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## LIQUID HOLDING CAPACITY AND STRUCTURAL CHANGES DURING HEATING OF FISH MUSCLE: COD (*Gadus morhua* L.) AND SALMON (*Salmo salar*)

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### Abstract

The loss of water and fat in cod and salmon muscle was studied as a function of heating temperature from 5-70°C. The liquid-holding capacity was measured by a low speed centrifugation net test leading to the separation of released liquid. To obtain a better understanding of the liquid-holding properties, the microscopic changes of the samples were evaluated by light microscopy. Two different preparation techniques were used. Cod lost twice as much water as salmon upon heating. After an initial delay, the water loss increased at 20-35°C, attained a maximum at 45-50°C, and thereafter decreased in both fish species. Salmon muscle was more heat-stable than cod muscle. Since the main structural changes appeared in the connective tissue at low temperatures (5-40°C), the water loss at these temperatures is probably mainly due to denaturation and melting of collagen. The maximum water loss was attained when the muscle cell shrank due to denaturation of myosin. The reduced water loss at higher temperatures (50-70°C) is probably caused by aggregates of sarcoplasmic proteins stabilizing the aqueous phase.

**Key Words:** Cod, salmon, fish muscle, heat-induced changes, liquid loss, microstructure, light microscopy.

### Introduction

Fish is usually consumed after heating and should preferably not only be tender but also juicy. Juiciness is related to water retention. Little fundamental work has been carried out with regard to the mechanism of liquid release from fish muscle upon heating. It has been reported, as reviewed by Aitken and Connell (1979), that the cooking losses vary greatly with the fish species and the cooking method. Aman (1983) found that the water-holding capacity of cooked mullet was related to solubility changes of the myofibrillar and sarcoplasmic proteins. Lipid and water together make up about 80% of the fish muscle (Suzuki, 1981). The free water in muscle, about 90% of the total water, is held by capillary and surface tension forces mainly in intracellular locations. The water-holding capacity of muscle is highly influenced by structural changes in the proteins: fibril swelling-contraction and the distribution of fluid between intra- and extracellular locations (Offer and Trinick, 1983; Schnepf, 1989). Thus, a proper understanding of the water-holding characteristics cannot be obtained without a knowledge of the microstructure of the material (Hermansson, 1986).

The flesh of teleost fish is constructed of adjacent muscle blocks, called myotomes, separated from each other by sheets of collagenous tissue called myocommata. Within each myotome, the muscle fibers run approximately parallel to each other, linked at each end to the myocommata. In fish, this junction is formed by fine collagenous processors which have their origin in myocommata and which then proceed as sheaths to surround each muscle fiber (Love, 1988). The ultrastructure of the myofibers was found to be similar to that of mammalian meat (Schaller and Powrie, 1972; Howgate, 1979; Bello *et al.*, 1981; Lampila and Brown, 1986; Shindo *et al.*, 1986). The cell envelopes and the junctions between the muscle fibers and connective tissue have been studied by scanning- and transmission electron microscopy (Almás, 1982; Bremner and Hallett, 1985). The cell envelope is composed of three distinct layers. Innermost is the sarcolemma, which forms a continuous boundary to the cell. External to this is the amorphous

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basement membrane. Outermost is a fibrous layer approximately 600-1100 nm thick, which consists of fine collagenous (reticular) fibrils, i.e., the endomysium. The endomysium runs into the surrounding perimysium, which in fish, as mentioned above, emerges from the myocommata. In this paper, no distinction is made between the endomysial and perimysial layer but they are referred to together as the pericellular layer.

When mammalian meat is heated, the fibers shrink in the transverse direction at 40-60°C (Offer *et al.*, 1989). This widens the gap between the fibers and their surrounding endomysium and increases the amount of water released from the muscle. In meat, the collagen network shrinks at 60-70°C due to denaturation. This shrinkage will greatly increase the pressure on the aqueous solution, causing it to be expelled more rapidly (Hamm, 1985; Wilding *et al.*, 1986). However, fish collagen (3% of total muscle protein), which contains less proline and hydroxyproline than mammalian collagen, is less thermally stable and more easily soluble than mammalian collagen. Thus, in fish muscle, the temperature at which thermal shrinkage and denaturation of collagen occur is generally lower than in mammalian muscle (Sikorski *et al.*, 1984). To our knowledge, very little attention has been paid so far to the role of collagen in determining the water-binding properties and cooking loss of fish.

Relatively few reports have dealt with heat-induced structural changes in fish muscle. Charley and Goertz (as reviewed by Howgate, 1979) reported that light microscopy revealed some disintegration of fibers in salmon pieces heated to 70°C. In addition, fibrous connective tissue became granulated and jelly-like whilst a considerable dispersion of fat globules occurred within the tissue. Schaller and Powrie (1972) found, by scanning electron microscopy, that the visible damage of the myofibers were more apparent in heat-treated trout compared to beef and chicken. Corresponding observations were found in thermally processed tuna muscle by Lampila and Brown (1986). Sarcoplasmic proteins, and probably collagenous material and other constituents were present in the interstices between the shrunken muscle fibers and the endomysium when the tuna muscle was heated to 60°C. Hatae *et al.* (1984, 1990) and Kanoh *et al.* (1988) compared heat-induced textural and optical microstructural changes of muscle fibers from five different fish species and dark and white muscle from yellowfin tuna, respectively. Their results indicated that drip loss and textural differences between fish species and dark and white muscle when heated are due to the different amount of sarcoplasmic proteins coagulated in the interstitial spaces and/or differences in the fiber diameter. In these previous works, the water-holding capacity and morphological properties of fish muscle have been meas-

ured either on raw fish or on cooked fish. No studies have so far concentrated on the structural changes within the muscle during heating.

