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Assessment of the Textural Variations of Muscle Tissues of *Labeo rohita* and *Scoliodon sorrokawah* with Emphasis on Their Collagen Content

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Abstract

Structural proteins specifically myofibrillar protein and collagen play a very significant role in determining the texture of the product. Shark (*Scoliodon sorrokawah*) is a cartilaginous fish having high collagen content and its crude protein content (22.01%) is comparatively higher than rohu (*Labeo rohita*) viz. 17.73%. The present study investigates the rheological differences between the two species differing in their collagen content. During freezing, an increase in the toughness was observed which was possibly due to the aggregation of myofibrillar proteins and collagen. Histographs and texture profile analysis data obtained indicated a two – phase toughening process in both the species. In both unfrozen and frozen samples, a two to three fold increase in toughening occurred during cooking. Though the first phase of hardening on cooking (50°C) was similar in both species, the second phase of hardening varied in frozen samples as 70°C and 75°C for fresh and frozen shark and 70°C and 80°C for fresh and frozen rohu respectively. These slight variations in the two species might be possibly due to the difference in their connective tissue protein content, distribution and properties. In raw muscle tissue, the toughness is very much a product of hardening of the connective tissue proteins. During the second phase of tissue hardening the weight loss and cooking shrinkage was observed which was possibly due to the loss of water holding capacity and conformational changes in the protein configuration.

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Introduction

Texture is the key quality factor determining the consumer acceptability of muscle foods. Fish texture is affected by intrinsic and extrinsic factors. The quality of raw material, chemical composition and preservation practice employed are known to determine the end product quality, as a slight discrepancy in them might cause irreversible loss of quality, giving poor quality to the product due to the reaction between proteins and oxidized lipids (Ingolfsdottir 1997). Freezing is a prominently adopted processing and preservation practice for maintaining the nutritional and eating quality of the fishery product. Long–term frozen storage might lead to marked increase in toughness and dryness of the tissue (Dunajski 1980). Measuring the electrical and biochemical properties of the muscle tissue could determine changes in fish freshness. Structural proteins namely, myofibrillar proteins and collagen affect the rheological characteristics of the muscle tissue. Tenderness depends on the contraction state of the myofibrillar structure. Fish is softer and is characterized by lesser amount of connective tissue and lower degree of cross-linkages (Venogopal and Shahidi 1996). The contribution of connective tissue proteins depends on the content and age of the species. Collagen is located in extra cellular matrix and plays a significant role in determining the textural properties in the muscle tissue. In fish muscle it exists as a fibrous sheet, myocommata holding the blocks of muscle tissue (myotomes) intact. It is species specific and varies with age, sex, starvation, maturity etc. Hassan and Mathew (1996) reported the collagen content and their characterization in few selected fish species along the Indian coast. Sato et al. (1986) also reported the characterization of collagen in several fish species. They found that collagen is closely related to the firmness of raw fish meat; the higher the collagen content, the firmer the raw muscle tissue, although such a relation between collagen and cooked meat of fish was hardly recognized (Dunajski 1980).

The present study investigates the textural variations in rohu (*Labeo rohita*, Clupeidae) and shark (*Scoliodon sorrokawah*, Scoliodontidae) at different cooking temperatures with emphasis on the collagen and their influence on rheological characteristics. The study considers the textural disparity between the two species with varying collagen content.

Materials and Methods

Sample collection

Rohu (*Labeo rohita*) measuring 50–55 cm were collected fresh from culture farms, iced and brought to the laboratory within two hours, cleaned and eviscerated. Shark (*Scoliodon sorrokawah*) measuring 70–90 cm, was collected from the Cochin Harbor situated on the Southwest coast of India. They were stored in ice, immediately brought to the laboratory and were eviscerated and cleaned. For the frozen study, the cleaned and eviscerated samples of rohu and shark were individually quick frozen in a tunnel freezer at -40°C for one hour, packed and stored in cold storage at -18°C.

