5. 4. 3. Results and discussion

5. 4. 3. 1. Rohu

Suwari gels set for 30 min showed only a marginal (46 g. cm to 57 g. cm) increase in gel strength upto 40°C but increased by two and three folds on incubating at 50°C and 60°C (118 g. cm and 185 g. cm respectively) whereas in gels set for 60 min the value peaked at 50°C (164 g. cm) and dropped at 60°C (124 g. cm) - Fig 2. The suwari gels did not set at 25°C (Table 1), and formed a sticky mass with relatively low rheological properties. As a result, folding tests could not be performed for these samples. However, gels showed a higher gel strength for samples set at 50°C for 30 min and this increased further at 60°C. The unfolding of native myosin in rohu, catla and mrigal takes place at temperatures above 40°C (as shown in Chapter 4) and the interaction between the exposed groups lead to the increased gel strength at these temperatures. Increasing setting time to 60 min increased the gel strength further at 50°C but not at 60°C showing signs

of *modori*. The gels showed good elasticity and foldability at these higher temperatures.

In the case of cooked gels, maximum gel strength was noticed with the gel set at 25°C for 30 min (Fig 2). The gel strength decreased at 35°C and then again increased upto 60°C. Duration of setting had a positive effect on gel strength at 40°C and above but not at 60°C. At 60°C, however, the gel strength, decreased with 60 min setting.

The compressibility (Fig 3) of the suwari set gels were low at 25°C, 35°C and 40°C irrespective of duration of incubation but increased with temperature. Higher compressibility values were noticed at higher temperatures of 50 and 60°C. The compressibility of the cooked gels increased by more than four folds at 25°C at both setting periods (30 and 60 min) compared to uncooked gels and there was not much change with temperature.

5. 4. 3. 2. Mrigal

The pattern of setting followed almost the same trend as seen in the case of rohu. In suwari gels set for 30 min, gel strength (Fig 4) showed no increase upto 40°C but increased on incubating at 50 and 60°C. Increased setting time (60 min) did not increase the gel strength at any temperature but decreased the gel strength at 60°C. This can be interpreted as the appearance of *modori* phenomena at 60°C on long

incubation (60 min). The suwari gels did not set at 25°C (Table 2), and formed a sticky mass with relatively low compressibility (Fig 5) even for a higher time of setting. The folding tests could not be performed for these samples. Slow setting was noticed at 30 and 40°C. But only gels set at 50 or 60°C irrespective of setting time (30/60 min) showed good setting ability and the gel formed showed a super grade "AA" with good elastic property.

In the case of cooked gels, maximum gel strength was noticed for that set at 25°C for 30 min (Fig 4). The gel strength decreased with increasing temperature and with duration of setting upto 50°C.

The compressibility of suwari gels as in the case of rohu showed lesser values but increased with temperature and duration of setting upto 60°C (Fig 5). In the case of cooked gels there was a two and half times increase in the compressibility at 25°C for both setting period and further increased with temperature and duration of setting.

5. 4. 3. 3. Catla

The gelling characteristics of catla flesh were the same as seen in the case of rohu and mrigal. The gel strength did not show any increase upto 40°C but increased on further increase of temperature (Fig 6). In contrast to the decrease in gel strength noticed with increase in setting time (60 min) at higher temperatures in rohu and mrigal, catla showed

increased gel strength with longer setting time. The folding test results showed good elasticity for gels set at even 40°C unlike the other two cases where maximum foldability was noticed at 50°C making a significant difference in the gelling characteristics (Table 3).

The compressibility of the suwari set gels increased with both temperature and duration of setting upto 40°C except for the gels set for 30 min. above 40°C (Fig 7). In the case of cooked gels, the compressibility of the gels set for 30 min. increased with temperature upto 40°C and then decreased. Prolonged set time (60 min) also produced similar pattern but the decrease started at 35°C as against the other case.

In most marine fishes, setting at lower temperature, especially at 40°C prior to cooking at 90°C produced gel of greater strength than that is cooked at 90°C without pre-incubation (Lanier, 1986) and is a property almost unique to fish (Suzuki, 1981). This type of pre-incubation did not produce any effect on the rheological properties of mammalian and avian flesh (Montejano *et al.*, 1984). The setting at higher temperature is related to the transition in rheological properties while low temperature setting is related to the action of transglutaminase enzyme (Lanier, 1994; An *et al.*, 1996). There was no setting on incubation at lower temperatures (25°C, 35°C and 40°C) in rohu, catla and mrigal and this appears to indicate that at lower temperatures the

actomyosin from these fishes are not unfolding to the extent required to bring about the cross linkages that will facilitate the development of the gel network. This indirectly indicates the better stability of actomyosin at lower temperatures from these species and also to the possibility of either the absence or low activity of transglutaminase in major carp muscles. Similar type of non-setting of salted salmon paste at 25°C and 40°C was reported and was related to the absence of factors responsible for gel setting in salmon flesh (Nishimoto *et. al.*, 1988). It is also reported that the gel forming ability of muscle proteins from poor setting fish can be enhanced by combining with that from good setting fish at appropriate temperatures of unfolding of protein chain (Chan and Gill, 1994).

The gel setting studies with sardine flesh (Alvarez and Tejada, 1997) showed that the gel formation is weak at 25°C and required longer setting time. It was further shown that the prolonged setting at 40°C only weakened the gel structure even with cooking that followed. At lower temperature, the orientation of proteins to form gels is only just beginning and gel formed did not reach optimum texture in the given time of gelation of 30°C or 60°C (Alvarez and Tejada, 1997).

Besides the temperature, which plays a crucial role in gel formation, the proteolytic activity of the fish flesh also plays an important role. Proteolytic degradation of proteins by heat stable

alkaline proteases affects the functionality of fish proteins, precisely the gel forming ability of the protein (Lin and Lanier, 1989). These types of proteases have been found in most of the fish species including threadfin bream (Toyohara *et al.,* 1990), carps (Makinodan *et al.*, 1969) Atlantic croaker (Lin and Lanier, 1989), white croaker (Makinodan *et al.*, 1987) mullet (Deng, 1981) and Atlantic menhaden (Boye and Lanier, 1988). These proteases in most of the fishes originate from the gut and in some cases like in Alaska pollock are endogenous to the meat with optimum activity in the range of 60°C to 75°C (Lee, 1986).

In the case of catla there was no proteolytic degradation of gel at 50 or 60°C but in the case of rohu and mrigal though suwari setting at 50/60°C showed an increase compared to lower temperatures, the subsequent cooking resulted in the marginal decrease in the gel strength at 50/60°C. This could be correlated to the autolytic activity of major carp muscles discussed in Chapter 3. No or marginally low autolytic activity was noticed in all the three major carps at 55°C and at pH values 5 to 7. This probably indicates that the *modori* phenomenan was initiated in rohu and mrigal at 60°C on prolonged setting but in the case of catla no such phenomenon was noticed upto 60°C. When Pacific whiting muscle was incubated at 60°C for 30 min before cooking at 90°C, most of the myosin heavy chain was degraded affecting its gel strength (Morrissey *et al.,* 1993) and was mediated by cathepsin L

(Seymour *et al.*, 1994; An *et al.*, 1994b). The activity of different proteolytic enzymes varies with different stages of the life and the chum salmon flesh during the spawning stages contains high cathepsins and alkaline protease activity (Konagaya, 1985; Nomata *et al.*, 1985).

From the experiments on the gel forming ability of flesh from rohu, catla and mrigal it was found that the washed meat from these fish species exhibit good gelling properties. The salted meat from rohu, catla and mrigal do not gel at low temperatures, a property generally noticed in marine species. Good setting was noticed at 50°C and 60°C for 30 min of setting time in rohu and mrigal while in catla good elastic gels were formed at 40°C itself. Increasing setting time positively influenced gel strength only in catla. There was decrease in gel strength on prolonged setting (60 min) at 60°C signifying the initiation of *modori* by the alkaline proteases in rohu and mrigal. In catla, no such decrease was noticed upto 60°C.

Table 1. Folding test characteristics of heat-induced gels in rohu

Temperature / time schedule (°C/ min)	Folding test grade	Characteristic
25/30	Not set,	soft and sticky
25/30; 90/30	Not set	soft not sticky
25/60	Not set	soft and sticky
25/60; 90/30	B	breaks on 2nd folding; soft not sticky
35/30	B	breaks on 2nd folding; soft and sticky
35/30; 90/30	A	breaks on 2nd folding; soft and moderately firm
35/60	B	breaks on 2nd folding; soft and sticky
35/60; 90/30	A	Breaks on 2nd folding; soft and moderately firm
40/30	A	breaks on 2nd folding
40/30; 90/30	A	breaks on 2nd folding very slowly
40/60	A	breaks on 2nd folding
40/60; 90/30	A	breaks on 2nd folding very slowly
50/30	AA	soft, firm, chewable
50/30; 90/30	AA	soft, firm, chewable
50/60	AA	soft, firm, chewable; more elastic
50/60; 90/30	AA	soft, firm, chewable; more elastic
60/30	AA	soft, firm, chewable
60/30; 90/30	AA	soft, firm, chewable
60/60	AA	soft, firm, chewable; more elastic
60/60; 90/30	AA	soft, firm, chewable; more elastic
70/30	A	breaks on 2nd fold; soft and less elastic
70/30; 90/30	B	breaks on 1st fold; soft and less elastic

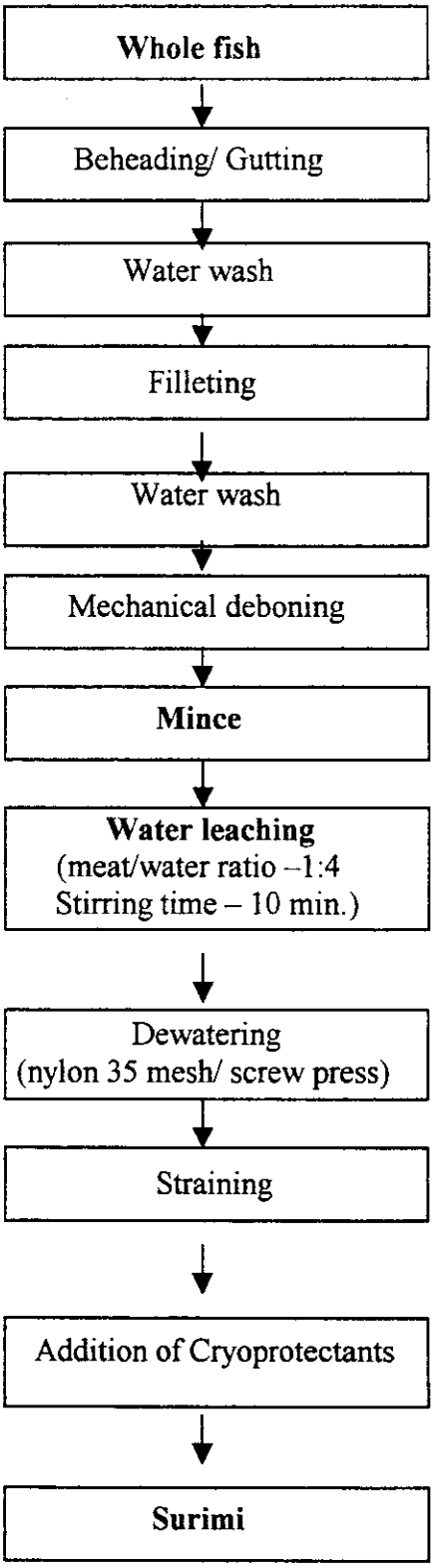
Table 2. Folding test characteristics of heat-induced gels in mrigal

Temperature / time schedule (°C/ min)	Folding test grade	Characteristic
25/30	Not set	gel not set, soft and sticky
25/30; 90/30	Not set	breaks on folding, soft and sticky
25/60	Not set	gel not set; soft and sticky
25/60; 90/30	B	breaks on 2nd folding; soft and sticky
35/30	B	breaks on folding; soft and sticky
35/30; 90/30	A	breaks on 2nd folding; soft and moderately firm
35/60	B	breaks on folding; soft and sticky
35/60; 90/30	A	Breaks on 2nd folding; soft and moderately firm
40/30	A	breaks on 2nd folding
40/30; 90/30	A	breaks on 2nd folding very slow
40/60	A	breaks on 2nd folding
40/60; 90/30	A	breaks on 2nd folding very slow
50/30	AA	soft, firm, chewable
50/30; 90/30	AA	soft, firm, chewable
50/60	AA	soft, firm, chewable; more elastic
50/60; 90/30	AA	soft, firm, chewable; more elastic
60/30	AA	soft, firm, chewable; more elastic
60/30; 90/30	AA	soft, firm, chewable; more elastic
60/60	AA	soft, firm, chewable; more elastic
60/60; 90/30	AA	soft, firm, chewable; more elastic

