CHAPTER 5

HISTOCHEMICAL STUDY OF MUSCLE TISSUE WITH EMPHASIS ON COLLAGEN
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5.1. INTRODUCTION

Histochemistry is an effective tool used for the direct diagnosis of chemical changes occurring in tissues at cellular and sub cellular levels. The normal method of assay in histochemistry involves development of color to flag the biochemical compounds that are used as the biomarkers. In the present study myofibrillar and collagen are used as the biomarkers. The staining techniques developed and employed were sufficient to assess the intensity of reactions during cooking and freezing as evidenced by the histochemical reactions. Essentially the method involves identifying qualitatively and to some extent quantitatively the occurrence of the biochemical markers. Many publications are available that provide information on histochemistry proving the significance of such studies. The method seems to be most reliable in establishing and bringing forth the various changes in the tissue. From the previous chapters it was observed that there were quantitative and qualitative variations in the collagen and myofibrillar protein of the three species that had a significant role on the muscle texture. The distribution and arrangement pattern of these structural proteins could have a key role in determining the texture. Hence histochemical studies of the musculature pattern of the three species under study are essential. In view of this investigations are carried out on the following aspects of the tissue.

1. Histochemical differences in the musculature pattern of rohu, squid and shark with emphasis on collagen.

2. Histochemical changes in the muscle proteins of these species at different heating regimen in both unfrozen and frozen samples.

5.2. REVIEW OF LITERATURE

The histological and histochemical studies were found to be highly beneficial in biology. Histochemical studies were helpful in understanding the content and accumulation of carbohydrates, general lipids, neutral lipids, proteins and RNA in relation to the gonad development and maturation.

The textural and nutritive value of seafood products are duly influenced by the composition and the structure of the muscle proteins (Goll et al., 1977). Firmness of raw
muscle tissue is an important index for freshness detection that were informed to decrease rapidly after death and further storage (Ando et al., 1995) causing quality deterioration. Firmness of raw muscle was found to be directly related to the content of collagen. In addition, it has been established that weakening of pericellular connective tissue collagen cause post-mortem softening of fish muscle (Sato et al., 1997). Extensive degradation of connective tissue in fish and loss of connective tissue structure was reviewed by Ando (1999). Collectively, these results indicated that most of the changes in fish texture were associated with connective tissue changes and attachments of fibers to connective tissue. Similarly in gaping, deterioration of fish muscle and disintegration of collagen fibrils were reported. Collagen is important in fish muscle firmness but there are limited reports on the effects of fish muscle collagen during frozen storage. There have been a few studies on degradation of collagen during freezing of squid (Loligo and Illex species). Additionally, collagen fibers and cell membranes were not studied earlier in squid muscle tissue. Therefore, effects of collagen are still unclear in softening of the squid (Loligo duvaucelli) muscle.

In fish it was observed that the fine collagen fibrils that arise from myocommata progressively deteriorate during frozen storage (Bremner, 1999). Ando (1999) demonstrated that post mortem tenderization of rainbow trout muscle was due to the disintegration of collagen fibers in the pericellular connective tissues. Ando et al. (1999) reported that with frozen storage the solubility of muscle collagen decreased, probably due to the cleavage of the triple helical region by a collagenase – like enzyme.

Histological observations of cooked prawns revealed the different connective tissue layer at relatively high temperatures (70 to 90°C) although endomysium showed partial disintegration (Nip and Moy, 1988). They also observed that prawn meat became firm and solid with further cooking and was unpalatable when the core temperature reached above 100°C. The heat processing with boiling water made prawn meat firmer. Nip and Moy (1979) reported damages to the tissue during freezing probably due to the formation of ice crystals between the fibers. Nip and Moy (1988) examined the various changes in the appearance of prawn meat during heat processing with macroscopic changes. For prawn meat processed at 90°C separation between meats was observed that could be due to the
excessive shrinkage of the muscle protein. Deformation value tended to increase during heat processing showing high values at 70°C and 90°C in contrast to raw meat.

A considerable amount of studies concerning the effects of temperature on tenderization of muscle food has been performed and reported by many scientists (Davey and Gilbert, 1976 and Bouton et al., 1975) of which most of the reports were related to red meat and mammalian muscle and only little work has been done on thermally induced changes on texture of fish and fishery products.

5.3. MATERIALS AND METHODS

5.3.1. Raw material collection and sample preparation

The samples of rohu (Labeo rohita), squid (Loligo duvaucelli) and shark (Scoliodon sorrokawah) were collected and prepared as detailed in 2.3.1 and 2.3.2 for the following analysis.