Determination of physico–chemical composition

Calibrated Cyberscan pH 500 (digital pH meter, MERCK) was used to determine the pH by immersing the probe in the slurry in a ratio of 1: 9 (sample: water). The methodology of Regenstein and Regenstein (1984) was used to measure expressible moisture (EM). One gram of tissue (1 cm^3) was placed in between the two clean–dried filter papers and a pressure of 10 kg/cm^2 was applied for 10 sec. The weight difference calculated in percentage reflected the expressible moisture. Water binding potential (WBP) was measured following the method of Borresen (1980). Moisture, crude protein, crude lipid and ash content in the fish samples were analyzed by AOAC (1990).

Protein fractionation of fish muscle

Fractionation of the muscle tissue was carried out in accordance with the methodology of Hashimoto et al. (1979); while the methodology of Mizuta et al. (1994) was followed to extract pepsin soluble collagen from the stroma protein. The flowchart depicting the protocol of fractionation is shown in figure 1. The whole of the fractionation procedure was performed at low temperatures of 3°C to 4°C. A 10 g tissue was homogenized with 10 vol of 0.05 M phosphate buffer (pH 7.5) by using a tissue homogenizer (Yorco Micro Tissue Homogenizer). The homogenate was centrifuged at 3000–4000 rpm for 10 min in a refrigerated centrifuge (MB–20 Super Speed Refrigerated Centrifuge). The supernatant obtained consisting of sarcoplasmic protein and non–protein nitrogen was treated with