Both cod and salmon are highly valued as food. The present work was undertaken to elucidate the relationship between microstructure and the liquid-holding properties of cod and salmon muscle. Owing to the low thermal stability of fish muscle proteins, emphasis has been laid on liquid-holding properties as a function of temperatures from 5 to 70°C. The microscopic changes of the samples were evaluated by light microscopy. To assess these changes, both cryo-sections and plastic sections of the samples were performed. To our knowledge, micrographs of fish muscle prepared with these techniques have not been published before.

## Materials and Methods

### Materials

Farmed cod and salmon were slaughtered and kept in ice for 2 days, until the resolution of *rigor mortis* and the muscle had softened, and then filleted. Each specimen was collected from the same sea netpen to minimize differences in feeding conditions. The fish used, 18 salmon and 30 cod, were of almost the same size and chemical composition. The average length of the cod was  $0.72 \pm 0.05$  m and that of the salmon  $0.61 \pm 0.06$  m, whereas the average weight of the headed and gutted cod was 2660  $\pm$  470 g and that of the salmon 2460  $\pm$  660 g. Cod white muscle meat contained on average 81  $\pm$  0.5% water, 18.4  $\pm$  0.6% protein and had a pH of 6.22  $\pm$  0.04. Salmon contained 69.8  $\pm$  1.3% water, 19.3  $\pm$  0.2% protein, 10.5  $\pm$  1.5% fat, and had a pH of 6.41  $\pm$  0.05. These relatively low pH values are due to high concentrations of glycogen in the farmed fish muscles at the time of slaughter (Rustad, 1992). The proteins were determined by the Kjeldahl-method, and fat by the Soxhlet-method.

### Liquid-holding capacity

Fillets from 5 specimens of cod and from 3 specimens of salmon were coarsely chopped in a precooled Stephan mixer (12) for 3 x 5 seconds. The temperature of the minces after chopping were 4-5°C. The liquid loss, measured as the percent of weight released per 15 gram of sample, was determined according to the net test described by Hermansson (1986). The samples were either directly centrifuged at 5°C or heated, in a metal tube with a rubber stopper at each end, at a rate of 1°C/min to the required temperature, held at that temperature for 10 minutes and then cooled to 10°C by keeping the metal tubes under running tap water for 30 minutes. The samples were transferred to centrifugation tubes and thereafter centrifuged at 10°C. The samples were heated to 20, 30, 35, 40, 45, 50, 55, 60 and 70°C.

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The temperature in the core of the sample was monitored during heating, by a temperature sensor brought through a whole in the middle of the upper rubber stopper of the metal tube. According to the net test, the fat and water loss was determined after mild centrifugation ( $210 \times g$  for 15 minutes). Mean values were calculated from 4 trials of 6 replicates at each temperature.

### Preparation of samples for microscopy

Both whole muscle and coarsely chopped muscle preparations were investigated. Blocks of whole muscle of approximately 15 g were taken from the fillets at the level of the dorsal fin and heated, as described above, parallel with 15 g of the coarsely chopped muscle. Samples were processed after 30 minutes of cooling to  $10^{\circ}\text{C}$ , except the  $5^{\circ}\text{C}$ -samples, which were either removed directly from the fillet or taken immediately after chopping. To assess the microscopic changes during heating, two different preparation techniques for light microscopy were performed. Both techniques were used for all samples, and the few micrographs presented here have been chosen out of many. The prepared samples were examined with a Nikon Macrophot Fx microscope.

**Cryo-sections.** Whole muscle blocks and blocks of chopped muscle ( $0.5 \text{ cm}^3$ ) were frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  prior to sectioning. The samples were sectioned frozen at  $-22^{\circ}\text{C}$ . A Leitz cryostat was used, and  $10 \mu\text{m}$  thick sections were cut, fixed in Bouin's fluid and thereafter stained with aniline blue and Orange G (Hermansson and Jordansson, personal communication).

**Plastic-sections.** Fixation was carried out in 2.5%/1.0% glutaraldehyde/formaldehyde with 0.2% glucose in Ringer's buffer (pH 6.8), dehydrated with ethanol and embedded in Historesin (LKB) as recommended by the manufacturer. The sections were cut  $1.5 \mu\text{m}$  thick in a Reichert-Jung Ultracut E using glass knives. The sections were transferred onto glass slides and stained with a modified Polychrome-method (Hermansson and Kidman, personal communication).