Table 3. Folding test characteristics of heat-induced gels in catla

Temperature / time schedule (°C/ min)	Folding test grade	Characteristic
25/30	Not set	soft and sticky
25/30; 90/30	AA	soft not sticky
25/60	Not set	soft and sticky
25/60; 90/30	AA	soft not sticky
35/30	Not set	breaks on folding soft and sticky
35/30; 90/30	AA	soft and moderately firm
35/60	Not set	breaks on 1st folding soft and sticky
35/60; 90/30	AA	soft and moderately firm
40/30	B	bends but breaks on 1st fold
40/30; 90/30	AA	soft, firm, crisp, chewable and elastic
40/60	AA	soft, firm, crisp, chewable and elastic
40/60; 90/30	AA	soft, firm, crisp, chewable and elastic
50/30	AA	soft, firm, crisp, chewable and elastic
50/30; 90/30	AA	soft, firm, crisp, chewable and elastic
50/60	AA	soft, firm, crisp, chewable and elastic
50/60; 90/30	AA	soft, firm, crisp, chewable and elastic
60/30	AA	soft, firm, crisp, chewable and elastic
60/30; 90/30	AA	soft, firm, crisp, chewable and elastic
60/60	AA	soft, firm, crisp, chewable and elastic
60/60; 90/30	AA	soft, firm, crisp, chewable and elastic

Fig 1. Scheme for the processing of major carps for surimi preparation



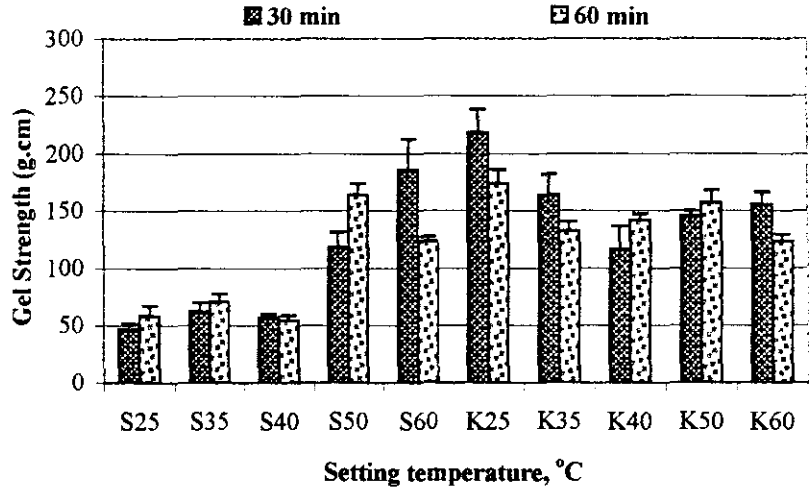


Fig 2 Gel Strength of rohu suwari (S) and kamaboko (K) gels prepared at different setting temperatures and time

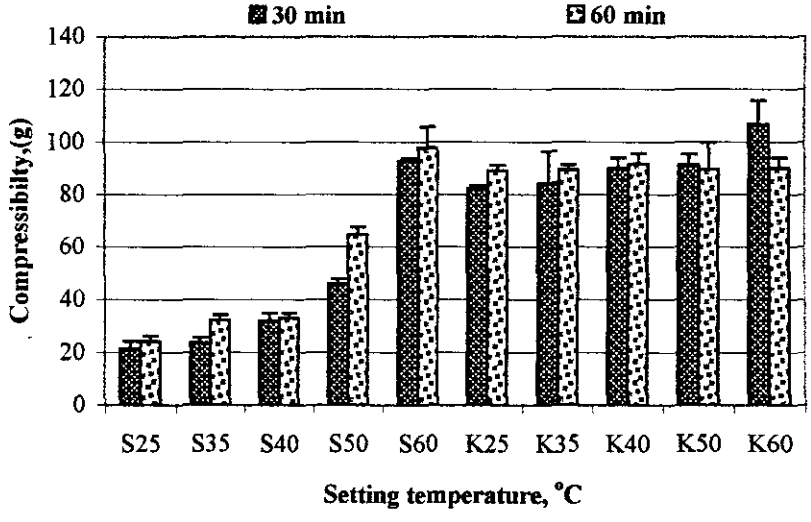


Fig 3 Compressibility of rohu suwari (S) and kamaboko (K) gels prepared at different setting temperature and setting time

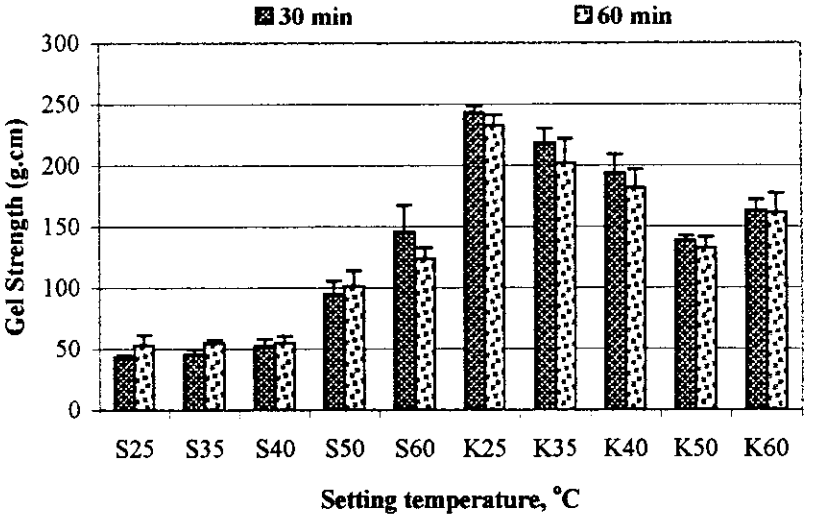


Fig 4 Gel Strength of mrigal suwari (S) and kamaboko (K) gels prepared at different setting temperatures and time

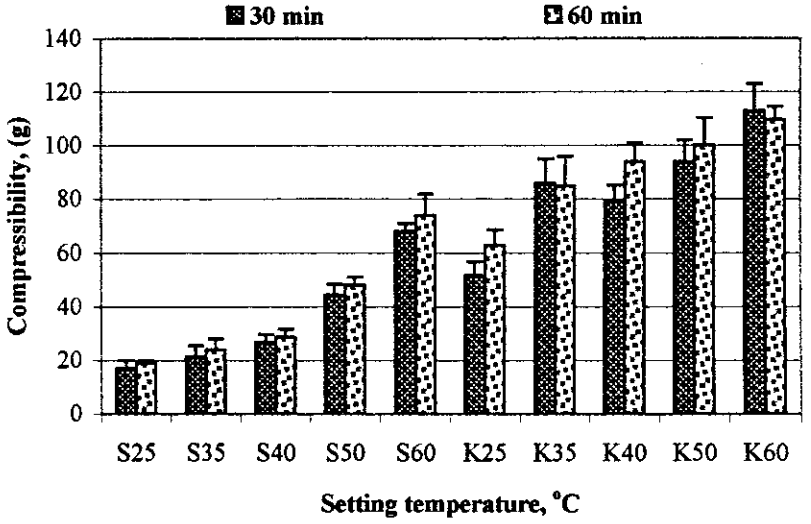


Fig 5 Compressibility of mrigal suwari (S) and kamaboko (K) gels prepared at different setting temperature and setting time

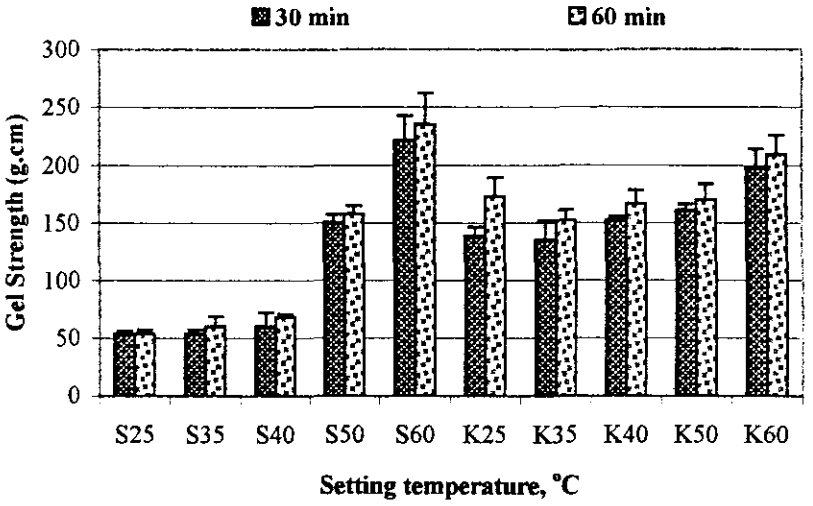


Fig 6 Gel Strength of catla suwari (S) and kamaboko (K) gels prepared at different setting temperatures and time

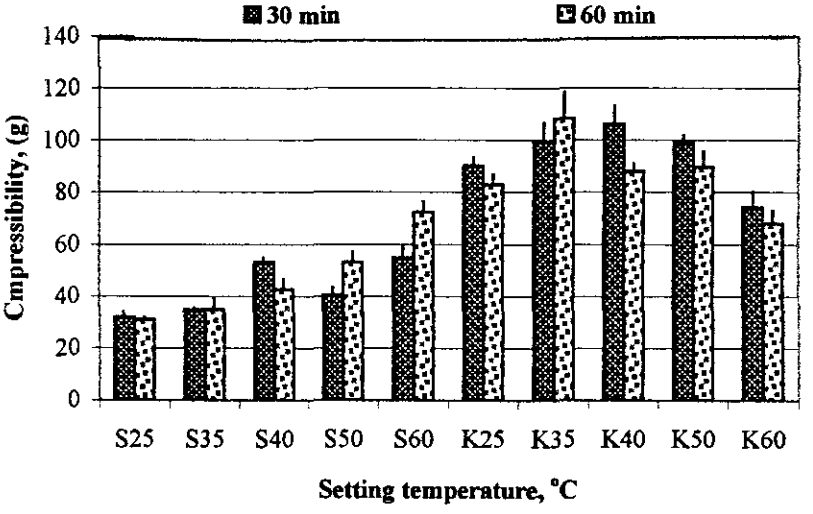


Fig 7 Compressibility of catla suwari (S) and kamaboko (K) gels prepared at different setting temperature and setting time

Chapter – 5

Section - 5

Comparison of rheological properties of surimi from different sizes of major carps

5. 5. 1. Introduction

The availability of fish in the market is highly variable and it is difficult most often to get uniform sized fish for processing. The day's harvest may be big or small size fish with different maturity stages and the processor has to depend on this variability for processing. There are differences in the proximate composition of fish as it grows and several changes in the physiology of the fish are likely to take place. The fish of one particular stage may or may not be suited for a particular type of processing. Reports indicate the effect of spawning season on the biochemistry, physicochemical and functional properties of fish proteins, particularly on the actomyosin, the active component responsible for the functional properties of surimi based products (Castle and Bishop, 1973; Beas *et al.*, 1988; Crupkin *et al.*, 1988; Roura *et al.*, 1990; Roura and Crupkin, 1995; Montecchia *et al.*, 1997).

It has been found that major carp meat have the essential characteristics required for surimi based products (Chapter 5. 2). Carps grow to big sizes and changes in composition take place during growth. However, there is not much data on the functional qualities of the meat with changes in size and this necessitates the need for the present study. Hence the experiment is designed to compare the washed mince (surimi) prepared from fish of two sizes so as to understand the differences, if any, in the gel forming characteristics of the fish based on size.

5. 5. 2. Materials and Methods

Freshwater major carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*) of two different sizes (Table 1) obtained in farm fresh condition, were partially iced over night to resolve rigor and the post rigor fish were used for the preparation of surimi as shown in Fig.1 in Chapter 5. 4. The gonadosomatic index (GSI) for the fish was calculated by a method described in Chapter 4. The experiments were carried out three times with different lots of fish. The frozen fish were stored in a deep freezer at –35°C pending analysis. Each sample was a pooled composite of three fish and the experiments were conducted in triplicate.