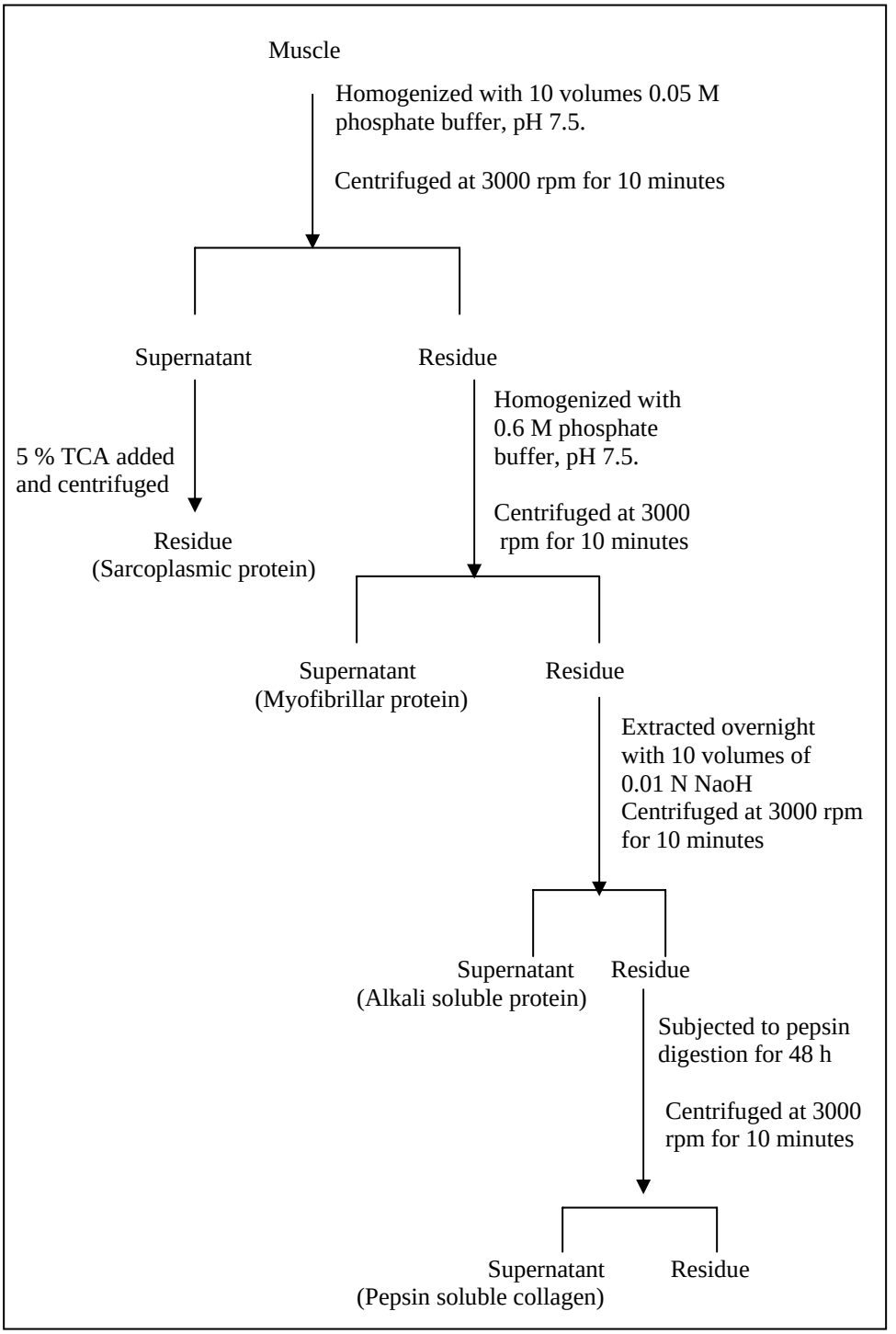

Fig 1. Protocol for protein fractionation

5% tricholoaceteic acid. The residue obtained from this was considered as sarcoplasmic protein (SP) fraction. The residue after the first fractionation was further homogenized with 10 vol of 0.6 M phosphate buffer (pH 7.5) and centrifuged at 3000–4000 rpm for 10 min. The supernatant was considered as myofibrillar protein (MY) fraction. The residue obtained was subjected to exhaustive extraction with 0.1 N NaOH for 24 h under continuous stirring. The supernatant obtained after centrifugation was used as alkali soluble protein (ASP) fraction. The final residue was suspended in 0.5 M acetic acid and digested for 48 hours with enzyme pepsin (SIGMA) at an enzyme: substrate ratio of 1: 20 (w/w). The collagen extracted in supernatant was considered as pepsin soluble collagen (PSC) fraction.

Texture profile analysis

Unfrozen and frozen samples of rohu and shark of 2 cm^3 size were wrapped in aluminum foil and cooked at different temperatures ranging from 45°C to 90°C for 1 min. The test was repeated in replicates of five for each sample. A six-member panel performed sensory evaluation and 7 – point scoring was used for evaluation of the overall acceptability (Borderias et al. 1983).

The instrumental texture analysis was carried out using Texture Analyzer (Lloyd Instruments, UK, model LRX PLUS). A small, flat–faced cylindrical probe of 50 mm diameter compressed the bite size of piece twice in a reciprocating motion. Test speed was maintained at 12 mm/min and trigger force at 0.5 kgf. Double compression makes it possible to perform texture profile analysis (Bourne 1978). From the plot of force–time curve hardness 1 (H1), hardness 2 (H2), cohesiveness (C) and stiffness (S) were evaluated.

Histochemical analysis

Fresh and frozen samples of rohu and shark (2 cm^3) were cut into ½ cm blocks and fixed overnight in Bouin's fixative, followed by dehydration in serially diluted ethanol (70–96%). The samples then embedded in paraffin wax (melting point 60°C) were cut into sections of 8 µm using rotary microtome (SIPCON SP 1120 Rotary Microtome, India). Double staining using Weigert's Haematoxylin stain and Van Geison's stain was performed. The myofibrils were stained yellow and collagen to red and were then observed under Nikon Eclipse E-200 compound light microscopes and photographed.