## Results and Discussion

### Liquid-holding capacity

Numerous factors, both intrinsic and extrinsic, will influence the liquid-holding capacity (LHC) of the muscle tissue. To collect data on LHC sample characteristics, handling procedures and methodology need to be accurate and reproducible. Not only do the chemical composition and pH of the fish muscle influencing the LHC vary from individual to individual, but the chemical composition differs within the head and tail regions of the fillet (Love, 1988). To avoid these differences, the liquid-holding capacity was measured in coarsely

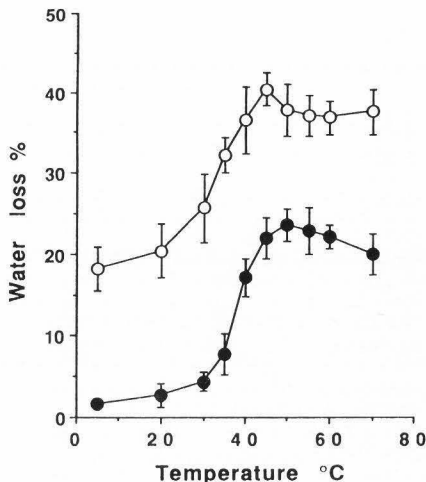


Figure 1. Water loss (% by weight) as a function of heating temperature of coarsely chopped salmon (solid circles) muscle and cod muscle (hollow circles) according to the net test. The bars give  $\pm$  standard deviation.

chopped fillets from several individuals in each trial. Figure 1 shows the water loss measured by the net test for cod and salmon as a function of heating temperature. The water loss was almost constant for both fish species between 5 and  $30^{\circ}\text{C}$ . After the initial delay, the water loss increased rapidly as a function of temperature, reaching a maximum at  $45$ - $50^{\circ}\text{C}$ , thereafter the water loss of both fish species seemed to decrease. Aitken and Connell (1979) reported that fish steamed for up to 45 minutes lost between 20 and 30% liquid, and that the loss from cod appeared to begin at around  $32^{\circ}\text{C}$  after an initial delay.

Generally, the liquid loss is more than twice as high for the chopped cod muscle than for the chopped salmon muscle regardless of the treatment. The protein content of the salmon muscle was slightly higher than that of the cod muscle, but correction for the water loss due to the protein content does not essentially affect the above result. The main water loss for cod started at about  $30^{\circ}\text{C}$  and for salmon at about  $35^{\circ}\text{C}$ . The maximum water loss was observed at a lower temperature ( $45^{\circ}\text{C}$ ) for cod muscle than for salmon muscle ( $50^{\circ}\text{C}$ ). These results may indicate that, under the experimental conditions used, the muscle proteins in salmon are more stable than in cod. According to Hermansson (1986) and Honikel (1989), the pH of the muscle will highly influence its

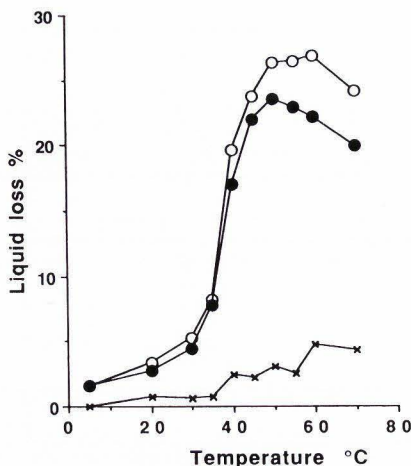


Figure 2. Liquid loss (% by weight) as a function of heating temperature of coarsely chopped salmon muscle. Total liquid loss (hollow circles), water loss (solid circles), fat loss (crosses).

water-holding capacity. A pH closer to the iso-electric point of the myofibrillar protein will increase the protein-protein attraction due to less negative charges of the amino acids of these proteins. The water-holding capacity is consequently reduced. The average pH of cod and salmon was 6.22 and 6.41, respectively. Some of the observed differences between cod and salmon muscle in the ability to hold water may therefore be explained by the different pH values of the samples.

The total liquid loss of salmon was separated into a water fraction and a fat fraction which were weighed. Figure 2 shows the total liquid loss, water and fat loss as a function of temperature. The main liquid loss is water. Easily separable fat in the liquid fraction was first observed at 40°C. At 60°C, about 40% of the total fat content in the muscle was lost.

#### Heat-induced structural changes

Heat-induced structural changes in muscle from cod and salmon, related to the liquid losses, have been studied as a function of temperature. Samples were taken at various stages of the heat treatment from 5 to 70°C. Coarsely chopped muscles were solidified in parallel by freezing or by plastic embedding. Structural changes observed with the two techniques were compared. To find out if the structural changes observed in coarsely

#### Figure Legends for Color Plates

Figure 3 (on page 167). Cryo-sections of coarsely chopped cod muscle at 5°C (a) and heated to 30°C (b), 40°C (c), 45°C (d), 50°C (e), 55°C (f), 60°C (g) and 70°C (h). The muscle proteins are yellow and collagen is blue.

Figure 4 (on page 168). Cryo- and plastic sections of coarsely chopped salmon muscle. Cryo-sections of unheated muscle, 5°C (a) and heated to 40°C (b), 50°C (c) and 60°C (d). The muscle proteins are yellow and collagen is blue. Plastic sections of unheated muscle, 5°C (e) and heated to 40°C (f), 50°C (g) and 60°C (h). The muscle proteins are bluish-red and collagen is blue. The arrowheads in Figs.4(e), (f) and (g) point to positions of emulsified fat.

