

Microstructure and texture of fresh and smoked Atlantic salmon, *Salmo salar* L., fillets from fish reared and slaughtered under different conditions

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Abstract

This study was carried out to examine differences in microstructure and texture of fresh and smoked farmed Atlantic salmon *Salmo salar* L. fillets with different biological characteristics. The left fillet was analysed fresh and compared with the salted and smoked right fillet from each individual fish. Light microscopy and image analysing techniques were used to study both transversal and longitudinal sections of the muscle. The fish muscle fibres shrank during the salting and smoking process, but sarcomere length did not change. After smoking, a considerable number of fat globules were dispersed among the muscle fibres. The biological characteristics studied were based upon diploid and triploid fish held in both sea cages and land-based tanks. Different starvation times were used and one group was stressed during slaughter. The cross-sectional area of muscle fibres from triploid fish was found to be larger than from diploid fish, both in fresh and smoked fillets.

Introduction

Cold smoking is the traditional smoking process for Atlantic salmon *Salmo salar* L. The cold smoking process is a combination of salting prior to smoking and smoking at temperatures between 20 and 30 °C. The process brings about changes in flavour and texture. Variation in factors throughout the process, e.g. temperature and time, affect the quality and yield of the product (Chan, Toledo & Deng

1975; Sink 1979; Skrede & Storebakken 1986; Choubert, Blanc & Courvalin 1992; Ramachandran & Terushige 1995). The commonly observed loss in weight during the process, according to the industry, is due both to dehydration of the muscle and to lipids leaching from the muscle. According to a review by Howgate (1979), the loss of weight due to dehydration in the smoking process is around 10–25%, depending upon the origin of the raw material and the final product characteristics. A low content of salt is known to improve the yield and liquid-holding capacity of fish muscle. Duerr & Dyer (1952) reported that 9% sodium chloride is a critical concentration regarding the uptake of both salt and water, and further salt uptake leads to a process of water removal.

Limited information is available in the literature on the effect of smoking processes on the fillet quality, especially on texture and microstructure of fish muscle. Bromlei (1949) (as reported by Howgate 1979) studied microscopic changes in both hot and cold smoked fish. However, a number of papers have been published on the effects of salts on water retention and/or structural properties of either fish or mammalian meat (Regenstein, Jauregui & Baker 1984; Wilding, Hedges & Lillford 1986; Shomer, Weinberg, & Vasiliver 1987; Ofstad, Kidman, Myklebust, Olsen, & Hermansson 1995).

Fibre types present in Atlantic salmon muscle have been established by Higgins (1990). The muscle was found to be composed of three types of fibre: white fibres, red fibres and pink fibres. Red fibres were in a well-defined band on either side of

the lateral line and were generally smaller in area than the white fibres. A thin, diffuse layer of pink fibres occurred along the red–white boundary. Hatae, Yoshimatsu & Matsumoto (1990) observed that cooked muscle from fish species with a smaller diameter of fibre showed higher strength in textural firmness. They observed that the textural difference between various species was related to the difference in sarcoplasmic protein content and/or fibre diameter. According to Stickland (1983), the mean cross-sectional area for white muscle fibres in rainbow trout *Oncorhynchus mykiss* (Walbaum) (50 cm in length) was 10 000 μm^2 . Fibre diameters in cooked muscle of different fish species were estimated by Hurling, Rodell & Hunt (1996) and the cross-sectional area for cooked cod *Gadus morhua* L. muscle fibres was c. 20 000 μm^2 .

Sarcomere lengths in fish muscle have been reported by Shindo *et al.* (1986) to be 1.7 μm in rainbow trout, 2.2 μm in tilapia *Sarotherodon niloticus* L. and 1.9 μm in mackerel *Scomber japonicus* L. Howgate (1979) measured the sarcomere length of cod muscle to be 1.5 μm . Knowledge about the interaction between microstructure and texture in relation to process parameters could possibly be useful when selecting raw material for processing and to optimize processing conditions, hence leading to improved product quality.

The objective of this study was to visualize by light microscope the difference in the muscle structure and mechanical properties of fresh and smoked salmon fillets in relationship to different biological characteristics of the fish material. The different biological characteristics studied were triploid and diploid fish reared both in land-based tanks and sea cages using different starvation times prior to harvest. One group was stressed during the slaughter process. Triploid fish were investigated because they are sometimes required in farms; they

are sexually sterile and are therefore unable to mate with wild salmon if they escape from fish farms. The main focus was on changes in muscle structure due to shrinkage of muscle fibres, sarcomere length, distribution of lipids, and mechanical properties of both fresh and smoked salmon fillets.