Results

Table 1 shows the proximate composition and major protein fractions of rohu and shark. A high content of crude protein (22.01%) was obtained in shark compared to rohu (17.73%). The high non-protein content like urea contributes to the high crude protein content in shark. The connective tissue protein was also high in shark. Table 2 shows the pH, expressible moisture (EM) and water binding potential (WBP) of unfrozen and frozen samples of rohu (*Labeo rohita*) and shark (*Scoliodon sorrokawah*) cooked at different temperatures. The pH of unfrozen sample of shark ranged from 6.3 to 6.5 and after freezing it was lowered to 5.7. Rohu showed a similar pattern of variation in pH with cooking. It could be due to the enzymatic reactions taking place in the muscle tissues during frozen storage conditions. Expressible moisture and water binding potential affect the tenderness of the tissue. Changes in water holding capacity is closely related to pH of the muscle. Water binding potential (WBP) was observed highest at 65°C for shark and in rohu, it was highest at 50°C. Table 3 shows the textural parameters evaluated instrumentally and the organoleptic scoring obtained for rohu and shark. Hardness of unfrozen uncooked shark was observed to be 5.13 mm and was reduced to 4.33 mm at 50°C. The hardness at 70°C was approximately half of the value obtained at 50°C. The hardness in rohu was 4.71 mm for fresh uncooked and was increased to 4.99 mm at 50°C. After freezing, hardness in shark increased to 6.41 mm possibly due to the freeze-denaturation of the muscle proteins. At 70°C, the shark had a hardness of 1.75 mm with optimum cooked texture while in rohu the optimum cooked texture was obtained at 80°C with a hardness of 1.26 mm. These changes in the behavior of muscle tissue might be due to the difference in collagen content, composition and their localization. In rohu the myosin is responsible for the thermal gel formation. Plate 1 shows the histograph of rohu and shark. Two phases of hardening is observed in both the species. The first phase of hardening is observed at 50°C for both the species. In the unfrozen samples the second phase of hardening was observed to occur at 70°C that shifted to 80°C and 75°C in rohu and shark, respectively. The first phase of hardening indicated the partial denaturation of collagen fibrils and penetration of heat to the myotomes causing the conformational changes of the myofibrillar proteins at 50°C (Plate 1). The sparser the collagen content, the easier is the heat penetration. Due to the denaturation of the collagen fibrils the

myotome bundles move apart. Further myofibrillar proteins were also observed to move apart due to collagen denaturation resulting in the formation of large pores in the muscle tissue. At 70°C, in unfrozen rohu the myofibrils tend to retain its compact nature and integrity possibly due to the gelatinization and shrinkage of collagen fibrils. A similar change is also visible in the frozen sample of shark (at 50°C) and rohu (80°C). A resemblance in the effect of temperature in both species was observed but with storage time there was a shift in the optimum cooked texture of rohu and shark to 75°C and 80°C that could be possibly due to the difference in the connective tissue protein content, amino acid profile and their possible freeze denaturation. Freezing also affected the textural changes by increasing the ionic concentration of liquid and breakage of electrostatic bonds between the proteins.

Table 1 Proximate composition and major protein fractions of rohu and shark

Biochemical composition	Rohu	Shark
Moisture, %	75.46	72.46
Crude lipid, %	0.82	0.90
Ash, %	1.48	2.36
Crude protein, %	17.73	22.01
Sarcoplasmic protein, % of total protein	13.51	16.07
Myofibrillar protein, % of total protein	77.33	45.62
Alkali soluble protein, % of total protein	14.54	15.56
Total collagen, % of total protein	0.76	24.85
Pepsin soluble collagen, % of total collagen	0.24	13.69

Values are average of three independent estimations.

Table 2. The pH, expressible moisture (EM) and water binding potential (WBP) of fresh and frozen samples of rohu and shark cooked at different temperatures

Temperature (ºC)	pH				EM (%)				WBP (g)			
	Rohu		Shark		Rohu		Shark		Rohu		Shark	
	0	*3*	*0*	*3*	*0*	*3*	*0*	*3*	*0*	*3*	*0*	*3*
Uncooked	6.30	5.70	6.40	5.70	72.42	72.93	74.19	85.02	1.84	1.59	1.68	1.68
45	6.30	5.90	6.30	5.90	70.69	68.10	71.73	76.12	1.57	1.46	1.46	1.46
50	6.30	6.30	6.30	6.30	74.61	74.84	75.39	88.74	2.46	2.21	1.82	1.82
55	6.30	5.90	6.50	5.90	68.35	67.15	67.10	75.05	1.97	1.24	1.90	1.90
60	6.30	5.70	6.40	5.70	79.23	74.42	77.49	73.54	1.97	2.08	1.97	1.97
65	6.30	5.80	6.30	5.80	75.02	72.56	74.23	80.69	1.92	1.83	2.07	2.07
70	6.30	5.80	6.50	5.80	75.04	65.56	73.90	74.28	1.92	1.44	1.71	1.71
75	6.50	5.80	6.40	5.80	78.58	72.77	76.42	79.67	1.67	1.36	1.49	1.49
80	6.30	5.70	6.30	5.70	79.19	61.83	79.02	74.72	1.39	1.57	1.26	1.26
90	6.40	5.70	6.30	5.70	76.33	65.04	75.20	80.51	1.94	1.16	1.21	1.21