Figure 5 (on page 169). Plastic sections of whole muscle of cod at 5°C (a, e) and heat-treated to 40°C (b), 45°C (c), 60°C (d) and 70°C (f, g). Differential interference contrast optics is used in Fig. 5g. The muscle protein is bluish-red and collagen is blue. The arrowheads in Fig. 5b point to positions of gaps in the pericellular layer, in Fig. 5d point to detachment of the peripheral myofibers from the sarcolemma, and in Figs. 5f and 5g point to ruptures in the cellular membrane.

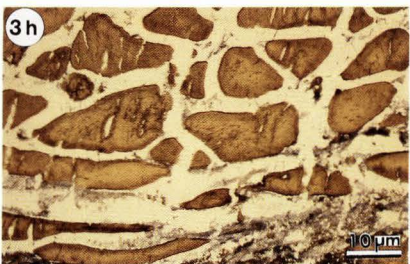
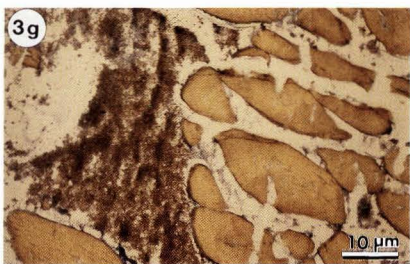
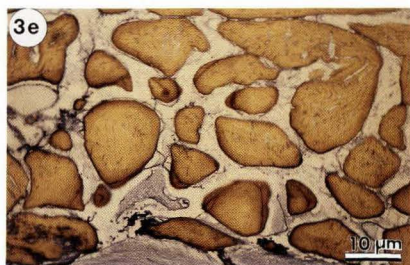
chopped model-systems were representative of the changes in intact muscle, whole muscles were embedded in plastic and examined. The micrographs presented were chosen out of many and illustrate the most typical heat-induced changes. Cod and salmon muscle underwent almost the same structural changes. Therefore micrographs of one of the two species were chosen.

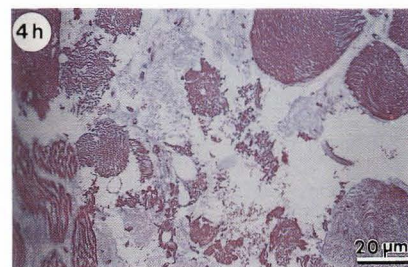
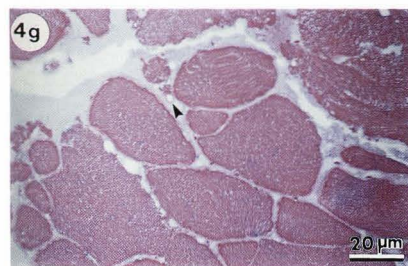
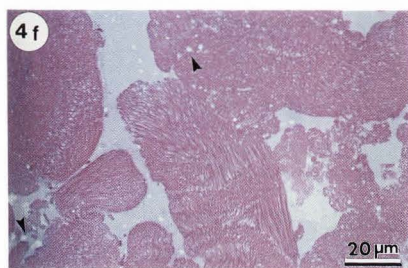
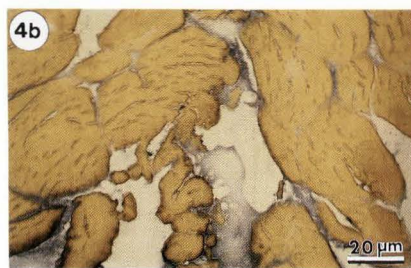
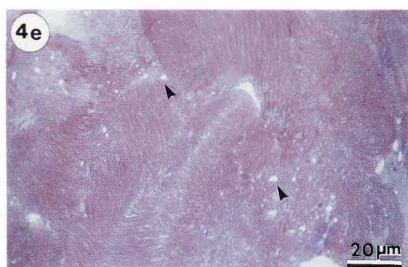
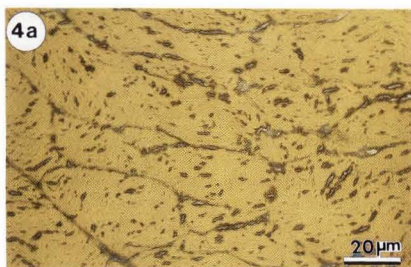
#### Structural changes of coarsely chopped muscles.

Structural changes as a function of temperature in cryo-sections of coarsely chopped cod and salmon muscle are shown in Figures 3a-h and 4a-d, respectively. In the aniline/orange G method, muscle fibers are stained yellow and collagen is stained blue. All the micrographs show both relatively intact muscle cells and cells disintegrated by chopping. Pieces of myocommata (dark blue) can be seen in Figures 3a, b, e and h.