5. 5. 2. 1. Procedure for rheological studies

The heat-induced gels were prepared from the washed and cryoprotectants added washed mince or surimi by the method as described in Materials and Methods (Chapter 2).

The kamaboko gel strength and gel compressibility were studied by the method described under Material and Methods (Chapter 2).

5. 5. 3. Results and Discussion

The gonadosomatic index of the small and big fish among rohu, catla and mrigal (Table 1) showed that for small fish the GSI from rohu and catla were almost comparable but that of mrigal showed slightly

higher value and it might be related to its physiology. For the bigger fish, however, the value decreased depending on the weight of the fish.

The composition of the fish showed some variation between the small and big fish. As explained in Chapter 3., the main difference is seen in the fat content. In rohu the difference was only marginal while in catla and mrigal the fat content doubled with increase in size of the fish. However, the fat content was less than 2% and could be grouped in the category of lean fish. The lean fish have got the advantage over the fatty fish in its suitability for the production of surimi and surimi based products. There is also a minor change in the distribution of nitrogenous fraction with changes, particularly in the salt soluble nitrogenous fractions as the fish increases in size.

The gel strength of the gels prepared from these fish was compared (Fig 1) and it was seen that the mrigal surimi was found to have higher gel strength for both the sizes, followed by catla and rohu. Among the two size groups, the strength of the gels made from the mince from big fish was marginally higher in all the three fish. The one-way ANOVA, however, showed a highly significant difference (0.01% level) in the gel strength of both small and big fish in rohu, catla and mrigal (Table 2) and the Duncan's analysis supported the difference (Table 3).

As for compressibility (Fig 2), the value almost remained same for rohu but in the case of catla and mrigal the compressibility

decreased. Hence, the compressibility did not clearly define any merit or demerit among the fish types. One-way ANOVA showed that compressibility among big fish were significant at 1% level while among small fish at 0.1% level (Table 4). Duncan's multiple analysis showed significant difference in compressibility in rohu from the other two among the big fishes and in mrigal among the small fishes (Table 5).

The fish meat during the feeding season was reported to have low moisture and high protein and hence exhibit high gel forming ability (Lee, 1984). During and after spawning, the decreased gel forming ability of some fish meat was associated with the increased muscle proteolytic activity leading to the breakdown of proteins (Lee, 1992). High muscle pH and high water content are also found to be responsible for the decreased gel forming ability during spawning season (Suzuki, 1981; Kurokawa, 1983).

Besides this, another factor that plays a role in reducing the gel forming ability of fish meat, particularly cultured fish, is the feeding habit. Starvation in fish was associated with decreased protein and lipid. This type of protein depletion is reported to have a negative effect on the gel forming ability of fish muscle proteins (Wendakoon and Shimizu, 1991). At the same time surimi prepared from tilapia meat fed on phytoplankton was found to have a better gel forming ability compared to the one fed on fish or krill meal (Wendakoon and Shimizu, 1991).

They also reported on the increased myosin degrading activity in tilapia muscle associated with prolonged feeding of fishmeal.

Thus, the size of the fish, whether 400 or 1200 g. showed only a marginal effect on the gelling properties of the surimi produced. The mince from both size groups produced good quality surimi in all the three fishes. Hence it is only the season, whether spawning or not, and the feeding habits which have a direct effect on the composition of the fish meat. The processing conditions have a role to play on the gelling properties of fish meat and the size of the fish did not have any effect on the gelling property of the fish meat.

Table 1. Comparison of gonadosomatic index in small and big sized fish in rohu, catla and mrigal

Fish		Weight (g) of whole fish	Weight of Gonad (g)	Gonadosomatic index
Rohu	Small	560	2.25	0.403
	Big	1200	4.01	0.335
Catla	Small	560	2.10	0.396
	Big	1350	3.98	0.296
Mrigal	Small	560	2.78	0.499
	Big	1600	4.35	0.273

Table 2. Oneway ANOVA of gel strength data between rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
Big fish	Between Groups	137438.0	2	68719.00	246.599	.000
	Within Groups	1672.000	6	278.667		
	Total	139110.0	8			
Small fish	Between Groups	140994.0	2	70497.00	405.155	.000
	Within Groups	1044.000	6	174.000		
	Total	142038.0	8			

Table 3. Duncan's analysis of gel strength data from rohu, catla and mrigal

Big fish

Duncan[a]

		Subset for alpha = .05		
A	N	1	2	3
rohu	3	245.0000		
catla	3		440.0000	
mrigal	3			543.0000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Small fish

Duncan[a]

		Subset for alpha = .05		
A	N	1	2	3
rohu	3	210.0000		
catla	3		402.0000	
mrigal	3			513.0000
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Table 4. Oneway ANOVA of compressibility date from rohu, catla and mrigal

		Sum of Squares	df	Mean Square	F	Sig.
Big fish comp	Between Groups	15176.00	2	7588.000	32.567	.001
	Within Groups	1398.000	6	233.000		
	Total	16574.00	8			
Small fish comp	Between Groups	4718.000	2	2359.000	11.360	.009
	Within Groups	1246.000	6	207.667		
	Total	5964.000	8			

Table 5. Duncan analysis of compressibility from rohu, catla and mrigal

Big fish

Duncan[a]

		Subset for alpha = .05	
A	N	1	2
catla	3	114.0000	
mrigal	3	136.0000	
rohu	3		210.0000
Sig.		.128	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

Small fish

Duncan[a]

		Subset for alpha = .05	
A	N	1	2
rohu	3	204.0000	
catla	3	207.0000	
mrigal	3		254.0000
Sig.		.807	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000

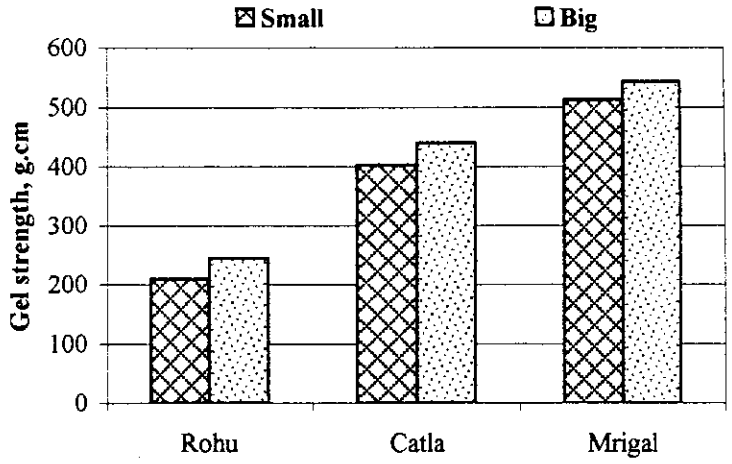


Fig 1. Comparisan of gel strength between two sized fishes in major carps

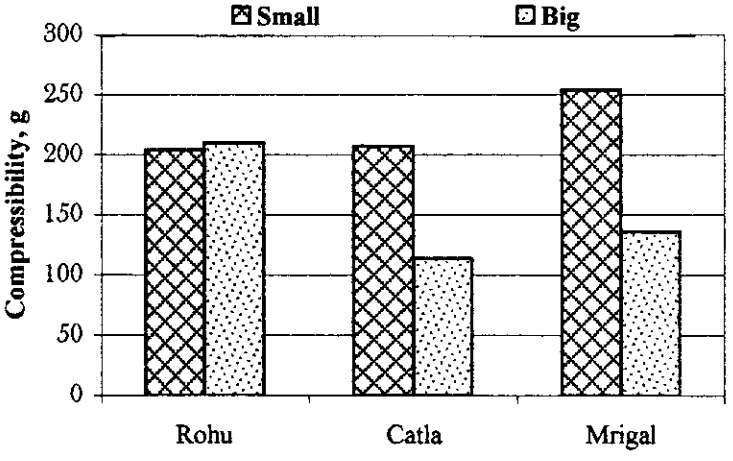


Fig 2. Comparisan of compressibility between two sized fishes in major carps

Chapter – 6

Frozen storage characteristics of fish meat and washed fish meat from major carps

6. 1. Introduction

Freezing is one of the important methods of preserving fish for long periods and frozen fish have become an important commodity both for domestic and export market in a number of developing countries. The frozen fish has got the advantage of large-scale distribution compared to ice stored fish and thus the cost of production compares well with iced fish.

When fish is held in frozen condition for a long period, it undergoes deteriorative changes in both texture and flavour due to changes in lipids and proteins. In the case of fish mince these changes take place at a rapid rate due to the very nature of fish mince. It is known that gadoid fishes develop toughness more rapidly than flat fishes (Sikorski *et al.*, 1976) and that fatty fishes like mackerel and herring produce rancid flavours more rapidly than white fish species (Hardy, 1980). The changes in the myofibrillar proteins are reflected as changes in texture and that the development of toughness is related to the insolubilisation of this protein (Laird *et. al.*, 1979). The functional properties of fish proteins are determined by its myofibrillar protein content (Goll *et al.*, 1977) and in fish meat this assumes significance due to high content of myofibrillar proteins and to the presence of relatively low content of connective tissue.

Thus, the deteriorative changes in texture during storage are related to the protein denaturation during frozen storage (Dyer, 1951;

Sikorski *et al.*, 1976; Shenouda, 1980; Jiang and Lee, 1985) and play a role in affecting the functional properties of the protein-based product. The textural changes is related to the interaction of formaldehyde, produced from the degradation of trimethylamine oxide (TMAO) by the action of specific enzymes present in dark muscles and the visceral organs of gadoid fishes, with the protein leading to their insolubilisation (Svensson, 1989). Many biochemical changes such as hydrolysis of lipids, oxidation of fat, development of peptides and free amino acids, changes in nucleotide profile, etc are involved in the fish muscle post mortem. The lipids and their derivatives, free amino acids and peptides are reported to affect the stability of proteins during frozen storage (Sikorski *et al.*, 1976; Shenouda, 1980; Jiang and Lee, 1985; Sankar and Nair, 1988; Sankar and Raghunath, 1995). These types of interactions are of great importance in the preparation and storage of quality products like surimi. It is well known that frozen storage reduces the gel forming ability of surimi (Kurokawa, 1979; Holmquist *et al.*, 1984; Scout *et al.*, 1988; MacDonald *et al.*, 1992) and the changes in the gel forming characteristics of surimi is attributed to the denaturation of myofibrillar proteins (Dyer, 1951; Noguchi and Matsumoto, 1970).

The quality deterioration of fish mince takes place both during freezing and frozen storage. Washing the mince, except catla mince, is reported to increase the freezing stability but caused deterioration on storage i.e. decreased storage tolerance (Shimizu and Fugita, 1985).

Lots of work has been done on the freezing and frozen storage of cold temperate and tropical fish species especially from the marine sector and relatively little work has been done on the freshwater fish. Frozen storage characteristics of whole individually frozen major carps have been studied (Devadasan *et al.*, 1978). Utilisation of minced fish in the form of frozen blocks offers additional large potential use in the preparation of value added fish products. The frozen stability of the minced fish would be important in establishing its reliability as raw material. Not much work has been carried out in India over the years, to study the changes associated with processing conditions in these species.

6. 2. Objectives

The present study is, therefore envisaged to,

- evaluate the quality of fish mince and surimi from the three major carps during frozen storage,
- evaluate the reliability of salt extractable protein, actomyosin and Ca^{2+} ATPase activity as indices of kamaboko quality during frozen storage of surimi.

6. 3. Materials and Methods

6. 3. 1. Preparation of myofibrillar protein concentrate

Freshwater major carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala;* average size of 650 g) obtained in farm fresh condition, were

iced over night to resolve rigor and the post rigor fishes were used for the preparation of surimi as shown in Fig.1. The unwashed mince and the washed and dewatered mince with and without cryoprotectants (8% Sucrose & 0.2% Tripolyphosphate) were frozen in a contact plate freezer immediately. The frozen fishes were stored in a deep freezer at –35°C and samples were taken periodically. The experiments were repeated three times with different lots of fishes.

6. 3. 2. Analytical methods

The different analytical procedures followed include estimation of moisture and total nitrogen (2. 4. 1), Total Salt Extractable Protein – SEP (2. 4. 6), water extractable Sarcoplasmic Proteins – SPP (2. 4. 7), myofibrillar protein - MFP (2. 4. 8), actomyosin (2. 4. 10), adenosine triphosphatase - ATPase (2. 4. 12) and rheological (2. 4. 17) parameters as described under Materials and Methods (Chapter 2).