5.3.2. Histochemical studies

Reagents used:

- **Bouin’s fixative**

  Saturated picric acid solution: 75 ml

  Formalin (40%): 25 ml

  Glacial acetic acid: 5 ml

- **Weigert’s iron – haematoxylin stain**

  Solution A: A 1% solution of haematoxylin in absolute alcohol.

  Solution B: Distilled water – 100 ml

  Ferric chloride (30%) – 4 ml

  Conc. HCl – 1 ml

These are stock solutions. They were mixed at the time of use. The stain was prepared freshly every day. Before use, one part of Solution A was mixed with one part of Solution B and 2 parts of distilled water was added. It is differentiated very slowly by picric acid and is therefore ideal for using with Van - Geison’s staining method.
**Van Geison’s stain**

This consists of saturated solution of picric acid in water with 5 ml of a 1% aqueous solution of acid fuschin added to each 100 ml. It stains collagen fibers of connective tissue to bright red; and muscle fibers and epithelium to yellow.

The histochemical analysis was done according to Ando et al. (1999). 1 cm thick samples were fixed in Bouin’s fixative for a period of 24 h. The tissues being pliable were larger in size during first fixation and were cut into small pieces after ½ h of soaking. It was then then left in the Bouin’s solution for the remaining period. After fixation they were passed through a series of alcoholic dilutions (70%, 96% and absolute alcohol). The tissues were kept in 70% alcohol for a period of 24 h followed by serial transfer to 80% alcohol where the samples were kept for 30 min with two successive changes of the solution. Followed by this the samples were passed to 96% alcohol (30 min with two changes) and absolute alcohol for 20 min (with two changes). The dehydration was completed by a final dip in acetone for 1 min.

After complete dehydration, the samples were passed to xylene till they appeared transparent. The samples were treated with xylene and then passed to xylene – wax mixture (3:1 and 1:1) sparing 5 min at each step. This was for the infiltration of wax into the tissue cavities. After infiltration using xylene: wax mixture, the tissues were infiltrated further using pure wax with two changes for 10 min. The tissues were then embedded in paraffin wax with cresine (congealing point about 60°C) using L – blocks. The blocks were then sectioned to a thickness of 8 μm using microtome (SIPCON SP 1120 Rotary Microtome, India). The sections were fixed on clean dry slide using glue and dried before further staining.

The slides were dipped in xylene in a decreasing series of alcohol and were stained using Weigert’s – Haematoxylin stain for 5 min with an acid: alcohol wash for 30 sec. They were then passed to Van Geison’s stain for 5 min and dehydrated through a series of alcohol for 10 min followed by xylene for another 10 min. After staining the permanent slides were made using DPX mountant. The photographs of the sections were taken using camera attached light microscope (Nikon Eclipse E 200 compound light microscopes fitted with Nikon DN 100 Digital Net Camera).
5.4. RESULT

5.4.1. Freezing and cooking of the muscle tissue

Plates 5.1 to 5.18 show the photomicrographs of the samples. Though they are only a very small area of a section, they aid in portraying the post mortem histochemical changes in the tissue. In the muscle sections of fresh samples (plate 5.1, 5.7 and 5.13) the intrinsic conditions are good, the myofibril bundles are regular, closely packed and the cellular architecture is complete with only few extra cellular spaces. In the frozen stored samples (plate 5.3 to 5.6, 5.9 to 5.12 and 5.15 to 5.18) the sections showed large tissue voids probably due to the crystallization of tissue water and ice formation leading to cell structure disruption. This increases with extended period of frozen storage. There exists depletion in protein resulting in low water binding capacity causing the release of water into the surrounding tissue where it accumulates. The myofilaments are bathing in the sarcoplasmic fluid and gathered into myofibrils. In the cross – section these fibrils are of greatly varying sizes and shapes that varied with species. Between fibrils the sarcoplasmic reticulum forms inter – connected vesicles. Sarcolemma, the outer layer often disintegrates and plasmalemma split off from fiber. Fibers are separated by large spaces containing fragmentary and precipitated material. Sarcoplasmic reticulum that was swollen in fresh sample were disintegrated and transformed into smaller ones. Apart from contraction, the predominant change was the gradual increase in permeability of membraneous structures with frozen storage. Upon freezing the ammoniacal odor of the shark tissue was reduced comparatively making the tissue more preferable.