This study was part of a co-operative European research project among several research institutes in Iceland, Spain, Norway and France on the effects of interaction between raw material characteristics and smoking processes on the quality of smoked salmon.

Materials and methods

Fish samples

Samples of 105 salmon (4 kg \pm 150 g) were used for the study. Salmon were collected from either sea cages or tanks by the Institute of Marine Research, Matredal, Norway. Seven different groups of fish were used (15 fish in each group, Table 1). Four groups were triploid salmon and three groups were diploid fish. The starvation time prior to harvest was different between the groups (0, 7 and 30 days) and one group was stressed during slaughter, i.e. bled without anaesthesia in water depth of one-half body weight. All the fish were of the same genetic strain and the smolt producer was Matre Aquaculture Research Station, Matre, Norway. The fish were harvested by netting, anaesthetized to unconsciousness by CO₂ and bled by cutting the gill arches on one side. Bleeding was conducted in sea water. The dead fish were gutted and cleaned, and each individual fish was weighed and labelled with a tag. The fish were transported to Institut Francais de Recherche pour l'exploitation de la Mer (IFREMER) in Nantes, France. During transport, the fish were stored on ice in sealed boxes.

Group	Sample description
1 L-3n-7	Land-based farming: triploids, starved for 7 days
2 L-2n-7	Land-based farming: diploids, starved for 7 days
3 S-3n-7	Sea-cage farmed: triploids, starved for 7 days
4 S-2n-7	Sea-cage farmed: diploids, starved for 7 days
5 S-2n-7-Str	Sea-cage farmed: diploids, starved for 7 days, stressed during slaughter
6 S-3n-0	Sea-cage farmed: triploids, starved for 0 days
7 S-3n-30	Sea-cage farmed: triploids, starved for 30 days

Table 1 Description of the sampled groups

Processing

On day six after slaughter, the fish were hand-filleted and trimmed at IFREMER. The trimming removed the rib bones and visual adipose tissue. The right fillet was dry salted and smoked (20 °C) and the left fillet was used as samples of the raw material. Salting was carried out on grills of a trolley. Pure, refined, dry granulate salt was used for salting at 12 °C for 6 h to reach 2.5% salt content in the fillets. Then the fillets were rinsed and kept at 2 °C for 12 h. The weight of each fillet was recorded just before smoking. All fillets were placed on racks and smoked at the same time and under the same conditions. Beech wood chips were used for smoking. The smoking process started with drying in the smoking oven for 30 min at 20 °C. Smoking was carried out at 20 °C, a humidity of 65% and air velocity 2 m s^{-1} for 6 h. After smoking, the trolleys were stored at 2 °C until packing the next day. The weight of each fillet was recorded just before vacuum packing.

Preparation of samples

All fish were individually labelled. Fresh left fillets (15 fillets) were compared with salted and smoked right fillets (15 fillets) of the same fish. Fifteen individual fish were investigated in each group (seven groups). All samples were collected from the same location on each fillet, e.g. below the dorsal fin (see Fig. 1). The samples for this study were selected from the white muscle area (Higgins 1990). Two

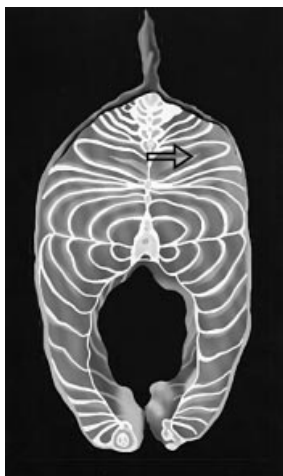


Figure 1 Samples were collected below the dorsal fin marked on the steak.

samples were collected for the microstructure study using a cork-boring knife and two samples for textural measurements. The samples were placed in plastic tubes containing O.C.T. compound (embedding medium, Tissue Tek, USA) and frozen in liquid nitrogen. Freezing (below $-80 \text{ }^{\circ}\text{C}$) occurred in approximately 40 s. The frozen specimens were stored at $-80 \text{ }^{\circ}\text{C}$ until sectioning.