6. 3. 3. Statistical analyses

The data were statistically analysed to test for correlation. A correlation matrix was made using the windows based SPSS statistical software.

6. 4. Results

Fig. 2 shows the unwashed meat, surimi and the kamaboko gel prepared from major carps as per the washing schedule and the single washing has improved the colour of the meat in all the three cases.

6. 4. 1. Changes in meat and washed meat during storage at – 35°C in rohu

The moisture content of the unwashed fish meat itself was slightly higher due to icing of the fish prior to processing (Fig 3). There was not much difference in the moisture content in the meat (81.93%) and washed meat (82.54%) during frozen storage. However, addition of cryoprotectants reduced the moisture content in both the cases by 5 - 6%. There was no apparent change in the total nitrogen but on calculating on dry weight basis there is decrease in the protein content of the washed mince, probably due to the elimination of water-soluble protein fraction during washing.

Effect of washing on the protein fraction of fish meat has been already been explained in detail in chapter 5. 2. The changes as a result of freezing and frozen storage only are discussed in this chapter. On frozen storage, SPP decreased by 8% (60 days) and 13% (60 days) respectively in meat in the absence and presence of cryoprotectants (Table 1). The SPP continued to decrease during the storage period and 61% and 58% of the original SPP protein were lost in both the samples

by the end of the storage period of 180 days. Only 50% of the original SPP was retained in the mince after washing as complete removal of SPP was not attempted as per the recommendations in Chapter 5. 2. In the case of washed meat with and without cryoprotectants a decrease of 41 and 38% respectively, were noticed during the same period of storage.

In the case of fish meat frozen without cryoprotectants, there was only a marginal loss in total SEP fraction upto 2% of the original in the first two months of storage and the loss slowly increased to 18% in 120 days and 34% in 180 days (Fig 4). The same trend was seen in the case of cryoprotectants added meat where a loss upto 29% was noticed for the same storage period of 180 days. In the case of washed mince 5%, 25% and 45% of the protein got insolubilised during the storage period of 60, 120 and 180 days respectively. The addition of cryoprotectants offered protection to some extent and the loss by insolubilisation was minimised and only 25% insolubilisation was noticed during the storage period.

MFP had under gone changes during storage and in the case of meat almost no change was noticed in the first sixty days (Table 1). About 11% insolubilisation was noticed in 120 days and at the end of 180 days, only 83% of the original MFP could be extracted. Addition of sucrose, sorbitol and tripolyphosphate, offered some protection to the MFP in whole meat. About 12% loss in MFP was noticed in 120 days,

which remained constant till the end of six-month storage at –35°C. In the case of washed meat without cryoprotectants, the extractability decreased by 3% (60 days), 24% (120 days) and 46% (180 days) of the original, while in the case of washed mince with cryoprotectants about 80% of the original MFP remained in soluble form. This shows that in major carps the presence of water-soluble fraction did not offer any negative effect on the MFP during frozen storage.

The myosin ATPase is believed to be an important index for the structural alteration in the protein molecule and was taken as an index for changes in the proteins. Immediately after freezing there was a slight loss of ATPase activity (Fig 5). During frozen storage (upto 180 days) the activity decreased in all the cases except in washed meat with cryoprotectants, where a comparatively higher activity was retained in the meat.

The kamaboko gel produced from washed meat showed good gel strength (Fig 6) compared to the unwashed meat. Removal of sarcoplasmic protein fraction recorded a 44% increase in gel strength, which gradually decreased during frozen storage. During frozen storage the gel strength of both unwashed and washed meat showed a decline. The major portion of the MFP fractions, though become inextractable during the storage, the gel strength was not affected. The compressibility data (Fig 7) also followed the same trend in all the samples irrespective of the presence or absence of cryoprotectants during the storage period.

This signifies that the MFP has not become really denatured but has undergone unfolding leading to partial denaturation but retained its functional characteristics.

All the parameters were highly negatively correlated to the storage time (Table 4) indicating that the values decreased with storage time. High correlation was seen between water soluble SPP and SEP, ATPase and compressibility and ATPase and SEP. The gel strength did not correlate with any of the parameters which could be due to the fact that the heat induced gels for rheological experiments were prepared without equalising the moisture content. The cryoprotectants added mince behaved almost similarly (Table 5). In the case of washed mince and cryoprotectant added washed mince (surimi), all parameters except compressibility showed good negative correlation with storage time as well as against each other (Tables 6 & 7).

6. 4. 2. Changes in meat and washed meat during storage at – 35°C in catla.

As seen in the case of rohu there is not much change in the moisture content in the different samples except with cryoprotectants added samples and this could be related to its higher water retention property of the meat (Fig 8). The moisture content increased from 79 to 84 percent. The increase was marginal i.e. only 3% in the case of meat, both washed and unwashed, in which cryoprotectants were added.

There is no apparent change in the total nitrogen but on calculating on dry weight basis there is decrease in the protein content of the washed mince, probably due to the elimination of water-soluble protein fraction during washing.

On frozen storage upto 60 days water soluble SPP showed a decrease of 10% in fish meat, which increased further to 33% and 46% by the end of 120 and 180 days respectively (Table 2). In the case of meat frozen with cryoprotectants the initial loss was just 7% but by 180 days the loss matched with that of unprotected sample. After washing the SPP content was only 41% of that present in the unwashed mince. In the case of washed mince without cryoprotectants, SPP decreased upto 25% of the original content in 180 days. The cryoprotectants offered marginal protection for SPP upto 60 days and then became ineffective.

In the case of fish meat frozen as such, there was only a marginal loss in total SEP upto 3% of the original during the first two months of storage and the loss slowly increased reaching a value of about 24% in six months (Fig 9). The presence of cryoprotectants did not really protect the fish protein in the unwashed meat. A loss upto 26% was noticed in the cryoprotectants added unwashed fish meat. The washed meat without cryoprotectants showed signs of structural alteration much earlier. After 2 months 11% loss in salt soluble fraction was noticed. Insolubilisation of SEP increased with storage and about 34% of the original soluble protein became insolubilised during frozen storage. The

cryoprotectants showed some effect on the catla myofibrillar proteins and the loss by insolubilisation was only 6%, 19% and 26% by the end of 2, 4 and 6 months of storage.

MFP is relatively unstable. However, there were no changes in the MFP content in catla during the first sixty days of storage (Table 2). Only a portion of MFP became inextractable afterwards and 11% of the original MFP became insoluble after storage of 180 days. Almost similar conditions prevailed in the case of the cryoprotectant added unwashed meat and sucrose, sorbitol and TPP did not seem to provide any protection. In the case of washed meat without cryoprotectants about 10% of the myofibrillar protein became insolubilised in the first two months of frozen storage. The quality of MFP continued to deteriorate and by the end of 180 days 36% of the original myofibrillar became inextractable due to structural alteration. In the case of cryoprotectants added washed fish meat about 72% remained unchanged demonstrating the cryoprotective action of additives.

Immediately after freezing there was a slight loss of myosin ATPase activity (Fig 10). During frozen storage the activity decreased and about 24% loss in activity was noticed at the end of 180 days in unprotected unwashed fish meat against 20% in cryoprotectants added fish meat. In the washed fish meat the activity was lost rather quickly in both protected and unprotected samples.

Kamaboko gel produced from washed meat showed a good gel strength compared to the unwashed meat. Removal of water-soluble fraction of the proteins increased the gel strength by almost 100%, which declined during frozen storage (Fig 11). The major portion of the myofibrillar protein, though became inextractable during storage, the rheological properties particularly the gel strength and folding test score are not really affected. The compressibility data also followed the same trend in all the samples irrespective of the presence or absence of cryoprotectants during the storage period (Fig 12). This may signify that the myofibrillar proteins have not become really denatured but have undergone unfolding and structural alteration but retained its functional characteristics.

The different parameters showed a high negative correlation with storage time and with each other excepting gel strength in frozen mince (Table 8). Cryoprotectants added (Table 9) mince behaved similarly and the compressibility could not be correlated with moisture and ATPase activity. The gel strength was not correlated with most of the parameters. Washed mince and surimi showed good correlation against storage time as also against each other (Table 10 & 11).

6. 4. 3. Changes in meat and washed meat during storage at – 35°C in mrigal

The changes in moisture followed similar pattern as seen in the case of rohu and catla (Fig 13). There was no apparent change in the total nitrogen but on calculating on dry weight basis there was increase in the protein content of the washed mince. The washed and cryoprotectants added meat had higher protein content.

On frozen storage at –35°C upto 60 days the SPP content of the meat showed (Table 3) 32 % loss, which further increased by more than two-folds in 180 days irrespective of the addition of cryoprotectants. As such only 48% of the water soluble protein was noticed in washed meat compared to unwashed meat and the insolubilisation of about 35% of the remaining sarcoplasmic portion was noticed during frozen storage upto six months.

In the case of frozen fish meat without cryoprotectant, there was an initial loss upto 20% of the original SEP after first two months of storage (Fig 14). The insolubilisation of SEP increased during further storage and 55% of the original SEP became solubilised by 6 months. The presence of cryoprotectants did not really have much effect on mrigal protein in the unwashed meat. The washed mince showed some stability. There was loss upto 23% in the first two months, which remained constant upto 4 months and then showed a marginal decrease of further 4% in six months. But in the case of washed meat with

cryoprotectants the loss during frozen storage was relatively low and only about 13% loss was noticed during the storage period of six months.

In unwashed meat, 13% of the relatively unstable MFP, had become insoluble in the first sixty days itself during storage at –35°C (Table 3). The insolubilisation continued and 45% of the original protein became inextractable in 180 days of frozen storage. Addition of cryoprotectant mixture had almost no effect on mrigal myofibrillar proteins. In the case of washed meat without cryoprotectants 25% insolubilisation of MFP was noticed in 60 days and no further change was noticed during the entire frozen storage period. But in the case of the cryoprotectants added washed meat more than 95% of the original MFP could be extracted even after six months of storage.

The index of protein denaturation, the ATPase activity was slightly lost immediately after freezing (Fig 15). During frozen storage about 15% of the activity was lost in the first 60 days of storage in the meat samples with and without cryoprotectants, which increased to 30 – 35% in six months. In the case of washed meat the loss was slightly more in the first 60 days compared to unwashed mince with cryoprotectants. However, after 180 days of storage 29 % of the activity was noted in both the cases.

The kamaboko gel produced from meat and washed meat showed good gel strength (fig 16) during storage period. Washed meat showed a

higher gel strength compared to the unwashed meat. However, gel strength values were above 200 g.cm during the storage period in all the cases. The compressibility (Fig 17) also remained uniform in all the samples irrespective of the presence or absence of cryoprotectants during the storage period. This may signify that the MFP had not become really denatured but have undergone unfolding and structural alteration but retained its functional characteristics.

All parameters excepting moisture and compressibility showed good correlation with storage time in the case of unwashed mince with and without cryoprotectants (Table 12 & 13). But almost all the parameters showed good correlation in the case of washed mince with and without cryoprotectants against all the parameters (Table 14 & 15).

6. 5. Discussion

The initial water content of the washed mince was slightly higher compared to the unwashed mince due to the effect of washing. Addition of cryoprotectants, however, decreased the water content by 4-5% in both the cases. The moisture content of both meat and washed meat more or less remained constant or increased only marginally during frozen storage in all the three fishes upto six months. This could be related to the water holding capacity of the protein. Similar results were reported during the frozen storage of hoki (MacDonald *et al.*, 1992). In the case of Alaska Pollock, the frozen storage was associated with a

decrease in moisture and was related to the decrease in the water holding capacity of the fish protein during frozen storage (Scott *et al.*, 1988).

The major changes take place in the mince and washed mince during frozen storage in the soluble protein fraction, which in turn affect the functional properties of the mince or washed mince. There was an initial increase in the soluble protein content immediately after freezing in all the four type of samples. This type of increase as a consequence of freezing is caused by the modification of chemical groups especially sulfhydryl groups (Monteccchia *et al.*, 1997), which in turn cause transitory modification of molecular conformation of the protein. Similar type increases were noticed in frozen stored squid mantle (Iguchi *et al.*, 1981).

Due to various reasons (chapter 3, Composition of major carps), single water washing was carried out and as a consequence only a portion of the water-soluble SPPs was removed during washing. SPPs being relatively unstable, the residual SPP present in the washed mince also underwent changes during frozen storage. Upto 60-70 percent losses of these proteins were noticed in 180 days in all the three fishes.