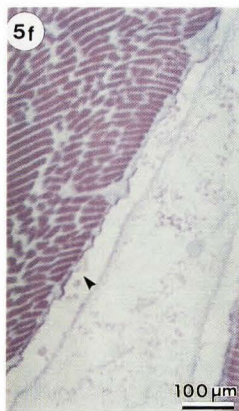
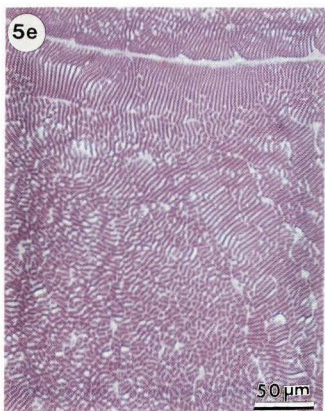
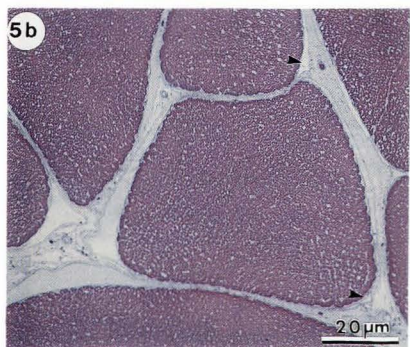
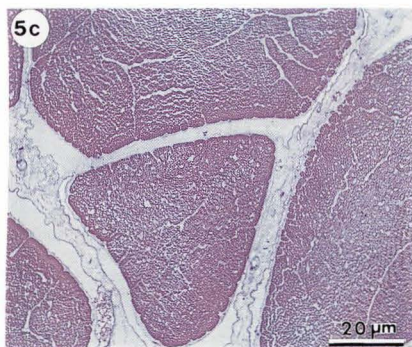
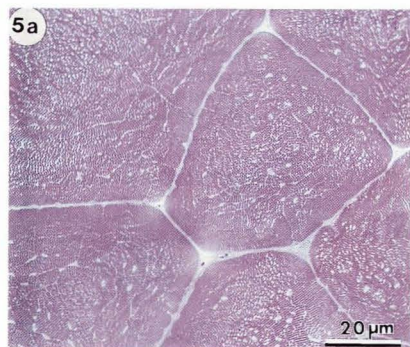
At 5°C cross-sectioned, intact muscle cells are surrounded by thin blue, collagenous sheets (Figs. 3a and 4a). However, gaps can be seen between the cells. This may be due to post-rigor spoilage, damage caused by chopping, or growth of ice crystals during freezing. In addition, swollen or melted collagen can be seen as a light blue film between the cod muscle cells in Figure 3a. The pericellular layers have begun to swell out and the intercellular collagenous film has aggregated when heated to 30°C (Fig. 3b). The water loss of cod has increased correspondingly, after an initial delay (Fig. 1).

Heat-Induced Liquid Loss and Structural Changes of Fish Muscle





Heat-Induced Liquid Loss and Structural Changes of Fish Muscle





Schaller and Powrie (1972) observed that the connective tissue between fibers from chicken muscle first underwent swelling before shrinkage occurred. At 40°C the water loss was considerable for both fish species. At this temperature, the collagenous layers have completely lost their linear appearance (Figs. 3c and 4b). The extracellular spaces have increased and breakage of the pericellular layers concomitant with shrinkage of the myofibrils has occurred at increased temperatures (Figs. 3d-h and 4c-d). Severe shrinkage, transverse to the fiber axis occurred at 45°C for cod (Fig. 3d) and at 50°C for salmon (Fig. 4c). Maximum water loss was obtained at these temperatures. Intracellular gaps appeared at 50°C (Figs. 3e-h, 4c, d). This is in accordance with the observations made from scanning electron microscopy (Schaller and Powrie, 1972; Lampila and Brown, 1986). Both transverse fractures of the myofibrils at the Z-disc and gaps at the level of the H-zone occurred in heat-treated trout muscle. The sarcomeres were much shorter than in unheated samples, and large spaces developed between each structural unit during thermal processing of tuna muscle.

The water loss increased as function of temperature and reached a maximum at 45 and 50°C for cod and salmon respectively. Above this temperature the water loss decreased. The average amount of solubilized proteins in the released liquid decreased at the same time; from 8.7% at 45°C to 4.2% at 70°C in the cod samples and from 9.3% at 50°C to 6.3% at 70°C in the salmon samples. Simultaneously, granulated material in the intercellular space of the muscle fibers became visible (Figs. 3e-h, 4c-d). The color of the granulated material is blue-yellowish or brownish, as expected if it is a mixed phase of collagenous (gelatin) and sarcoplasmic proteins, as previously proposed (Schaller and Powrie, 1972; Lampila and Brown, 1986; Hatae *et al.*, 1990). With specific fat staining (not shown) it was possible to see that a considerable amount of fat globules was dispersed within the intercellular gelatinized material of the salmon samples, as described by Howgate (1979).

The typical thread-like collagenous structure is, however, clearly evident in the piece of connective tissue, probably myocommata, shown in Figure 3e, whereas at 70°C (Fig. 3h), the myocommatal fraction has gelatinized. This indicates that the myocommatal fraction is more heat-resistant than the pericellular fraction. Differences in types of collagen within pericellular and myocommatal connective tissue may result in slightly different properties and stabilities of the collagen (Sato *et al.*, 1989). Histological and electrophoretic studies have previously shown that pericellular connective tissues and the fine network which connects myomers to myocommata were degraded and thin collagen fibrils in these tissues disappeared during chilled storage. On the other hand, myocommata and thick collagen fibers were still evident (Bremner and Hallet, 1985, 1986; Hallet and

Bremner, 1988; Ando *et al.* 1991; Sato *et al.*, 1991).