Values are average of five independent estimations. *0* – unfrozen, *3* – frozen stored for three months

Table 3 Texture profile analysis of rohu and shark cooked at different temperatures

Tempera-ture (ºC)	Rohu										Shark									
	Instrumental								Sensory		Instrumental								Sensory	
	H1 (mm)		H2 (mm)		C		S (kgf/mm)		Scoring		H1 (mm)		H2 (mm)		C		S (kgf/mm)		Scoring	
	0	*3*	*0*	*3*	*0*	*3*	*0*	*3*	*0*	*3*	*0*	*3*	*0*	*3*	*0*	*3*	*0*	*3*	*0*	*3*
Uncooked	4.71	5.12	3.77	3.88	0.21	0.14	6.28	6.34	ND	ND	5.13	4.33	3.87	3.53	0.13	0.20	6.60	5.45	ND	ND
45	2.51	4.61	2.04	3.49	0.19	0.12	1.57	4.89	4	4	0.89	5.42	0.72	4.40	0.10	0.22	2.76	5.64	4	4
50	4.99	5.86	4.16	4.54	0.26	0.19	**4.46**	**6.05**	5	4	4.33	6.41	3.19	5.08	0.14	0.23	**5.09**	**6.81**	5	4
55	3.82	3.27	3.02	2.65	0.22	0.17	3.82	3.04	5	4	0.97	2.42	0.77	1.97	0.10	0.18	3.28	3.55	5	4
60	4.33	2.02	3.20	1.53	0.16	0.13	3.94	3.82	6	4	0.87	1.56	0.72	1.22	0.11	0.14	3.04	1.68	6	4
65	1.20	2.14	1.02	1.68	0.24	0.12	1.83	3.69	6	5	0.95	1.51	0.80	1.18	0.15	0.15	4.18	1.29	6	5
70	2.17	1.61	1.87	1.27	0.26	0.14	2.46	2.37	5	5	1.93	1.65	1.69	1.31	0.28	0.16	**8.57**	1.93	5	5
75	1.26	1.37	0.96	1.09	0.13	0.12	**5.52**	2.74	5	5	1.10	1.75	0.92	1.35	0.18	0.18	3.85	**3.95**	5	5
80	1.22	1.26	0.99	0.95	0.14	0.12	2.06	**5.87**	5	5	1.04	1.37	0.89	1.08	0.19	0.14	3.36	1.26	5	5
90	2.16	1.45	1.92	1.18	0.29	0.18	2.47	2.38	5	5	2.04	1.53	1.76	1.24	0.29	0.16	2.94	1.80	5	5

Values are averages of three independent estimations. H1- hardness at first bite; H2- hardness at second bite; C- cohesiveness; S- stiffness; *0* – unfrozen, *3* – frozen stored for three months. ND – not detected.