Cryosectioning

The specimens were sectioned ($10 \mu\text{m}$) frozen at $-27 \text{ }^{\circ}\text{C}$ in a cryostat (Leica CM1800, Heidelberg, Germany) for transverse and longitudinal cuts, and mounted on slides. The orange G and methyl blue staining method modified from Flint (1994) was used. The sections were stained for 5 min in orange G [0.5 g of orange G (C.I. 16230 certified Polysciences, Warrington, USA), 1 mL acetic acid dissolved in 99 mL distilled water and filtered]. The sections were washed with distilled water and then stained for 5 min in methyl blue solution [0.07 g methyl blue (C.I. 42780 acid blue 93 SIGMA, USA), 1 mL acetic acid dissolved in 99 mL distilled water for 30 min and filtered]. The stained samples were washed for 5 min with distilled water before mounting with Mountex (Histolab Products AB, Vastra Frolunda, Sweden). Using this modified staining method from Flint (1994), the muscle proteins stained orange and collagen stained blue.

Mounted sections were stained to visualize fat using the oil red O staining method modified from Flint (1994). The sections were stained with oil red O staining solution [0.5 g oil red O (C.I. 26125, Polyscience, Warrington, USA) in 100 mL isopropyl alcohol diluted with 70 mL cold distilled water] for 10 min at $4 \text{ }^{\circ}\text{C}$. Sections were then washed with cold water for 2 min. The samples were mounted with Aquatex (Merck, Darmstadt, Germany) and examined immediately. Using this modified staining method from Flint (1994), fat stained red.

Viewing and image processing by light microscope

The samples were examined in a microscope (Leica DML) at $100\times$, $200\times$, $400\times$ magnification. TV camera and LEICA Q500MC image processing analysis software (Cambridge, UK) were used for calculations of diameter, cross-sectional area and number of fibres in the images. Three pictures

including 60–100 fibres each were processed and calculated for each sample.

Longitudinal samples for sarcomere length

Both low temperature (some times termed 'cold-stage'), scanning electron microscopy (LT-SEM) and light microscopy were used for analysing longitudinal samples and calculation of sarcomere length. Samples for both light microscopy and LT-SEM were fixed using the same method. Fixation was carried out using glutaraldehyde and paraformaldehyde in a phosphate buffer that is a modified method by Carson (1997) as follows: Phosphate buffer: Monobasic sodium phosphate solution, NaH_2PO_4 (83 mL 2.26% solution) and sodium hydroxide, NaOH solution (17 mL 2.52% solution) was added. Paraformaldehyde (2 g) was dissolved in 100 mL phosphate buffer (0.2 M solution) at 60 °C, then 4 mL glutaraldehyde solution (50%) was added, and finally the pH of the solution was adjusted to pH 7.2 using 1 M NaOH.

Light microscope

The specimens were sectioned (8 μm) frozen at -27°C in a cryostat (Leica CM1800, Heidelberg, Germany) for longitudinal cuts. Samples were viewed in a microscope as described above.

LT-SEM

The SEM microscope from Cambridge Instruments 1988, was used. The cryo preparation system (CT 1500 from Oxford Instruments, Oxford, UK) is a special unit attached to the SEM for analysing biological specimens. The fixed samples were frozen in liquid nitrogen, then located in the specimen holder (temperature -194°C) where the samples were broken (not cut), freeze-dried and finally coated with gold prior to examination of the samples.

Textural measurements

The TA.XT2 Texture Analyzer was used (Stable Micro System, Surrey, UK) with a load cell of 25 kg. A blade (knife edge, 60°) of a thickness of 3.0 mm and width of 70 mm was used. The shear force was measured according to Sigurgisladottir (1998). The blade approach was applied by pressing the blade through the muscle, vertical to the muscle fibres.

Duplicate measurements were performed on each sample.

Statistics

Data sets were compared by multiple comparison ANOVA using a pairwise comparison by Sigmapstat 2.0 (Jandel Scientific Software, Ontario, Canada). The difference was deemed significant at $P < 0.05$.

Results and discussion

Cross-sections and shear force

Light micrographs of cross-sections of salmon muscle fibres from fresh and smoked fillets stained by orange G and methyl blue (collagen is blue coloured and muscle proteins are orange) are shown in Fig. 2A and B. Larger areas than shown in Fig. 2A and B were used to calculate the average cross-sectional areas.