During cooking the collagenous tissue acquired a granular appearance and the fibers fell apart in rohu. Sometimes frayed pieces of collagen were found over the surface of these sections (plate 5.3 to 5.6 and 5.15 to 5.18). Waves and zig – zag pattern were seen in the sections of cooked meat at higher temperatures. These passive contractions could be due to the active contraction of adjoining fibers and the contraction of collagen. The tissue heated at 40° to 60°C (plate 5.1, 5.7 and 5.13) had a wide intercellular gap region with high frequency that might be due to thermal denaturation and shrinkage of intercellular protein and dehydration of muscle fibers. The thin connective tissue of endomysium and
perimysium in muscle tissue heated at 55 to 60°C were observed to be thin although there were separations from the muscle fibers. The tissue heated at 70 to 90°C (plate 5.2, 5.8 and 5.14) showed images of remarkably reduced muscle fibers that shrink and gather in the gap region that were frequent around the muscle fibers. This could be due to the rapid effect of temperature on tissue during cooking at high temperatures. Structure of endomysium in the three species became quite unclear in the tissues cooked at 70 to 90°C although the perimysium was still evident. Structure of epimysium was also maintained. Shark has high collagen content compared to rohu and squid. On cooking collagen was converted to gelatin. At 50°C, since the connective tissue was distorted, the tissue became tender. At 70°C shark muscle tissue showed appealing texture probably due to the gelatin from collagen that held the myotomes together probably retaining the nutritional quality. Unlike rohu where tissue structure was completely distorted at 90°C, shark gelatin was able to hold together the muscle cells.

5.4.2. Musculature pattern

In rohu and shark the connective tissue consisting of collagen formed myocommata sheath that enveloped each myotome bundle and help in holding the myotome bundles together. The musculature pattern of the squid mantle tissue differed from that of rohu and shark as they are composed of several layers of fibers running transversally to each other and are covered with several sheets of connective tissue (plate 5.7). Muscle fiber layer of the mantle made up to about 98% of the mantle thickness. They were observed to consist of 0.1 – 0.2 mm thick bands with fibers running circumferentially sandwiched between thinner sheets of radial fibers. Four sheets of connective tissue cover the muscle fibers: the inner tunic and the visceral lining on the inner side of the body cavity and the outer tunic and outer lining beneath the skin. Just under the skin proper there were pigment cells containing dark red and brown melanin pigment cells, together with the skin.

5.5. DISCUSSION

Connective tissue of the fibrous structures primarily, consisting of collagen, could be categorized into epimysium, perimysium and endomysium as in rohu and shark that
differed from each other in being morphologically discrete collagen depots. Epimysium is the heavy sheath of connective tissue surrounding individual muscle tissue that thickens at both origin and insertion. Endomysium encircles individual muscle fiber and were found to superimpose the basement membrane, perimysium is the thin layer enveloping muscle bundles and muscle fibers (myofibers) and endomysium is the fine layer encasing the muscle fibers. Epimysium is normally removed while de – skinning and only the perimysium and endomysium connective tissues are suggested to be involved in the realistic toughness of meat tissue. Perimysium and epimysium comprise the intracellular connective tissue and were not separable in rohu and shark. The bulk (90%) of intracellular connective tissue consisted of perimysium and was probably the main contributor to meat texture (Light et al., 1985). In squid, perimysium was not clearly distinguishable. The connective tissues of the squid tunics had high collagen content (Ottwell and Hamann, 1979). With its complex muscle orientation and high collagen content raw squid was relatively tough and possessed rubbery texture. The muscle patterns of squid (Loligo duvaucelli) are comparable with the results of Llorea et al. (2001).

Freezing could alter the quality of frozen samples in comparison to the unfrozen fresh products. Physical and chemical changes in proteins that occur during frozen storage result in texture deterioration that negatively affect the functional, nutritional and sensory properties of the product. Studies showed that fiber separation occurred with increased period of frozen storage. The sample texture changed more extensively resulting from partial denaturation of proteins. Myosin possibly unfolds in frozen samples resulting in the exposure of hydrophobic groups that then cause protein aggregation resulting new cross – linkages during frozen storage. This kind of protein – protein interactions led to decreased water holding capacity and increased expressible moisture that is reported in chapter 2. The variations are in agreement with the findings of Xiong and Mikel (2001). Enzymatic activities can partially unfold the myosin in frozen – thawed samples as observed in rohu and shark (Shenouda, 1980). These interactions during frozen storage cause an increase in extracellular volume with fibers moving apart. On freezing, the ammonia – like components of urca in shark tissue were lost either due to leaching or through evaporation, rendering the shark muscle tissue more preferable. The voids formed in the
shark muscle tissue during freezing were less possibly due to the less spacing of myocommata during ice crystal formation.