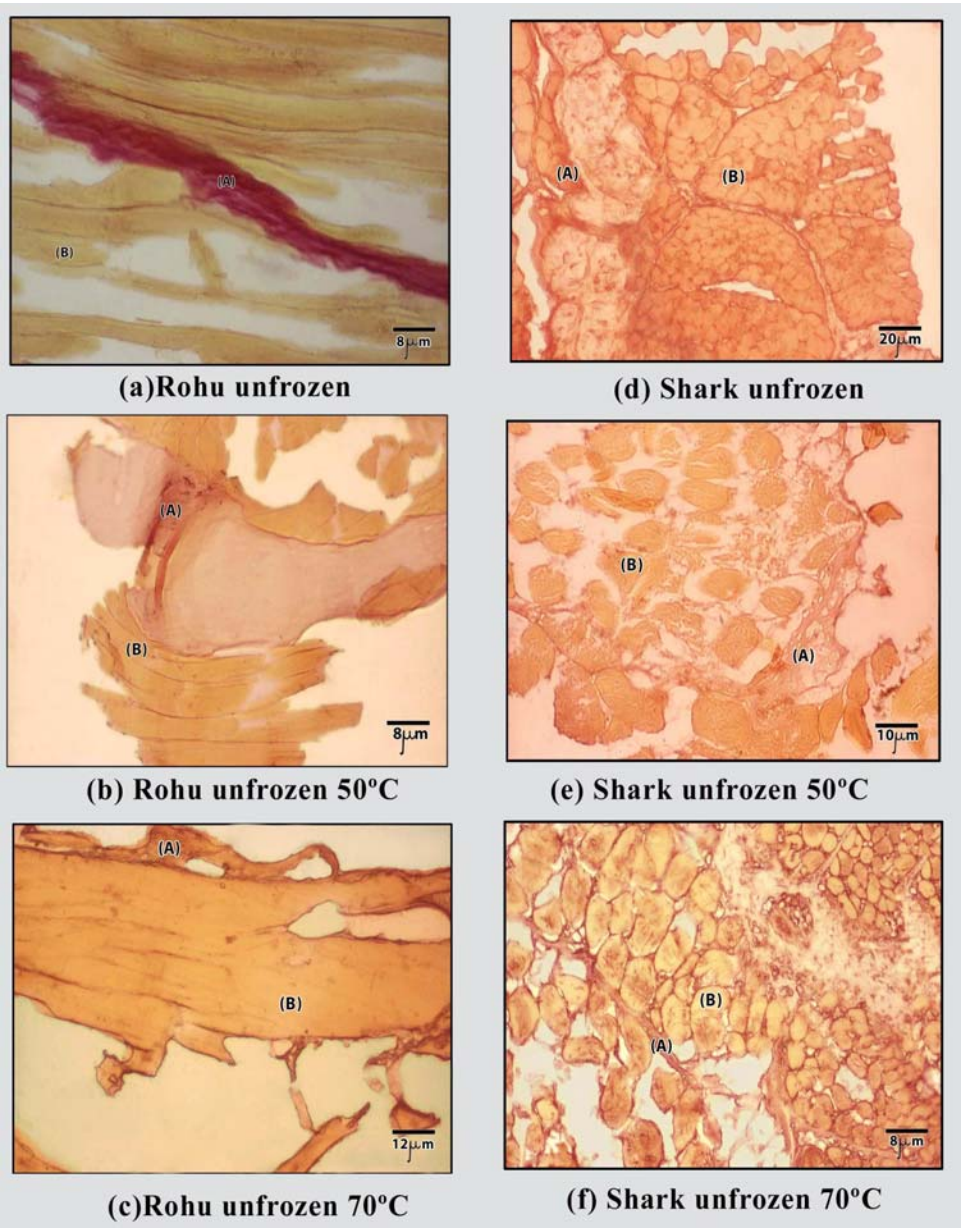

Plate 1a. Unfrozen samples of rohu and shark cooked at various temperatures
(A) Collagen, (B) Myofibrillar protein