**Comparison of preparation techniques.** Cryosections and plastic sections were made in parallel to rule out possible artifacts obtained by preparation. The advantage of cryosectioning is that it is relatively easy to perform and well suited for a large number of samples. The disadvantage is that the microstructure may be damaged due to growth of ice crystals during freezing. To avoid this, coarsely chopped muscle blocks of both salmon and cod were chemically fixed, dehydrated and embedded in historesin. This technique gave the best resolution for light microscopy, and no freeze damage or mechanical redistribution was induced in the sample. The chemical fixation and dehydration used in this technique may cause shrinkage or affect the macromolecular phase, but such effects could not be seen from the resolution given by light microscopy.

Cryo-sections and plastic sections of coarsely chopped salmon muscle are compared in Figures 4a-h. The myofibers are stained bluish-red, while the collagen tissue is stained blue in the plastic embedded samples. The sarcoplasmic proteins, visible at 60°C (Fig. 4h) within the collagenous matrix, are stained red. Similar structural changes due to temperature are observed for the two different preparation techniques. However, the collagenous phase is better preserved by the plastic embedding technique than by the cryo-sectioning. In Figures 4e-g gelatinized collagen appears as a continuous phase between the muscle cells. Gelatin, which is damaged by freezing, looks more ragged in the cryo-sectioned samples (Figs. 4b and c). Similar results were obtained with cod muscle.

According to the specific fat staining of the cryo-sectioned samples (not shown), the small uncolored spots encircled by a distinct blue line (indicated by arrowheads) may be emulsified fat (Figs. 4e-g). As far as we can see from these micrographs, there is no obvious correlation between the fat loss and the heat-induced structural changes in the salmon samples.

**Structural changes of whole muscle.** Some of the observed structural changes shown in Figures 3 and 4 may have been reinforced by the chopping, and a comparison was made between chopped and whole muscle. The microstructure of whole muscle of both cod and salmon was studied by sections solidified by plastic embedding. To visualize the observed tendencies, the most significant changes of whole cod muscle, as shown in Figures 5a-f, were chosen. Similar changes were observed for the salmon muscle.

A post-rigor transverse section of cod muscle, at the level of the dorsal fin, is shown in Figures 5a and e. The separation between cells is seen as small spaces with few and small gaps. The pericellular layer may be observed as a thin, threadlike sheet surrounding the cells. Myofibrils are clearly distinguished as individual units.

Myofibrils of the central portion of the muscle cell are shown as small and well separated spots. The typical arrangement of the myofibrils as bundles of ribbons around the periphery of the myofibers is easily seen at the higher magnification in Figure 5e. This is in accordance with the common pattern in fish muscle (Howgate, 1979; Bello *et al.*, 1981). No swelling of the connective tissue could be observed in the 5°C-sample, unlike that of the chopped sample (Fig. 3a). However, at 20°C (not shown) swollen/melted collagen could be observed in the whole muscle as well. The extracellular space was widened with increasing temperature, even though no shrinkage of the muscle cell could be observed. When heated to 40°C, the myofibrils still keep their peripheral ribbon-like shape, and the inner myofibrils also appear as small separated spots (Fig. 5b), but the extracellular space has increased considerably. The pericellular layer is disturbed and has lost its ordered configuration, probably due to denaturation and melting of collagen. Gaps (indicated by arrowheads) can be seen within the pericellular layer, probably between the basement membrane and the outer reticular, collagenous layer (Almás, 1982; Brenner and Hallet, 1985). The main transition temperature ( $T_{max}$ ) of collagen, measured by scanning differential calorimetry, in whole cod muscle is approximately 42°C at pH 7.0 (Hastings *et al.*, 1985). The water loss measured on heating to 40°C may therefore mainly be due to loss of configuration of the pericellular layer and breakdown of the connective tissue which then occurred. The relationship between the observed spoilage of the pericellular layer and the water loss between 5 and 40°C is, however, not quite clear. In mammalian meat, thermal shrinkage of the collagen fibers is proposed to expel intracellular water (Wilding *et al.*, 1986; Hamm, 1985). A similar shrinkage of the collagenous layer compressing the cells could not be observed at the temperatures of main liquid release from either cod or salmon muscle. According to Sikorski *et al.* (1984), the thermal contraction of fish collagen fibers is a rather rapid process taking place within a temperature range of 2 to 4°C between 40 and 45°C. The twisted blue threads in the extracellular matrix, as seen in Figure 5c, is then probably shrunken collagen fibers which upon further heating convert to gelatin. Shrinkage of collagen will, therefore, not explain the liquid released at temperatures below 40°C. Further studies must be carried out to demonstrate more clearly the contribution of the muscle collagen to the liquid released upon heating fish.

Hastings *et al.* (1985) measured the main transition of cod muscle myosin (pH 7.0) to occur at approximately 45°C, whereas  $T_{max}$  of actin was 75°C. Severe shrinkage of the myofibers occurred at 45°C, corresponding with maximum water loss. The muscle cell shrank transverse to the fiber axis and deep invaginations and intracellular cavities appeared where intracellular material may

have leaked (Fig. 5c). The observed shrinkage at 45°C is then probably caused by the denaturation of myosin. The increased water loss between 40 and 45°C (Fig. 1) may be explained by the reduced water-imbibing capacity of the tightly bound myofibrillar proteins, which causes the immobilized cellular water to move and flow out at low pressure (Offer and Trinick, 1983; Hamm, 1985). At 60°C (Fig. 5d), detachment of the peripheral myofibers from the sarcolemma (arrows) and some disintegration of the myofilamentous structure have occurred.