Cooking serves dual function of tenderizing the muscle by converting collagen to gelatin, and by toughening due to heat coagulation of the myofibrillar proteins. Hence, the muscle characteristics obviously depend on the relative effects of these two structural components. Diameters of collagen fibrils were smaller in the fishes than in the squid and been focused in as a protein that greatly contribute to meat texture (Olacchea et al., 1993). With frozen storage a partial disintegration of endomysium and perimysium was observed in the muscle, while the structure of the thick connective tissue epimysium was not much changed. On the other hand, a preferential decrease in the relative staining intensity of the components was detected for collagen from the muscle with further cooking which may be due to the conversion of collagen to gelatin. The disintegration of thin connective tissue could be due to some biochemical changes in the components (Ando et al., 1999). It was generally believed that collagen shrinks (toughening effect) at a temperature around 63°C and may be partially transformed to gelatin at higher temperatures that was almost similar to denaturation temperature (60°C) for squid as observed in the present study (Hamm, 1966). The denaturation and the shrinkage temperature for rohu (50°C) and shark (50°C) were further lower. With increasing the temperature from 50°C to 60°C the texture profile parameters decreased in samples than in fresh that was discussed in chapter 4. It could be due to additional factors involved in the cooking effect at these temperatures than just changes in connective tissue alone (Bouton et al., 1975). Shark has high collagen content that was converted to gelatin upon cooking (70°C) and held the muscle cells. It also helped in retaining the nutritional quality of the tissue. Even at 90°C the tissue structure was comparatively maintained than that of rohu. Epimysium was maintained in the tissue, though perimysium and endomysium showed slight denaturation with increased temperature of cooking.

Davey and Gilbert (1976) reported that certain proteolytic enzyme cathepsin C that is relatively heat stable could also support tenderization effect at 60°C. Rohu and shark cooked at 45°C and above 70°C had higher gel strength than at 60°C possibly due to muscle alkaline protease that was found in the fish flesh (Makinodan et al., 1963). The extent of protein – protein interaction in rohu actomyosin probably increased with increasing
temperature. The rate increases sharply with temperature increase from room temperature to 45°C. When heated at various temperatures ranging from 55°C to 80°C, the toughening increased initially and then decreased resulting in softening of the tissue. For meat processed at 90°C separation between meats was observed that could be due to the excessive shrinkage of the muscle protein. Deformation value tended to increase during heat processing showing high values at 70°C and 90°C in contrast to raw meat. At higher temperature, no tenderizing effect was observed in rohu and shark. This might be because of the loss of enzyme activity and only protein denaturation was having significant effect on texture activity (Deng, 1981). Upon cooking the losses of water from the cut ends of the meat were high. This was accompanied with shrinkage of meat in two phases. In squid at 45 to 60°C the shrinkage was primarily transverse to the fiber axis and at higher temperatures, 60 to 90°C the shrinkage were parallel to the fiber axis. This could possibly explain the toughening of the squid meat when compared to rohu and shark muscle tissue.