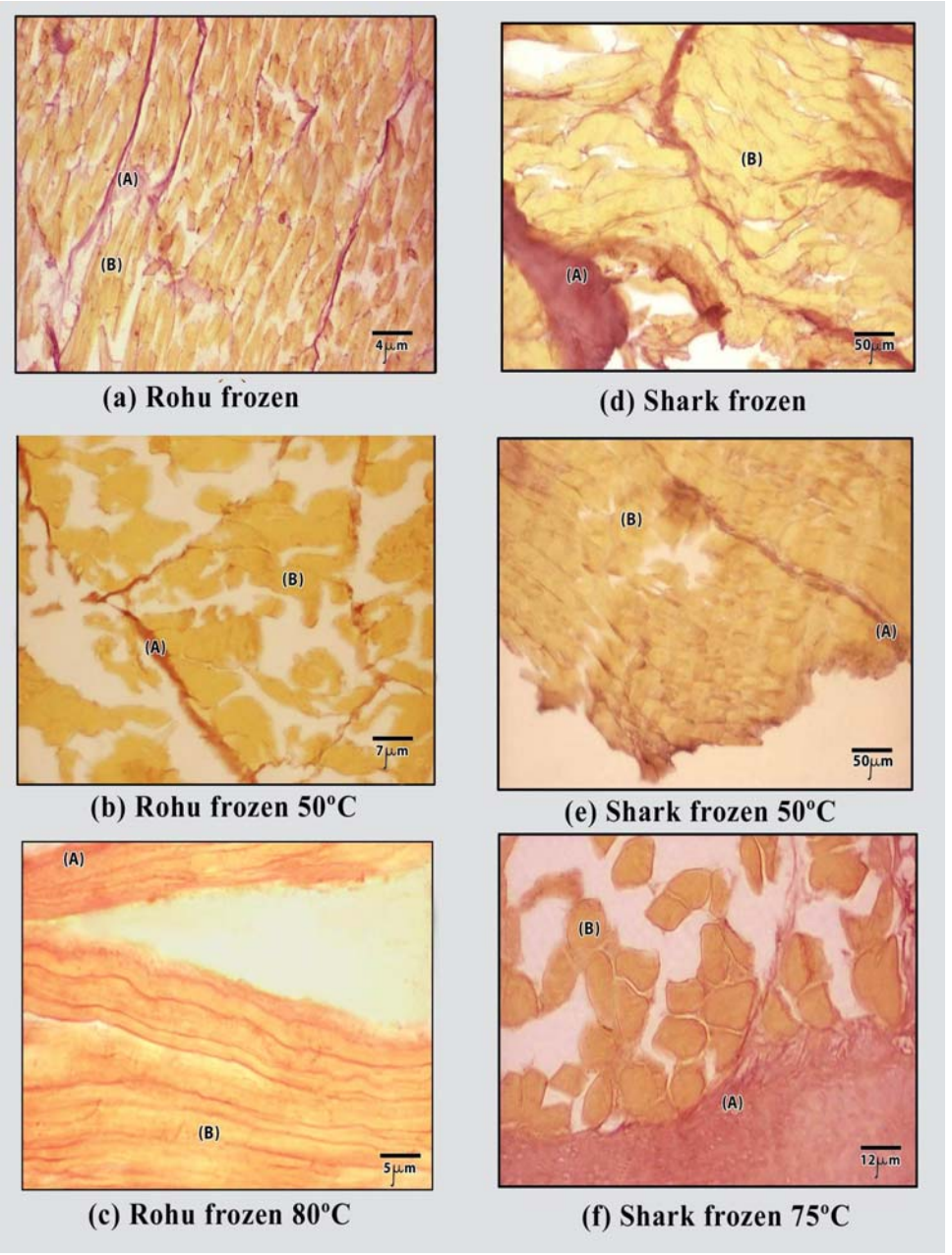

Plate 1b. Frozen samples of rohu and shark cooked at various temperatures
(A) Collagen, (B) Myofibrillar protein

Discussion

The changes observed in the pH of the muscle tissues of the two species with freezing and cooking could be possibly due to the enzymatic actions taking place in the muscle tissues during frozen storage conditions. Expressible moisture and water binding potential significantly affect the tenderness of the tissue. Changes in water holding capacity is closely related to pH of the muscle. Bouton et al. (1972) reported that changes in the water holding capacity (WHC) of tissue over the pH range 5.4 to 7.2, were large and linear for both raw and cooked bovine muscle. The water binding property is a function of protein functionality that is determined by freshness of species of fish and other processing variables (Douglas and Lee 1988). A certain amount of moisture is necessary for the adequate solubilization of the protein and for the formation of the gel networks.