The concentration of SEP was found to be highest in frozen mince with or without cryoprotectants followed by washed mince with or without cryoprotectants. This was not unexpected since the washing, besides removing the water extractable substances, is likely to denature some of the relatively high molecular weight proteins resulting in lower

concentration of SEP at the onset of the study. Similar results were reported during the preparation and frozen storage of surimi from red hake (Holmquist *et al.*, 1984). The SEP and hence the MFP had undergone changes during frozen storage in all the three fish species in the similar pattern. The decrease in SEP was greater than that of MFP and was related to the denaturation of SPP. Decreases during frozen storage have been reported in the actomyosin and Ca^{2+} ATPase of lizardfish surimi (Lan *et al.*, 1987) and in red hake fillets and mince meat (Holmquist, 1984). Greater inextractability of salt extractable proteins during the frozen storage of whole individually frozen major carps also have been reported (Devadasan *et al.*, 1978). The loss in protein extractability during frozen storage is caused by aggregate formation of components of MFP (Sikorski *et al.*, 1976; Jiang *et al.*, 1986) and this aggregation (denaturation) is responsible for the deterioration of gel forming ability and texture of protein and protein based products (Jiang *et al.*, 1986). The initial result of protein denaturation is the formation of soluble aggregates, which subsequently become insoluble and as a consequence the protein becomes in extractable (Montecchia *et al.*, 1997). On the basis of the frozen storage Alaska pollock surimi at different temperature, it was suggested that –30°C was the most suitable temperature for short-term storage of surimi and –40°C for long time storage upto one year (Shaben *et al.*, 1985).

During 180 days of storage at –35°C, about 65 – 75% of SEP in mince or 75-95% of SEP in washed and cryoprotectant added mince remained extractable. The major constituent of SEP is MFP. Among the factors involved in freeze denaturation are effect of ice crystal formation, binding of lipids and other oxidising products to proteins, oxidation and reaction of thiol groups and the reaction of amino acids with formaldehyde formed endogenously (Sikorski *et al.*, 1976; Shenouda, 1980; Suzuki, 1981). The muscle with higher free amino acids was reported to undergo greater denaturation and that muscles with high levels of histidine and lysine was relatively unstable (Jiang *et al.*, 1987). Further, it was also reported that the hydrophobic amino acid residues of white muscle proteins are more readily exposed on freezing (Niwa *et al.*, 1986) and these may be responsible for the interaction with other groups. As the nitrogenous compound TMAO, whose breakdown to dimethylamine and formaldehyde (FA) causes the insolubilisation of MFP in marine fishes, is almost absent (Shenouda, 1980) in freshwater fishes, the insolubilisation of protein through interaction with FA can be ruled out. The major carps, at about 500g-weight stage are mostly lean fishes with fat content less than 1%. Hence the possibility of interaction between free fatty acids, formed from phospholipid hydrolysis by endogenous enzymes, and protein is relatively high. These free fatty acids attach themselves to specific sites on the protein molecule creating

more hydrophobic regions surrounding the protein molecule making them insoluble (Sikorski *et al.*, 1976).

The ATPase activity decrease as a result of frozen storage followed the same trend in all the samples and in all the three fishes. The maximum loss of activity was noticed in unprotected mince, followed by mince with cryoprotectants, washed mince and washed mince with cryoprotectants. For rohu, 80% of the original activity was retained after 6 months of storage at –35°C and the losses were 76% and 73 % respectively for catla and mrigal. Loss of activity during frozen storage to the level of 50- 80% was reported in meat and in protein solutions (Buttkus, 1967; Scott *et al.*, 1988).

Washing to remove the water-soluble SPP increased the gel strength several folds in all the three fishes, which decreased with the length of storage. The strength of kamaboko gel prepared from washed and cryoprotectants added mince was higher than that of gel prepared from fish mince in rohu and mrigal. But in the case of catla, though the increase in gel strength as a result of washing was several folds higher, the gel strength quickly decreased on storage and reached a level almost comparable to that prepared from mince. The gel strength of surimi and surimi-based products were significantly affected by storage temperature (Hsu, 1990) and the decrease during frozen storage is influenced by the presence of SPP presumably by their denaturation rather than by the denaturation of MFP (Nishioka and Koneada, 1979). The decrease in gel

strength during frozen storage has been reported in seven species of fishes and the decrease is species dependent (Atsumi *et al.*, 1995). Though there was an increase in compressibility as a result of washing, there was no definite relation with frozen storage in all the three fishes.

Thus, the salt soluble protein and hence the MFP had undergone changes during frozen storage in all the three fish species more or less in the same pattern. The decrease in salt extractable proteins was greater than that of MFP and was related to the denaturation of SPP. About 65 – 75% of salt extractable proteins in mince or 75-95% in washed and cryoprotectants added mince remained extractable after 180 days of storage at –35°C. As the nitrogenous compound TMAO is almost absent in freshwater fishes, the insolubilisation of protein through interaction with formaldehyde is almost ruled out. But being lean fish, the interaction of fatty acid formed from phospholipid with protein was relatively high. The conformational changes taking place in protein molecules were supported by decrease in ATPase activity during the storage.

Washing to remove the water-soluble SPP increased the gel strength several folds in all the three fishes, which decreased with the length of storage. The surimi or washed mince with cryoprotectants from these fishes had good stability at –35°C upto six months without much alterations in composition and hence can be recommended for the development of surimi based product.

Table 1. Changes in the sarcoplasmic* and myofibrillar protein* fractions of meat and washed meat with and without cryoprotectants in rohu during the storage at –35°C

Storage	Meat		Meat +CP		Washed meat		Washed meat +CP	
Days	Sarcoplasmic protein	Myofibrillar protein	Sarcoplasmic protein	Myofibrillar protein	Sarcoplasmic protein	Myofibrillar protein	Sarcoplasmic protein	Myofibrillar protein
0	28.27 ± 3.78	44.77 ± 5.19	27.14 ± 6.30	46.71 ± 8.66	13.64 ± 1.38	38.59 ± 6.20	13.05 ± 2.63	38.93 ± 3.85
60	26.01 ± 6.98	45.54 ± 9.40	23.61 ± 4.55	45.81 ± 4.68	11.87 ± 3.92	37.75±10.81	11.54 ± 2.44	33.68 ± 2.32
120	19.81 ± 0.96	40.06 ± 6.44	16.18 ± 2.88	41.17 ± 7.62	10.01 ± 5.06	29.16 ± 4.18	9.24 ± 5.76	35.70 ± 7.65
180	10.99 ± 1.22	37.18± 10.79	11.25 ± 1.73	41.18 ± 5.02	8.01 ± 1.34	20.72 ± 5.29	7.99 ± 4.54	31.00 ± 8.28

* values are average of three experiments
values are on Dry Weight Basis
CP - cryoprotectant

Table 2. Changes in the sarcoplasmic* and myofibrillar protein* fractions of meat and washed meat with and without cryoprotectants in catla during the storage at –35°C

Storage	Meat		Meat +CP		Washed meat		Washed meat +CP	
Days	Sarcoplasmic protein	Myofibrillar protein	Sarcoplasmic protein	Myofibrillar protein	Sarcoplasmic protein	Myofibrillar protein	Sarcoplasmic protein	Myofibrillar protein
0	28.26 ± 1.16	50.26 ± 9.46	27.95 ± 3.06	48.96 ± 6.20	11.57 ± 2.71	47.05±10.61	11.08 ± 2.09	40.56 ± 2.85
60	25.43 ± 0.71	50.73 ± 6.70	25.49 ± 5.86	48.80 ± 3.61	9.71 ± 2.95	42.46 ± 6.01	10.95 ± 3.51	37.59 ± 1.58
120	18.93 ± 0.29	45.46 ± 9.67	20.01 ± 3.49	45.39 ± 4.27	9.07 ± 2.19	31.68 ± 5.68	9.35 ± 2.78	32.48 ± 3.12
180	15.26 ± 2.21	44.42 ± 7.11	15.37 ± 0.42	41.55 ± 4.46	8.68 ± 3.36	30.01 ± 5.28	8.20 ± 5.32	28.98 ± 6.14

* values are average of three experiments
values are on Dry Weight Basis
CP - cryoprotectant

Table 3. Changes in the sarcoplasmic* and myofibrillar protein* fractions of meat and washed meat with and without cryoprotectants in mrigal during the storage at –35°C

Storage	Meat		Meat +CP		Washed meat		Washed meat +CP	
Days	Sarcoplasmic protein	Myofibrillar protein	Sarcoplasmic protein	Myofibrillar protein	Sarcoplasmic protein	Myofibrillar protein	Sarcoplasmic protein	Myofibrillar protein
0	27.23 ± 2.42	49.37 ± 5.83	24.88 ± 3.37	48.40 ± 8.56	14.15 ± 1.79	41.88 ± 2.78	14.68 ± 0.64	41.00 ± 4.24
60	18.54 ± 2.53	42.74± 13.13	19.54 ± 4.68	39.58 ± 4.54	11.56 ± 2.00	31.58 ± 7.56	12.54 ± 0.92	40.75 ± 6.66
120	16.24 ± 2.10	39.30 ± 4.05	14.25 ± 2.72	37.89 ± 2.52	10.54 ± 1.65	31.48 ± 6.08	10.24 ± 0.87	41.02 ± 9.97
180	7.21 ± 1.50	27.26 ± 0.97	6.16 ± 2.91	27.55 ± 00.96	9.23 ± 2.81	31.12 ± 5.06	9.51 ± 0.88	38.93 ± 8.64

* values are average of three experiments
values are on Dry Weight Basis
CP - cryoprotectant

Table 4. Correlation matrix of results of chemical and physical tests on mince from rohu during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	-0.9713**	-0.9525**	-0.9293**	-0.9483**	-0.5171	-0.9665**
Moisture		0.3667	0.3445	0.762	0.1417	0.3417
Sarcoplasmic protein			0.9969***	0.8819*	0.7181	0.9883***
Salt extractable protein				0.8686*	0.7703	0.9743**
ATPase					0.5713	0.8612*
Gel strength						0.6093

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Table 5. Correlation matrix of results of chemical and physical tests on cryoprotectants added mince from rohu during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	0.4264	-0.9938***	-0.9907***	-0.9375**	0.0109	-0.9059*
Moisture		-0.3976	-0.3650	-0.2418	-0.5721	-0.7677
Sarcoplasmic protein			0.9477**	0.9691**	0.0533	0.8928*
Salt extractable protein				0.9761***	0.0415	0.8761*
ATPase					0.1102	0.7906
Gel strength						0.2996

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Table 6. Correlation matrix of results of chemical and physical tests on washed mince from rohu during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	0.8280*	-0.9959***	-0.9631**	-0.9788***	-0.9133*	-0.7599
Moisture		-0.8755*	-0.9452**	-0.8378*	-0.6801	-0.8373*
Sarcoplasmic protein			0.9834***	0.9804***	0.8948*	0.7871
Salt extractable protein				0.9665**	0.8227*	0.8003*
ATPase					0.8109*	0.6557
Gel strength						0.8486*

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Table 7. Correlation matrix of results of chemical and physical tests on cryoprotectants added washed mince (surimi) from rohu during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	-0.5201	-0.9968***	-0.9267**	-0.9898***	-0.9768***	-0.8549*
Moisture		0.5724	0.2298	0.4772	0.3341	-0.8373*
Sarcoplasmic protein			0.8136*	0.9905***	0.9576**	0.8439*
Salt extractable protein				0.8986*	0.9806***	0.8295*
ATPase					0.9668**	0.9093*
Gel strength						0.8929*

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Table 8. Correlation matrix of results of chemical and physical tests on mince from catla during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	0.9503**	-0.9906***	-0.9781***	-1.0000***	0.7792	0.9055**
Moisture		-0.9688**	-0.9692**	-0.9503**	0.6781	0.9559**
Sarcoplasmic protein			0.9973***	0.9906***	-0.8164*	-0.9547**
Salt extractable protein				0.9781***	-0.8343*	-0.9731**
ATPase					-0.7792	-0.9055*
Gel strength						0.8184*

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Table 9. Correlation matrix of results of chemical and physical tests on cryoprotectants added mince from catla during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	0.9904***	-0.9892***	-0.9850***	-0.9558**	-0.3710	0.8696*
Moisture		-0.9946***	-0.9970***	-0.9495**	-0.2573	0.9191**
Sarcoplasmic protein			0.9986***	0.9768***	0.3279	-0.8796*
Salt extractable protein				0.9669**	0.2778	-0.9017*
ATPase					0.4773	-0.7633
Gel strength						0.1355