5.6. CONCLUSION

The histochemical studies show that the musculature pattern of the squid mantle tissue differed from that of rohu and shark as they are composed of several layers of fibers running transversally to each other. These layers were covered with several sheets of connective tissue. The differences in the stain intensity confirm the decomposition of collagen to gelatin with increase in temperature of cooking thus decreasing the integrity between the muscle cells. Even though the intercellular spaces were narrow and collagen fibrils were scarcely visible in raw tissues they played a significant role in affecting the texture during frozen storage and cooking. The muscle pattern became clearer in frozen stored samples in comparison to raw samples of all the species possibly due to formation of intercellular spaces. Cell detachment occurred during cooking as a result of the decrease in the intercellular integrity due to loosening of collagen fibers probably resulting in the decrease of the binding force between cell membrane and collagen. The voids observed in the raw muscle tissue of squid and shark was less compared to cooked samples and those after frozen storage. The histochemical observations were confirmed the results of texture profile analysis discussed in the previous chapter. The firmness of cooked muscle was
weak but constant in squid and shark and could be explained by considering the occurrence of gelatinization of intercellular collagen. The solubility differences could be due to the differences in the intermolecular cross-links of collagen in the three species studied. The specific influences of connective tissues depend on the thickness, amount of collagen, their density and type of cross-links between fibrils. Specific patterns of collagen distribution and its localization in squid mantle have a profound role in textural changes during heat treatments require further studies in this respect.
Plate 5.1 Histograms of fresh samples of rohu, *Labeo rohita* cooked at different temperatures.
(a) fresh, (b) 45 °C, (c) 50 °C, (d) 55 °C, (e) 60 °C and (f) 65 °C.
(A)- Collagen, (B)- Myofibrillar protein.
Plate 5.2 Histographs of fresh samples of rohu, *Labeo rohita* cooked at different temperatures. (g) 70 °C, (h) 75 °C, (i) 80 °C, and (j) 90 °C. (A)- Collagen, (B)- Myofibrillar protein.
Plate 5.3 Histograms of frozen samples (three months) of rohu, *Labeo rohita* cooked at different temperatures. 
(a) frozen(three months), (b) 45 °C, (c) 50 °C, (d) 55 °C, (e) 60 °C and (f) 65 °C.

(A)- Collagen, (B)- Myofibrillar protein.
Plate 5.4 Histographs of frozen samples (three months) of rohu, *Labeo rohita* cooked at different temperatures.

(g) 70 °C, (h) 75 °C, (i) 80 °C, and (j) 90 °C.

(A)- Collagen, (B)- Myofibrillar protein.
Plate 5.5 Histographs of frozen samples (six months) of rohu, *Labeo rohita* cooked at different temperatures.

(a) frozen (six months), (b) 45 °C, (c) 50 °C, (d) 55 °C, (e) 60 °C and (f) 65 °C.

(A) - Collagen, (B) - Myofibrillar protein.
Plate 5.6 Histograms of frozen samples (six months) of rohu, *Labeo rohita* cooked at different temperatures. 
(g) 70 °C, (h) 75 °C, (i) 80 °C, and (j) 90 °C. 
(A)- Collagen, (B)- Myofibrillar protein.
Plate 5.7 Histograms of fresh samples of squid, *Loligo duvaucelli* cooked at different temperatures.
(a) fresh, (b) 45 °C, (c) 50 °C, (d) 55 °C, (e) 60 °C and (f) 65 °C.
(A)- Collagen, (B)- Myofibrillar protein.
Plate 5.8 Histograms of fresh samples of squid, *Loligo duvaucelli* cooked at different temperatures. (g) 70 °C, (h) 75 °C, (i) 80 °C, and (j) 90 °C. (A)- Collagen, (B)- Myofibrillar protein.
Plate 5.9 Histograms of frozen samples (three months) of squid, *Loligo duvaucelli* cooked at different temperatures. (a) frozen(three months), (b) 45 °C, (c) 50 °C, (d) 55 °C, (e) 60 °C and (f) 65 °C.

(A)- Collagen, (B)- Myofibrillar protein.
Plate 5.10 Histograms of frozen samples (three months) of squid, *Loligo duvaucelli* cooked at different temperatures. (g) 70 °C, (h) 75 °C, (i) 80 °C, and (j) 90 °C.

(A)- Collagen, (B)- Myofibrillar protein.
Plate 5.11 Histograms of frozen samples (six months) of squid, *Loligo duvaucelli* cooked at different temperatures. 

(a) frozen(six months), (b) 45 °C, (c) 50 °C, (d) 55 °C, (e) 60 °C and (f) 65 °C.

(A)- Collagen, (B)- Myofibrillar protein.
Plate 5.12 Histograms of frozen samples (six months) of squid, *Loligo duvaucelli* cooked at different temperatures. (g) 70 °C, (h) 75 °C, (i) 80 °C, and (j) 90 °C. (A)- Collagen, (B)- Myofibrillar protein.
Plate 5.13 Histograms of fresh samples of shark, *Scoliodon sorokawah* cooked at different temperatures. 

(a) *fresh*, (b) 45 °C, (c) 50 °C, (d) 55 °C, (e) 60 °C and (f) 65 °C.

(A)- Collagen, (B)- Myofibrillar protein.
Plate 5.14 Histographs of fresh samples of shark, *Scoliodon sorrokwah* cooked at different temperatures.

(g) 70 °C, (h) 75 °C, (i) 80 °C, and (j) 90 °C.