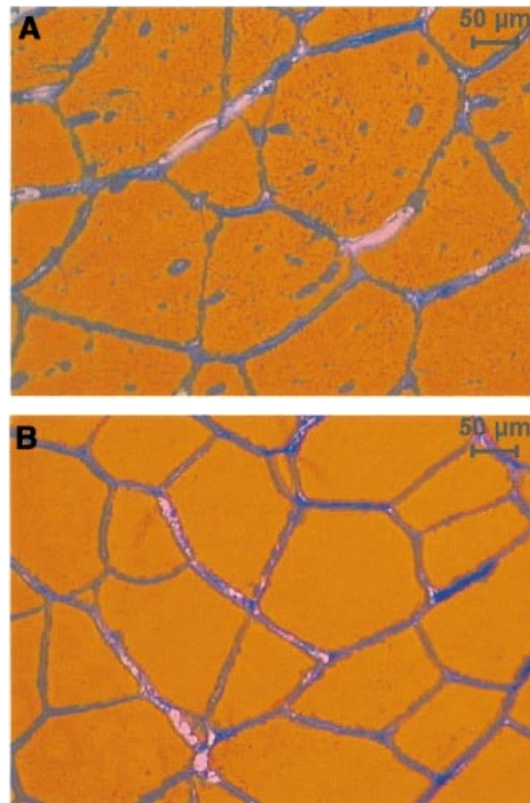


Figure 2 Transverse sections of muscle from salmon fillet both fresh (A) and smoked (B). The samples were stained using orange G and methylene blue. Muscle protein stains orange and collagen stains blue.

Figure 3 Cumulative number of muscle fibres as percentage of the total number of fibres in one picture plotted vs. cross-sectional area of fibres from both fresh and smoked salmon fillets. Data are from one fish sample as an example.

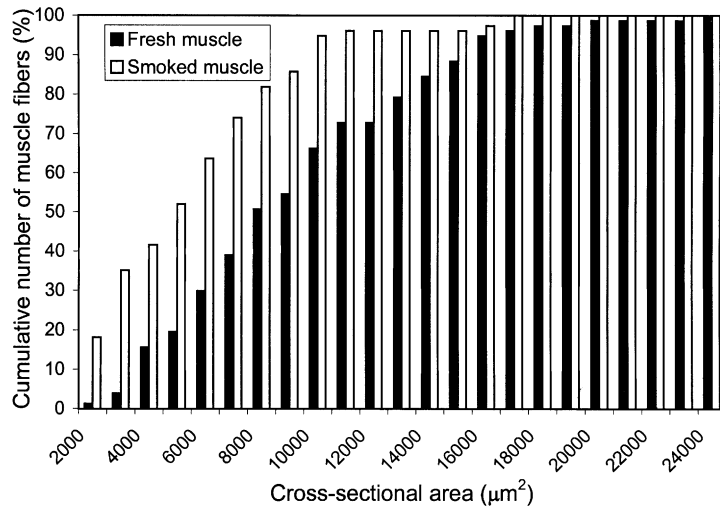
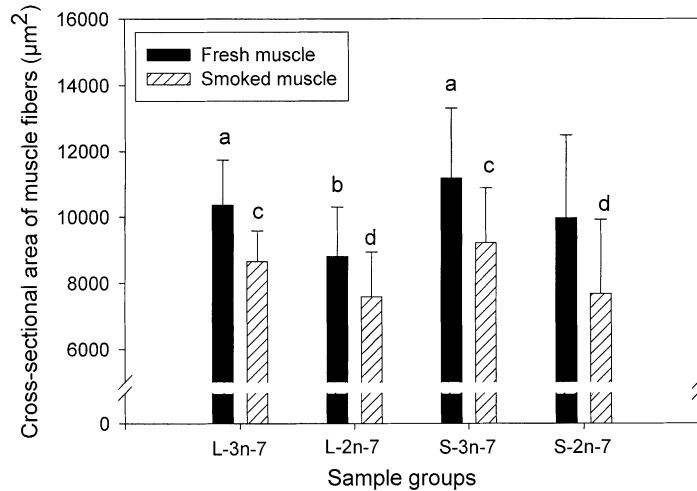


Figure 4 Average cross-sectional area of fresh and smoked muscle fibres from both triploid and diploid fish farmed in sea cages and in land-based tanks. Data are mean and standard deviation from 15 fish, where 60–100 fibres are used for calculation of the mean for each sample. There are significant differences ($P < 0.05$) between columns, indicated by the letters a and b for fresh muscle, and between c and d for smoked muscle. L-3n-7, land-based triploid; L-2n-7, land-based diploid; S-3n-7, sea-caged triploid; S-2n-7, sea-caged diploid.