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Table 10. Correlation matrix of results of chemical and physical tests on washed mince from catla during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	0.8991*	-0.9294**	-0.9715**	-0.9869***	-0.9390**	-0.9251**
Moisture		-0.9971***	-0.9296**	-0.9057*	-0.9656**	-0.7935
Sarcoplasmic protein			0.9986***	0.9307**	0.9725**	0.8230*
Salt extractable protein				0.9952***	0.9883***	0.9379**
ATPase					0.9700**	0.9669**
Gel strength						0.8935*

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Table 11. Correlation matrix of results of chemical and physical tests on cryoprotectants added washed mince (surimi) from catla during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	0.9823***	-0.9973***	-0.9962***	-0.8866*	-0.9209**	-0.9942***
Moisture		-0.9740**	-0.9963***	-0.7974	-0.8999*	-0.9785***
Sarcoplasmic protein			0.9957***	0.9124*	0.9725**	0.9983***
Salt extractable protein				0.8736*	0.9395**	0.9976***
ATPase					0.9211**	0.9037*
Gel strength						0.9569**

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Table 12. Correlation matrix of results of chemical and physical tests on mince from mrigal during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	0.3260	-0.9744**	-0.9716**	-0.9789***	-0.9165*	0.2467
Moisture		-0.4638	-0.5158	-0.7974	-0.0383	-0.8353*
Sarcoplasmic protein			0.9969***	0.9278**	0.8978*	0.0859
Salt extractable protein				0.9029*	0.8638*	0.0325
ATPase					0.9910***	-0.4496
Gel strength						0.4845

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Table 13. Correlation matrix of results of chemical and physical tests on cryoprotectants added mince from mrigal during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	-0.2110	-0.9575**	-0.9397**	-0.9691**	-0.0157	-0.1559
Moisture		-0.0763	-0.1143	0.4451	-0.9197**	0.8318*
Sarcoplasmic protein			0.9963***	0.8570*	0.2693	0.3816
Salt extractable protein				0.8298*	0.2771	0.3764
ATPase					-0.2112	0.0587
Gel strength						0.3031

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Table 14. Correlation matrix of results of chemical and physical tests on washed mince from mrigal during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	-0.4954	-0.9810***	-0.9440**	-0.9234**	-0.0157	0.8421*
Moisture		0.3259	0.7464	0.7155	-0.3470	0.0297
Sarcoplasmic protein			0.8716*	0.8659*	0.6643	-0.9112*
Salt extractable protein				0.9857***	0.2720	0.6175
ATPase					0.2115	-0.6902
Gel strength						-0.9124*

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Table 15. Correlation matrix of results of chemical and physical tests on cryoprotectants added washed mince (surimi) from mrigal during storage at –35°C for up to 6 months

	Moisture	Sarcoplasmic protein	Salt extractable protein	ATPase	Gel strength	Compressibility
Storage time	0.9864***	-0.9776***	-0.9957***	-0.9829***	-0.9782***	-0.4000
Moisture		-0.9988***	-0.9969***	-0.9857***	-0.9732**	-0.9874***
Sarcoplasmic protein			0.9989***	0.9795***	0.9641*	0.5825
Salt extractable protein				0.9912***	0.9833***	0.4816
ATPase					0.9978***	0.4914
Gel strength						0.4477

Test results were correlated against storage time and against each other
* P<0.05; ** P< 0.01; ***P< 0.001

Fig 1. Scheme for the processing of major carps for surimi preparation for frozen storage

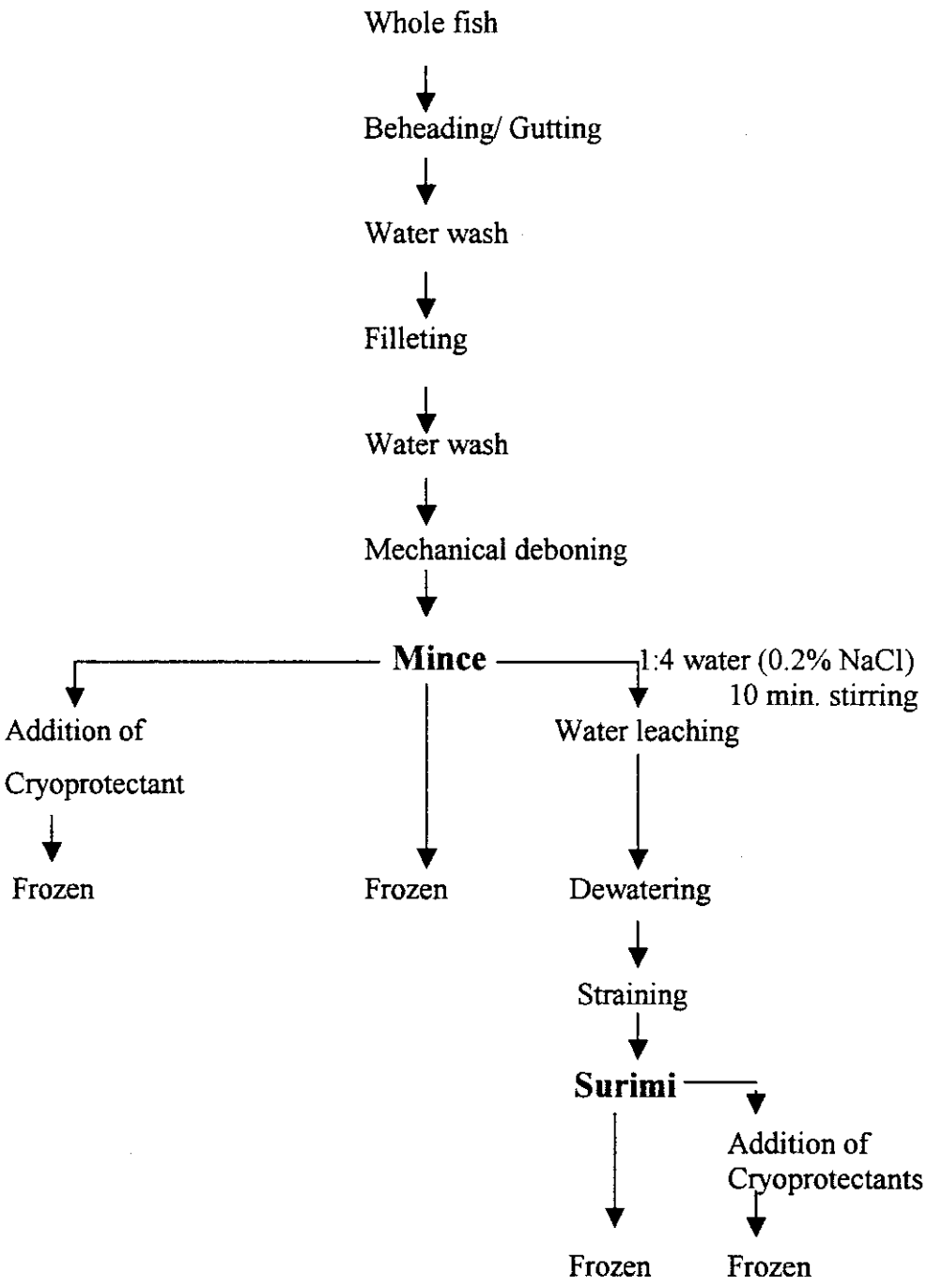
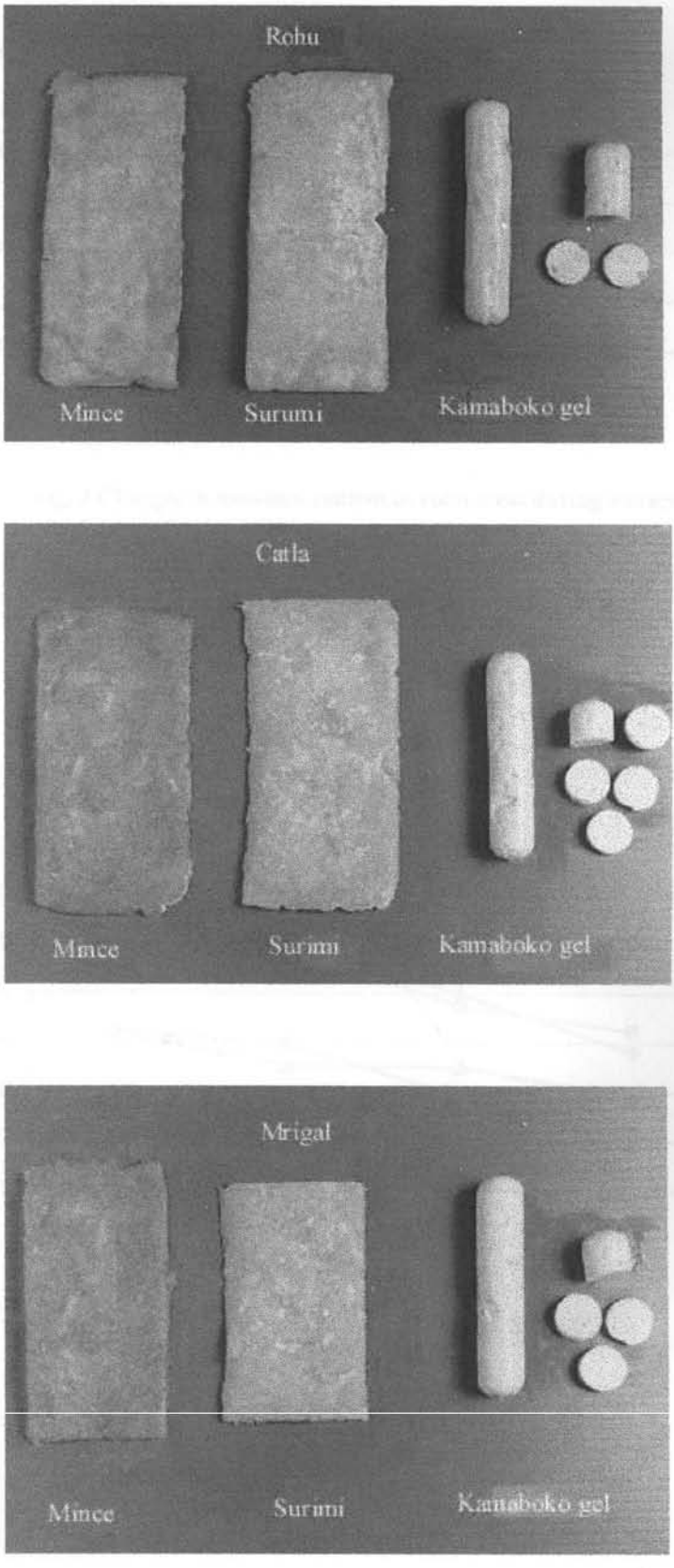


Fig. 2. Fish mince, surimi and kamaboko gel from major carps



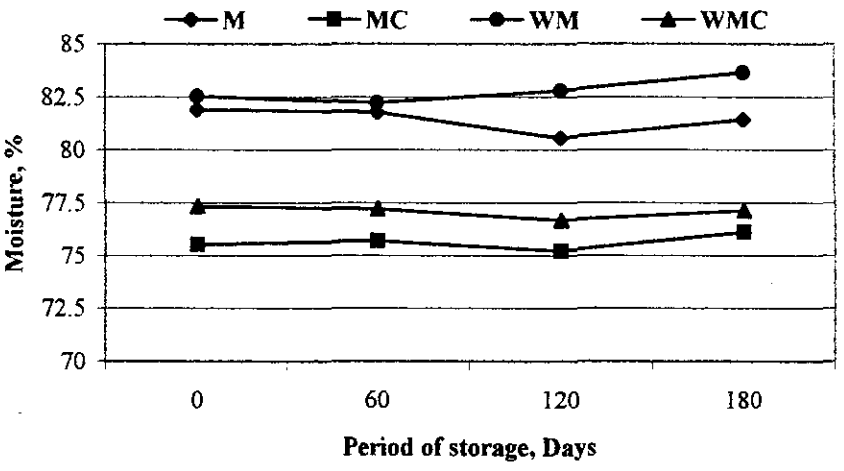


Fig. 3 Changes in moisture content in rohu meat during storage at - 35°C

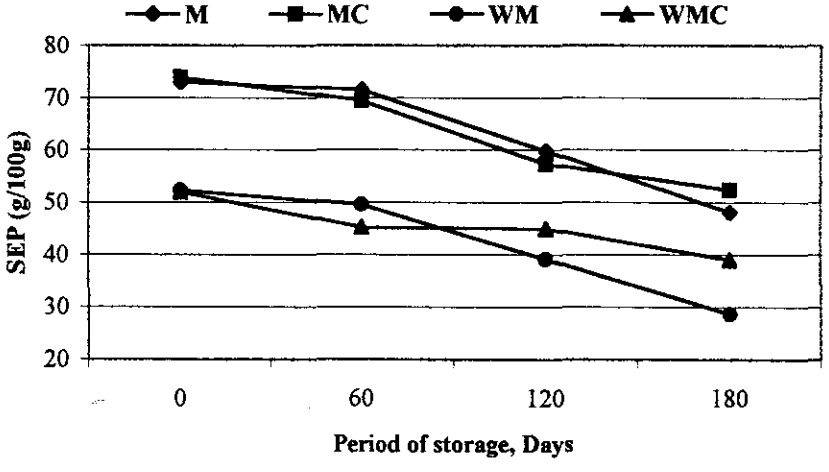


Fig. 4 Changes in the total salt extracable proteins (DWB) during storage at -35°C in rohu