(A)- Collagen, (B)- Myofibrillar protein.
Plate 5.15 Histograms of frozen samples (three months) of shark, *Scoliodon sorrokawah* cooked at different temperatures. (a) frozen(three months), (b) 45 °C, (c) 50 °C, (d) 55 °C, (e) 60 °C and (f) 65 °C.

(A)- Collagen, (B)- Myofibrillar protein.
Plate 5.16 Histograms of frozen samples (three months) of shark, *Scoliodon sorrokawah* cooked at different temperatures.

(g) 70 °C, (h) 75 °C, (i) 80 °C, and (j) 90 °C.

(A) Collagen, (B) Myofibrillar protein.
Plate 5.17 Histograms of frozen samples (six months) of shark, *Scoliodon sorrokawah* cooked at different temperatures. (a) frozen (six months), (b) 45 °C, (c) 50 °C, (d) 55 °C, (e) 60 °C and (f) 65 °C.

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Plate 5.18 Histograms of frozen samples (six months) of shark, *Scoliodon sorrokkawah* cooked at different temperatures. (g) 70 °C, (h) 75 °C, (i) 80 °C, and (j) 90 °C.

(A)- Collagen, (B)- Myofibrillar protein.
perimysium in muscle tissue heated at 55 to 60°C were observed to be thin although there were separations from the muscle fibers. The tissue heated at 70 to 90°C (plate 5.2, 5.8 and 5.14) showed images of remarkably reduced muscle fibers that shrink and gather in the gap region that were frequent around the muscle fibers. This could be due to the rapid effect of temperature on tissue during cooking at high temperatures. Structure of endomysium in the three species became quite unclear in the tissues cooked at 70 to 90°C although the perimysium was still evident. Structure of epimysium was also maintained. Shark has high collagen content compared to rohu and squid. On cooking collagen was converted to gelatin. At 50°C, since the connective tissue was distorted, the tissue became tender. At 70°C shark muscle tissue showed appealing texture probably due to the gelatin from collagen that held the myotomes together probably retaining the nutritional quality. Unlike rohu where tissue structure was completely distorted at 90°C, shark gelatin was able to hold together the muscle cells.

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temperature. The rate increases sharply with temperature increase from room temperature to 45°C. When heated at various temperatures ranging from 55°C to 80°C, the toughening increased initially and then decreased resulting in softening of the tissue. For meat processed at 90°C separation between meats was observed that could be due to the excessive shrinkage of the muscle protein. Deformation value tended to increase during heat processing showing high values at 70°C and 90°C in contrast to raw meat. At higher temperature, no tenderizing effect was observed in rohu and shark. This might be because of the loss of enzyme activity and only protein denaturation was having significant effect on texture activity (Deng, 1981). Upon cooking the losses of water from the cut ends of the meat were high. This was accompanied with shrinkage of meat in two phases. In squid at 45 to 60°C the shrinkage was primarily transverse to the fiber axis and at higher temperatures, 60 to 90°C the shrinkage were parallel to the fiber axis. This could possibly explain the toughening of the squid meat when compared to rohu and shark muscle tissue.

5.6. CONCLUSION

The histochemical studies show that the musculature pattern of the squid mantle tissue differed from that of rohu and shark as they are composed of several layers of fibers running transversally to each other. These layers were covered with several sheets of connective tissue. The differences in the stain intensity confirm the decomposition of collagen to gelatin with increase in temperature of cooking thus decreasing the integrity between the muscle cells. Even though the intercellular spaces were narrow and collagen fibrils were scarcely visible in raw tissues they played a significant role in affecting the texture during frozen storage and cooking. The muscle pattern became clearer in frozen stored samples in comparison to raw samples of all the species possibly due to formation of intercellular spaces. Cell detachment occurred during cooking as a result of the decrease in the intercellular integrity due to loosening of collagen fibers probably resulting in the decrease of the binding force between cell membrane and collagen. The voids observed in the raw muscle tissue of squid and shark was less compared to cooked samples and those after frozen storage. The histochemical observations were confirmed the results of texture profile analysis discussed in the previous chapter. The firmness of cooked muscle was
weak but constant in squid and shark and could be explained by considering the occurrence of gelatinization of intercellular collagen. The solubility differences could be due to the differences in the intermolecular cross – links of collagen in the three species studied. The specific influences of connective tissues depend on the thickness, amount of collagen, their density and type of cross – linkages between fibrils. Specific patterns of collagen distribution and its localization in squid mantle have a profound role in textural changes during heat treatments require further studies in this respect.