Cumulative distribution of the cross-sectional area of muscle fibres from fresh and smoked fillets is shown in Fig. 3. The cross-sectional area of muscle fibres from fresh and smoked salmon muscle ranged from 500 to 28000 µm². Figure 3 shows that higher number of fibres with small cross-sectional area were in the smoked muscle than in the fresh muscle. For example, proportion of fibres with cross-sectional area of 10000 µm² or smaller (cumulative approach) were 85% for the smoked muscle but only 55% for the fresh muscle. Figure 3 clearly shows therefore that the cross-sectional areas of muscle fibres from fresh fillets were larger than from smoked fillets from the same fish. The average cross-sectional area of muscle fibres from smoked fillets was significantly ($P < 0.05$) smaller than from the fresh fillets of the same fish in all

seven groups (Figs 4 and 5). Shear force was significantly ($P < 0.05$) higher in the smoked fillets than in the fresh (Figs 6 and 7). The smoking process consists of both a salting step and thereafter a smoking step that includes heating at 20 °C, and both steps affected the muscle structure and mechanical properties. Salt is known to have an impact on the properties of muscle fibre protein and is probably responsible for some of the effects seen on the texture and microstructure of the smoked samples in this study. Shomer *et al.* (1987) demonstrated the swelling of myofibrils at 0.3% NaCl treatment which increased at 1.5% NaCl. Wilding *et al.* (1986) has also studied the effect of salts on swelling of meat. Swelling in hypertonic salt solutions such as 0.6 M KCl was from two to three times the original fibre transverse diameter. Ofstad

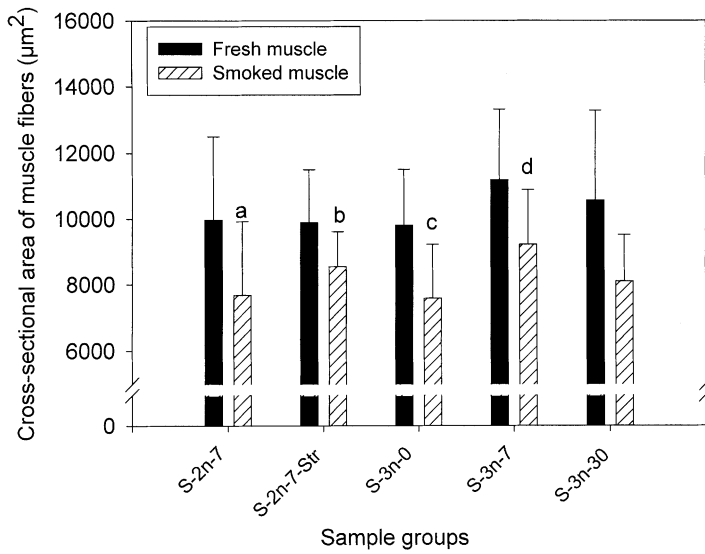


Figure 5 Average cross-sectional area of fresh and smoked muscle fibres from stressed salmon and salmon starved for 0, 7 and 30 days. Data are mean and standard deviation from 15 fish, where 60–100 fibres are used for calculation of the mean for each sample. There are significant differences ($P < 0.05$) between columns indicated by the letters a and b for fresh muscle, and between c and d for smoked muscle. S-2n-7, sea-caged diploids; S-2n-7-str, stressed sea-caged diploids; S-3n-0, not starved sea-caged triploids; S-3n-7, 7-days starved sea-caged triploids; S-3n-30, 30-days starved sea-caged triploids.