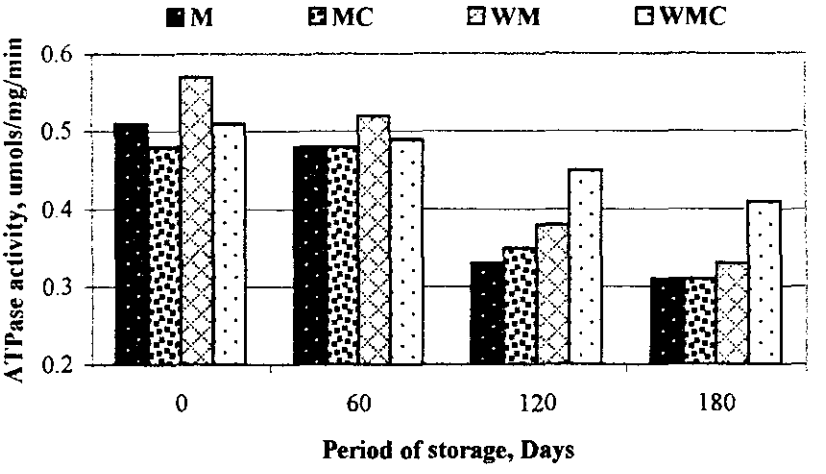


Fig. 5 Changes in actomyosin ATPase activity in rohu meat during storage at -35°C

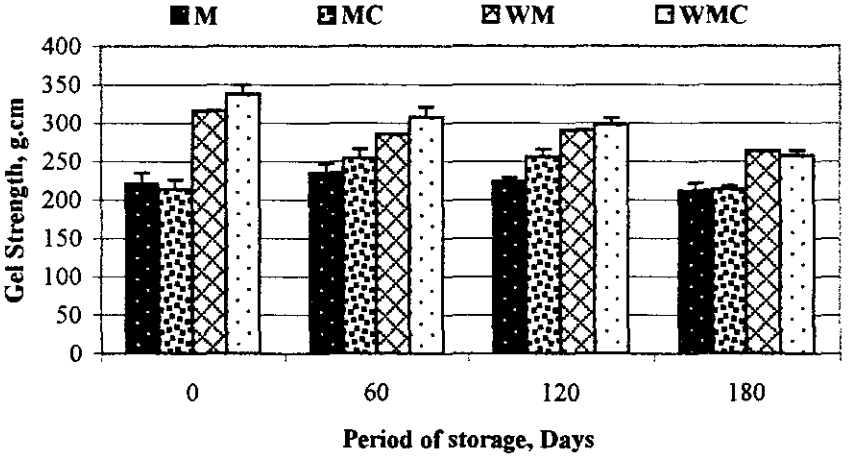


Fig. 6 Changes in gel strength of kamaboko gels made from rohu meat during storage at -35°C

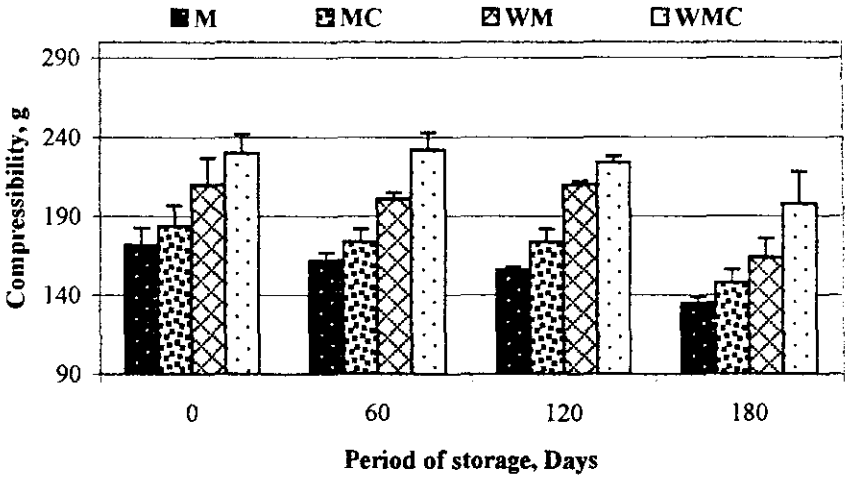


Fig. 7 Changes in compressibility of kamaboko gels made from rohu meat during storage at -35°C

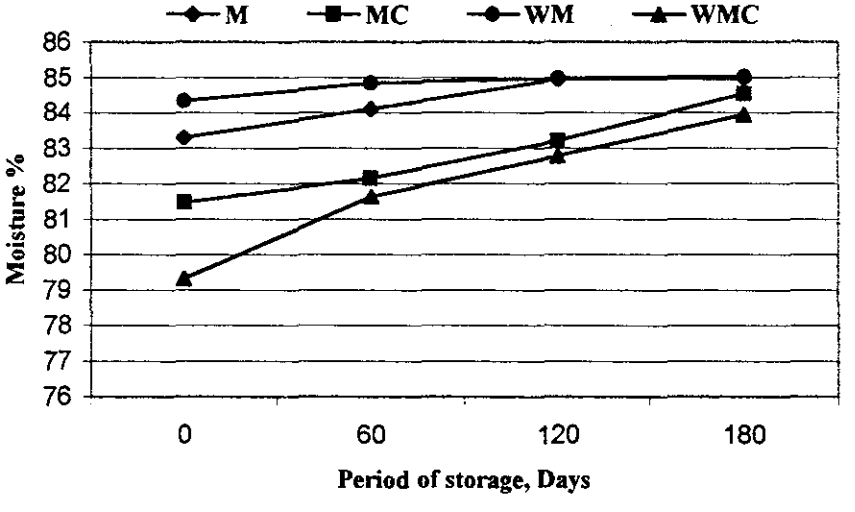


Fig. 8 Changes in the moisture in catla meat during storage at - 35°C

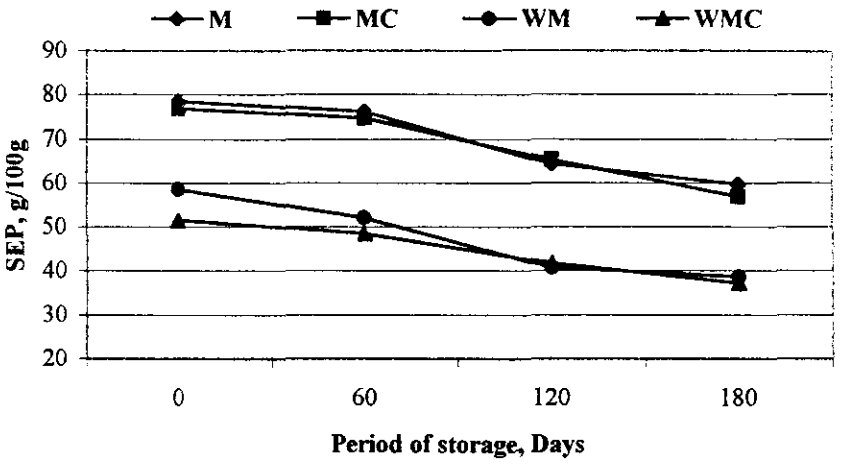


Fig. 9 Changes in total salt extractable protein (DWB) in catla meat during storage at -35°C

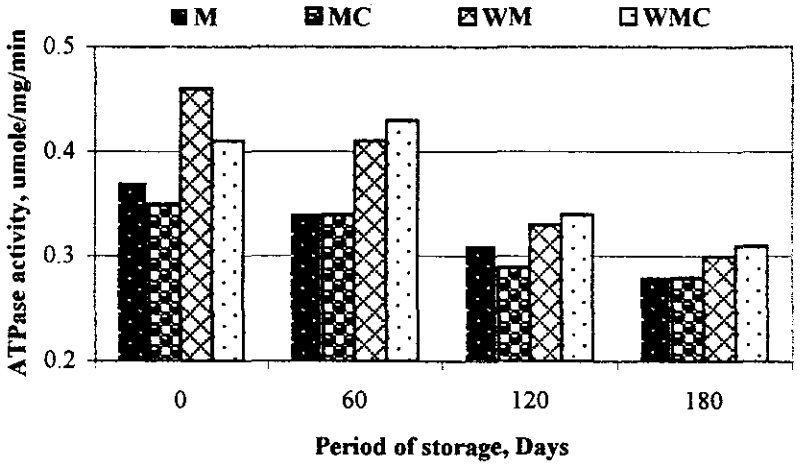


Fig. 10 Changes in myosin ATPase activity in catla meat during storage at -35°C

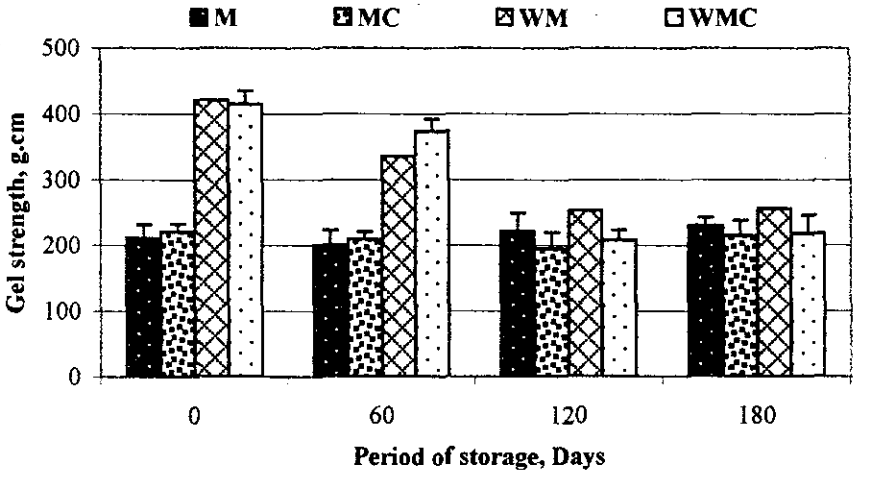


Fig. 11 Changes in gel strength of kamaboko gels made from catla meat during storage at -35°C

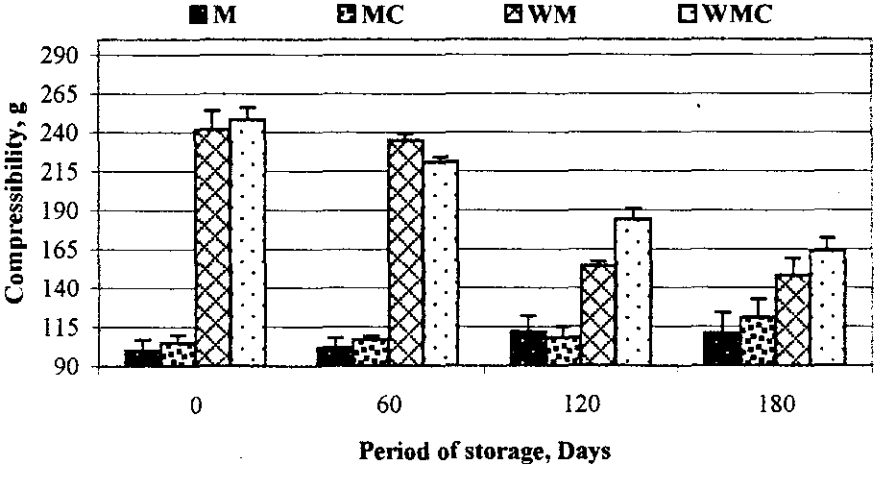


Fig. 12 Changs in the compressibility of kamaboko gels made from catla meat during storage at -35°C