Coagulated sarcoplasmic proteins can be seen, as red granulated material, in Figures 5c, d, f and g. The amount of extracellular red granulates increased with increasing temperature. This in accordance with the transition of sarcoplasmic proteins occurring at 45, 57 and 67°C (Hastings *et al.*, 1985). At a higher magnification of muscle heated at 70°C (Figs. 5f and g), it may look as there are ruptures (indicated by arrowheads) in the cellular membranes. Spaces inside the myofibers separate groups of myofibrils, and there is a clear connection between these large spaces and the intra- and extracellular spaces by small canals. Granulated sarcoplasmic proteins can be observed both in the inter- and intracellular space when using differential interference contrast optics as shown in Figure 5g. Lampila and Brown (1986) proposed that a rupture of the cellular membranes, or a weakening of the same occurring during heat treatment, might facilitate the outward movement of intracellular constituents and enhance the degree of granulation. As previously mentioned, the water loss decreased concomitantly with the appearance of the interstitial granulated material. The reason for the reduced water loss may be that aggregates of sarcoplasmic proteins and collagen are able to hold water and/or plug the intercellular capillaries, thus preventing water from being released during centrifugation.

According to the micrographs in Figures 3, 4 and 5, the structural changes caused by heat treatment are quite similar in coarsely chopped and whole muscle regardless of the preparation technique. Structural changes happened at lower temperatures for the cod muscle than the salmon muscle in accordance with the water loss. From our work, it is not possible to explain the differences in liquid losses between cod and salmon muscles.

The content of collagen in fish muscle varies considerably from species to species, and even within a species there is a seasonal variation as the body reserves are depleted during spawning and migration (Love, 1988). There is a significant effect of collagen on liquid released upon heating which might affect the sensory properties of cooked fish. Further studies should be carried out to study the relationship between microstructure and functional properties of fish products caused by structural changes of collagen during processing under various conditions.

**Conclusions**

The similarities between the plastic sections and the cryo-sections imply that the heat-induced morphological changes discussed above are true and not induced during preparation. Hence, there is a relationship between the water-holding capacity of the fish muscle and the tissue-specific structural changes which occurred during heating. At low temperatures, concomitantly with increasing water loss, the pericellular connective tissue underwent the most striking change. Maximum water loss was attained correspondingly with the appearance of transverse shrinkage of the muscle cells, intercellular gaps and widening of the extracellular spaces. The water loss decreased as a function of temperature when extracellular, granulated material became visible.

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#### Discussion with Reviewers

**P. Wilding:** In their discussion the authors state there is a significant effect of collagen on liquid released upon heating thus affecting the sensory properties of cooked fish. However, in this paper the liquid loss following heating was measured by applying a centripetal force and therefore may not equate with losses that occur during cooking. Therefore, how relevant is the data presented here to the sensory properties of cooked fish?

**Authors:** The water loss was determined after a mild centrifugation which apparently did little damage to the gross structure of the samples. Hermansson and Ström (1989) have shown that the moisture loss which takes place spontaneously on heat treatment and cooling of meat model systems are smaller, but corresponds with the moisture loss after the mild centrifugation included in the net test. The relationship between water loss and sensory properties should be studied further.

**L.E. Lampila:** What is the fat content of the cod muscle?

**Authors:** The fat content of wild cod muscle is about 0.7%. Most of it is phospholipids in membranes (Love, 1988). The fat content of farmed cod muscle is the same as in the muscle of wild cod (unpublished results).

**L.E. Lampila:** Could the authors use their observations to measure the degree of swelling of collagen and its subsequent shrinkage at higher temperatures and to measure the degree of muscle cell shrinkage through the thermal transitions (5°C to 70°C).

**Authors:** We can not use our material to measure these parameters. The material is not homogeneous; the heated samples consists of muscles of 5 different individuals and are taken from slightly different areas of the fish. Thus, the micrographs shown at different temperatures are from

different cells. According to Love (1988) both the fiber diameter and the amount of connective tissue will vary along the fillet and between the individuals. To measure the degree of swelling/shrinkage of collagen and the degree of muscle cell shrinkage during heat treatment would either require several hundred samples to be significant (Kryvi *et al.*, 1985), or that we were able to follow the structural changes of the same cells during heating.

**L.E. Lampila:** If there is physical damage due to pressure from swelling at lower temperatures; then is it possible that its effect is exacerbated as more protein denaturation occurs with rising temperatures?

**Authors:** Yes, it is reasonable to believe that all weak areas will be more easily damaged at higher temperatures.

**L.E. Lampila:** Would the main transition temperatures of collagen, myosin and actin be different in the cod studied (pH 6.22) from those observed by Hastings *et al.* [(1985), (pH 7.0)]?