Gopakumar (1997) reported the proximate composition in different species of marine, fresh water and brackish water fish. The biochemical compositions in fish muscle vary with sex, size, stages of maturity and season. Starvation could result in decrease of protein and lipid content coupled with an increase in the water content. Shark was observed to possess high protein content compared to rohu. Shark muscle contains high urea content. Among the different fractions of the protein, the connective tissue protein was high in shark. In cartilaginous fish, the collagen content is comparatively higher than the carps (Hassan and Mathew 1996). Collagen is prominently concentrated in skin, fins and skeleton. Sato et al. (1986) reported the detailed characterization of fish collagen in several species in relation to their swimming mode.

Structural proteins namely myofibrillar proteins and collagen determine the functional and rheological characteristics of the muscle tissue (Dunajski 1980). The tenderness of muscle tissue (the shear strength) depends on the contraction status of myofibrillar proteins (Cuq et al. 1996). These changes could be due to the collagen content and their localized distribution. Collagen was observed to be sparse in rohu and hence, the heat penetration to myotome bundles and their denaturation was comparatively easier than shark. The texture of cooked meat is tacit to be affected by the solubilized protein, gelatin or a shrunken fiber derived from the heat denatured muscle collagen. Hence, it is reasonable to speculate that muscle collagen is equally responsible for the texture of raw and cooked fish meat. Fish collagen contains less proline and hydroxyproline than mammalian collagen making the tissue more susceptible to conformational changes and

thermal shrinkage of the proteins at low temperature (Sato et al. 1986). These changes in the behavior of the muscle tissue with respect to species and temperature could be due to the difference in the collagen content, composition and localization. Connective tissue protein content and the age of the species affect the textural properties (Kruggel and Field 1971). Fish myosin has different physicochemical properties like stability in association with pH and temperature that result in gelation. In rohu, the myosin is accountable for thermal gel formation. Ko and Hwang (1995) and Karthikeyan et al. (2004) reported the contribution of sarcoplasmic proteins in improving the thermal gelation of myofibrillar proteins in milkfish. When tissue is cooked, collagen is converted to gelatin. Both the insoluble and soluble forms of collagen are of great importance in determining the overall toughness of the tissue. Post mortem changes in textural characteristics are indicated by changes in myofibrillar proteins and connective tissue protein. Loss of fiber compact structure and increase in extracellular space between the fibers were developed (Ingolfdottir 1997). Yield of intramuscular collagen decreased with time of post mortem processing (Papa et al. 1997). According to him, stress on collagen fibers altered some of the physical characteristics of collagen including thermal shrinkage temperature, collagen cross linkages, thereby affecting muscle tenderness. Pfeiffer et al. (1972) reported that connective tissue influences the tenderness of muscle tissue but an increase in the tenderness with post-mortem aging involves changes in the myofibrillar proteins. He also reported that yield of intra muscular collagen decreased with time of post-mortem processing. Stress affects the collagen cross-linkages and muscle tenderness. Collagenases present and operative at neutral and alkaline pH were also known to affect the texture (Romuald et al. 2005). The number and strength of cross linkages in the collagen influence the tissue tenderness. Increased toughness of the meat could be explained by an increase in collagen cross-links (Kruggel and Field 1971). The musculature pattern of rohu and shark were almost similar with sheets of collagen fibrils dominating the myocommata enveloping the myotome bundles. Shark connective tissue vary from rohu as shark has high collagen content in the epimysium that envelops the myotome bundles and this could possibly result in variations in the optimum cooked temperature.

Myofibrillar proteins and collagen fibers play a primary role in determining the textural properties of the product. The proportion of collagen, composition and their localization have an important function in texture determination. Shark (13.69 % of total protein) has a higher collagen content than rohu (0.24% of total protein). Histographs obtained sup-

ported the results obtained by the texture profile analysis indicating that the best cooked texture for both species in fresh condition was at 70°C with maximum juiciness. The first phase of tissue hardening in these samples was noticed at 50°C. Collagen fibrils were susceptible to the freeze denaturation and hence affected the shark muscle tissue in obtaining the optimum texture at 80°C after freezing. In frozen samples, proteins may have undergone aggregation and structural changes resulting in the slight increase in toughness in rohu and shark and a slight shift in the optimum texture of rohu to 80°C with frozen storage.

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