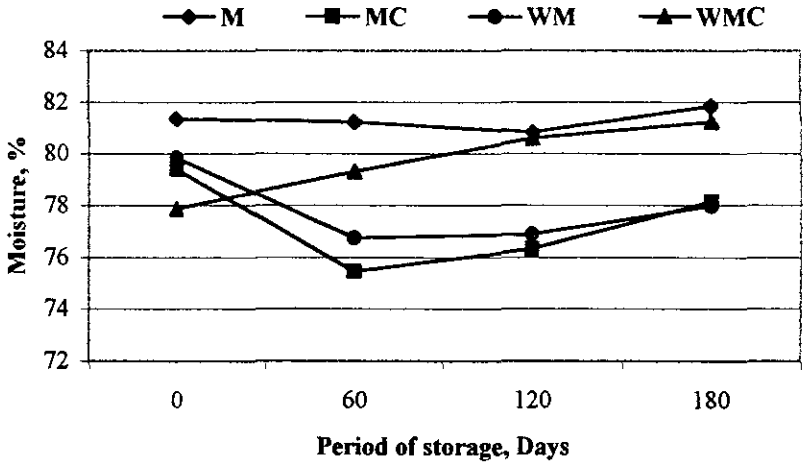


Fig. 13 Changes in moisture in mrigal meat during storage at -35°C

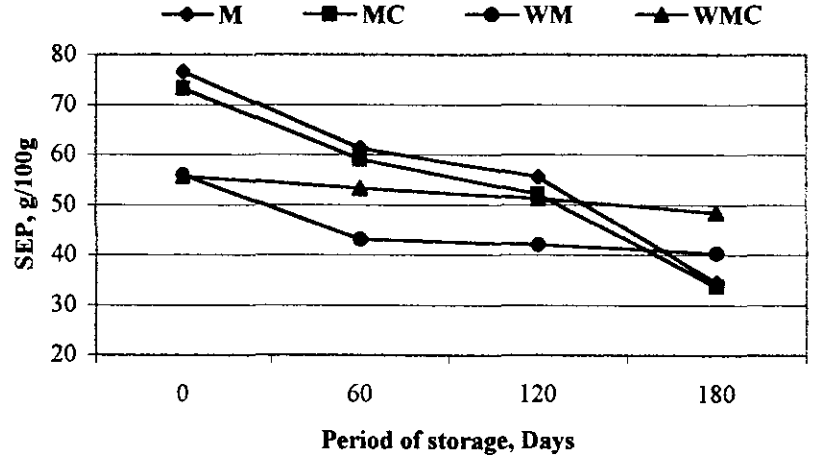


Fig. 14 Changes in salt extractable protein (DWB) in mrigal meat during storage at - 35°C

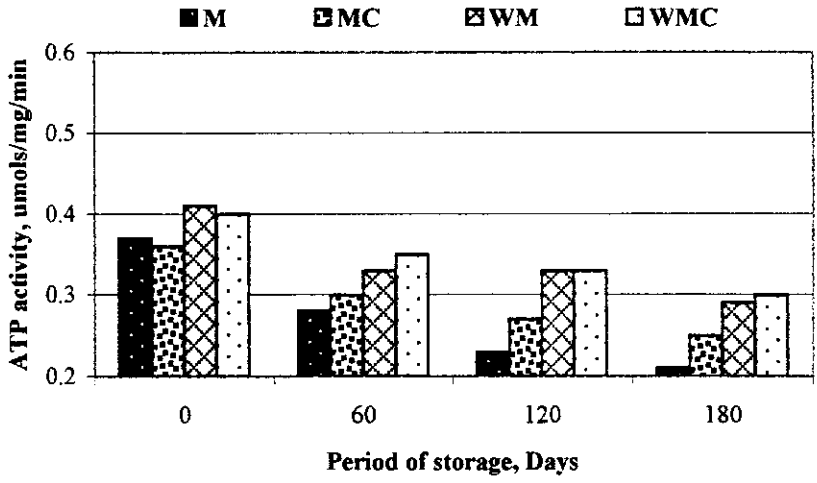


Fig. 15 Changes in the actomyosin ATPase activities in mrigal meat during storage at -35°C

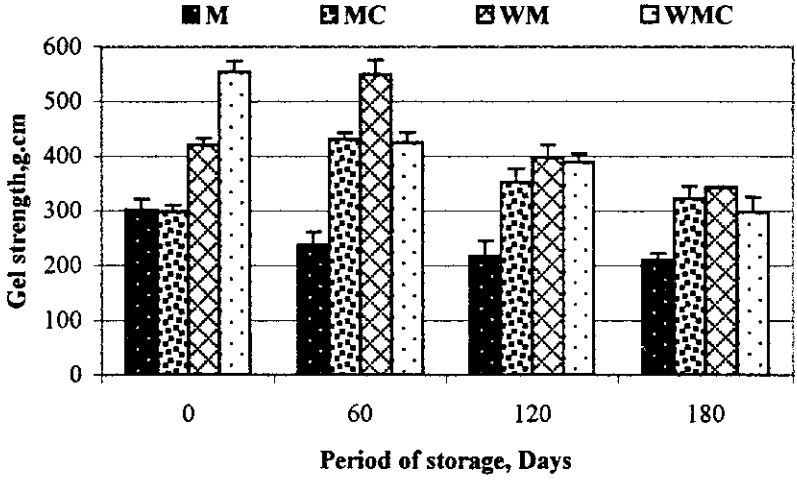


Fig. 16 Changes in gel strength of kamaboko made from mrigal meat during storage at -35°C

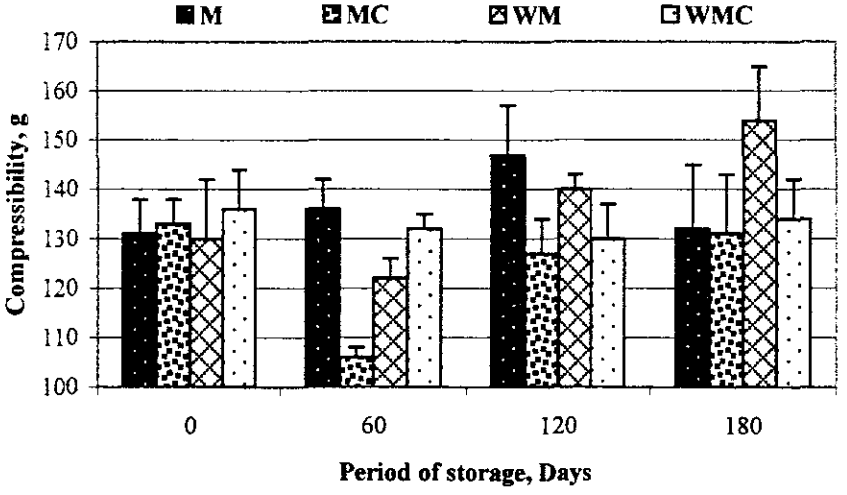


Fig. 17 Changes in compressibility of Kamaboko made from mrigal meat during storage at -35°C

Chapter – 7

Summary and Recommendations

Fish is the cheapest source of good quality animal protein readily available to the masses throughout the world and its demand is always on the increase. The aquaculture production of fish and shellfish contribute its share in the aquatic production.

In India, the freshwater fish is mainly used for direct consumption and is not used for product development. The marketing of freshwater fish is not streamlined and often, the catch is transported to distant places to dispose off at throwaway prices. In the absence of a proper utilisation strategy and non-availability of information on the biochemical aspects of the processing, this study assumes significance.

Surimi or myofibrillar protein concentrate is a mechanically deboned minced fish, washed to remove blood, fat, solubles and other odouriferous substances and stabilised by the addition of cryoprotectants. The absence of any flavour permits its utilisation as a vehicle for flavour addition in the production of analogue products. Most commonly used fishes for surimi production in India include the lean, white meat fishes like threadfin bream (*Nemipterus japonicus*), lizard fish (*Saurida thumbil*) and croaker (*Otolithus* sp.).

This study aims to evaluate the possibilities of utilising the freshwater major carps – rohu, catla and mrigal, which contribute to more than 95% of total freshwater fish production - for the preparation of fish mince and surimi.

Considering the composition of fish, the water-soluble nitrogen and salt soluble fraction constituted 21% and 55 – 60% of total nitrogen respectively. The non-protein nitrogen represented 12% while the insoluble connective tissue nitrogen contributed to 2-3 per cent of total nitrogen. Considering the fatty acid composition, the mono unsaturated fatty acid content of the major carps was higher than that of marine fishes. The content of the polyunsaturated fatty acid, particularly eicosapentaenoic acid (EPA) was very low. The essential amino acids contributed to 41 to 51% of the total amino acids in freshwater major carps. Aromatic amino acid content was slightly higher compared to marine fishes while proline showed a lesser content. There was not much of variation in the composition of meat but the autolytic activity of the meat showed some variation.

Marginal differences were noticed in the natural actomyosin properties between small and big sized fish in all the three fishes. When the natural actomyosin from rohu, catla and mrigal were heated to 30°C relatively little changes were noticed in any of the analysis. The natural actomyosin molecules began to unfold around 40°C and substantial unfolding occurred from 40°C to 60°C. As unfolding proceeded, the numbers of hydrophobic amino acid residues and number of sulfhydryl groups increased at the surface of the molecule. Comparing the actomyosin from the three major carps that from rohu was more stable followed by mrigal and catla.

There was considerable loss in salt extractable protein, with a marginal loss of myofibrillar protein in the first washing itself. The elasticity of the gel, the gel strength and the compressibility of the fish meat, however, increased as a result of washing. Mrigal had comparatively higher gel strength followed by catla and rohu. Washing resulted in the conformational changes in fish actomyosin to some extent, as shown by the decrease in the Ca^{2+} ATPase activity.

The sol prepared from fresh water fish showed some differences. The salted flesh from rohu, catla and mrigal do not gel at low temperatures, a property generally noticed in marine species. Good setting was noticed at 50° and 60 °C for 30 min of setting time in rohu and mrigal while in catla good elastic gels are formed at 40°C itself. Increasing setting time positively influenced gel strength only in catla. The compressibility of the gels showed marginal differences between the three fishes. Compressibility of the gels set at lower temperature increased upon cooking and the increase in compressibility with increasing temperature and duration of setting showed difference among the fish. There was no decrease in gel strength at 50 or/and 60°C irrespective of length of setting time (30 or 60 min.) signifying the possible absence of the activity of *modori* causing proteases at the temperatures studied.

The size of the fish, whether 400 or 1200 g, showed only a marginal effect on the gelling properties of the surimi produced. The mince from both size groups produced good quality surimi in all the

three fishes. Hence it is the season (whether spawning or not), feeding habits (effecting the composition of the fish meat) and the processing conditions that affect the gelling properties of fish meat and the size of the fish did not seem to have any effect.

The salt soluble and hence the myofibrillar protein were found to have undergone changes during frozen storage in all the three fish species more or less in the same pattern. The decrease in salt extractable proteins was greater than that of myofibrillar protein and is related to the denaturation of sarcoplasmic proteins. About 65 – 75% of salt extractable proteins in mince or 75-95% in washed and cryoprotectants added mince remained extractable after 180 days of storage at –35°C. But being lean fish, the interaction of fatty acid formed from phospholipid with protein is the primary cause of protein denaturation. The conformational changes taking place in protein molecules were supported by decrease in ATPase activity during the storage.

Recommendations

- There are no interfering substances like TMAO in the meat of freshwater fish and the quality improvement as seen by colour, flavour and acceptability of mince and kamaboko produced, is noticed in one wash itself. Hence, a single washing with mince to water (containing 0.2% NaCl) ratio of 1:4 is recommended for

the processing of rohu, catla and mrigal mince for surimi preparation

- From the experiments on the gel forming ability of flesh from rohu, catla and mrigal it is concluded that the washed flesh from these fish species exhibit good gelling properties and can be used in the preparation of value added products. There is not much difference in the quality of surimi as far as size of the fish is concerned.
- Washing to remove the water-soluble SPP increases the gel strength several folds in all the three fishes, which decreases with the length of storage. The surimi or washed mince with cryoprotectants from these fishes have a good stability at –35°C upto six months without much alterations in composition and hence is recommended for the development of surimi based product.
- The yield and quality of surimi prepared from the major carps are found to be comparable with other conventional species used for surimi production and hence they also could also be used as raw material for commercial production of surimi.

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