**Authors:** According to Hastings and Rodger (1985), the transition temperatures of myosin and actin decreased progressively in the pH range 5.0 to 8.0. However, the differences between pH 6.0 and 7.0 are small and probably smaller than differences in  $T_{max}$  caused by different experimental procedures and raw materials.

**A. Bremner:** Were the fish at a comparable stage of development? Fast growing fish in aquaculture often exhibit soft or slack muscle tissue if harvested at a stage of a growth spurt. Would this account for the difference between species?

**Authors:** Both the cod and salmon were quite big fish which had been in cultivation for a long time, and had stabilized. However, the cod was mature, whereas, the salmon was not. The functional properties of the fish flesh will depend on the biological status of the fish (Love, 1988). The effect of development stage on the functional properties of the muscle should be investigated, but in our opinion it has to be done with the same fish species.

**P. Wilding:** The authors found a large difference in the water loss that occur when cod is heated compared to salmon flesh. Do the authors have any hypothesis concerning the origin of this difference in behaviour?

**A. Bremner:** Ando *et al.* (1992) reported detectable changes in the pericellular collagen of rainbow trout within 24 hours storage (5°C) after death. If similar changes are so rapid in salmon and cod then the timing of the sampling in experiments such as the one reported here becomes critical. Indeed, it is possible that the differences between species are a function of differing rates of deterioration in the pericellular collagen!

Another point concerns the nature of the pericellular layer, in particular the collagen. Although the shrinkage temperature of fish collagen (type I) is near 40°C the melting temperature is much lower, nearer 20°C and this may be sufficient to alter the physical properties of this layer which present a physical barrier to release of fluid. Furthermore, the collagen in the pericellular layer may, at least in part, be type V collagen. The melting and shrinkage temperatures for fish type V collagen are not yet known, but they may be lower than that for the type I which Almás (1982) reported in the muscle cell envelope of cod. Different proportions of the collagen species or in their molecular configurations in the two species may help account for the considerable differences in liquid loss on heating. Also, the chain structure of the collagen can alter the melting temperature. Human type V collagen with  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$  molecular chain structure exhibited temperature transitions about 2°C lower than those for the  $\alpha 1$ ,  $\alpha 2$  chain structure (Morris *et al.*, 1990).  
**Authors:** We appreciate these very useful comments. The swimming habit of cod and salmon is quite different and this might account for the different properties of the connective tissue. One possible explanation for the different water loss may be due to different amounts of the collagen types (type I and V) in the pericellular tissue which may affect the stability. We observed from the plastic-embedded muscle samples, that the structural changes of pericellular tissue occurred at higher temperatures in the salmon than in the cod-muscle as did the water loss. However, in salmon there is a lot of both intra- and extracellular fat in the muscle which also might influence on the water loss upon heating.

**A. Bremner:** Where do the authors consider the liquid came from? Is it from the sarcoplasm or does it arise from the loss of water binding properties of the actomyosin due to heat or to both these factors? Do the authors consider it possible to use assays of enzyme activity on liquid extracts possible in conjunction with electrophoresis to try to ascertain where the liquid originates.

The basal lamina (basement membrane) is the cell barrier responsible *in vivo* for controlling fluxes and ion movements in and out of the cells. Disruption by heat to the basement membrane, either in the type IV collagen or in the linking fibrillar structures or in the proteoglycans may alter the permeability of this barrier and allow fluid escape. Do the authors have material they could inspect by transmission electron microscopy (TEM) at high magnification to look for changes in the basal lamina and in the linking structures at various temperatures.

**Authors:** We think that the liquid loss is both from the sarcoplasm and from the liquid held by the myofibrils. The proportion between them might vary as a function of temperature. It may be possible to determine the origin of the released liquid by use of enzymatic assays or electrophoretic techniques. Presently we are investigating

these points by looking at the relationship between functional properties and structure of fish muscle. Inspection of the material with TEM is also a possibility.

**T.A. Gill:** What proof do we have that sarcoplasm stains red? It would appear to me that it is impossible to determine whether the red material is aggregated sarcoplasm or fragments of myofibrils.

**Authors:** The Polychrome-method stains both myofibrillar and sarcoplasmic proteins bluish-red. We quite agree that it is difficult to determine whether the red material is aggregated sarcoplasm or fragments of myofibrils, particularly in the coarsely chopped and heated salmon sample in Figure 4h. Schaller and Powrie (1972) have shown that when the sarcoplasm was removed from intact beef muscle by centrifugation or extraction, no granular material was found in the heated tissue. Heated beef sarcoplasm had a granular appearance; the diameters of the granules were similar to those in heated intact beef muscle. A similar observation was done of heated tuna muscle by Lampila and Brown (1986). We therefore believe that the red, granulated materials seen in Figures 4 and 5 are either aggregated sarcoplasmic proteins or a mixture of sarcoplasmic proteins and, as suggested by you, fragments of myofibrils.

**T.A. Gill:** Could it be that heating results in thermal gelation of some of the cellular proteins and that the gel matrix so formed holds water?

**Authors:** Heating will result in gelation of the myofibrillar proteins which are mainly located within the myofibrils at this low ionic strength. The aggregates of released sarcoplasmic proteins and fragments of myofibrils mixed within gelatin, probably stabilize the total structure.

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