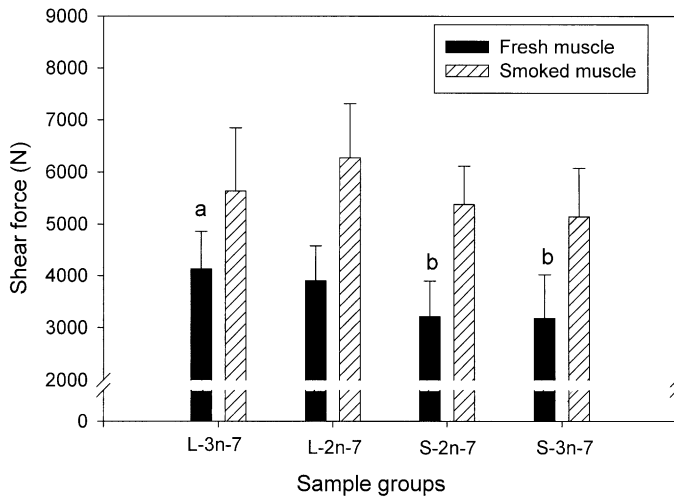


Figure 6 Shear force (N) measurements of fresh and smoked muscle fibres from both triploid and diploid fish farmed in sea cages and in land-based tanks. Data are mean and standard deviation from 15 fish, analysed in duplicate. There are significant differences ($P < 0.05$) between columns indicated by different letters. L-3n-7, land-based triploid; L-2n-7, land-based diploid; S-3n-7, sea-caged triploid; S-2n-7, sea-caged diploid.

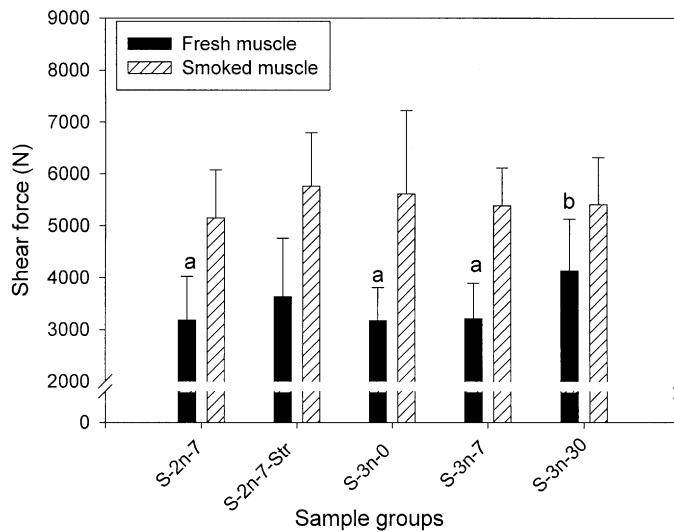


Figure 7 Shear force (N) measurements of fresh and smoked muscle fibres from stressed salmon and salmon starved for 0, 7 and 30 days. Data are mean and standard deviation from 15 fish, analysed in duplicate. There are significant differences ($P < 0.05$) between columns indicated by different letters. S-2n-7, sea-caged diploids; S-2n-7-str, stressed sea-caged diploids; S-3n-0, not starved sea-caged triploids; S-3n-7, 7-days starved sea-caged triploids; S-3n-30, 30-days starved sea-caged triploids.

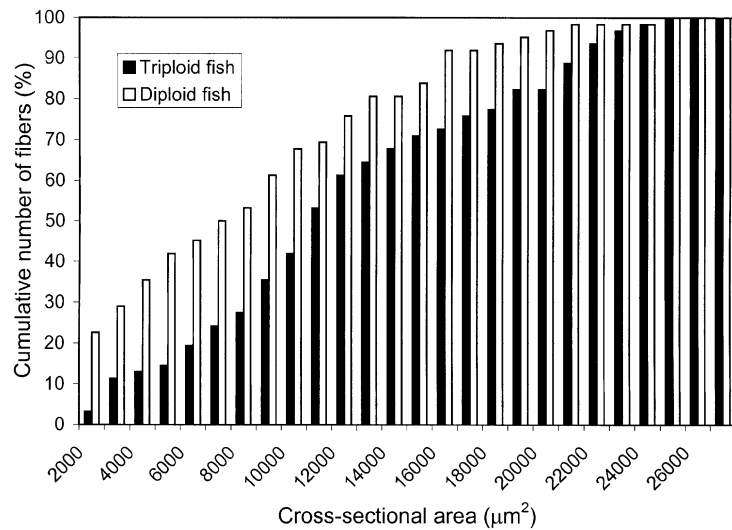


Figure 8 Cumulative number of muscle fibres as percentage of the total number of fibres in one picture plotted vs. cross-sectional area of fibres from both triploid and diploid salmon fillets. Data was collected from one fish sample as an example.

et al. (1995) and Regenstein *et al.* (1984) have stated that salts in low concentrations (1–2%) improve the water-binding properties in muscle. The final salt content in the fillets was approximately 2.5%.

The average cross-sectional areas of fresh muscle fibres were larger in groups including triploid fish (L-3n-7, S-3n-7) than in groups including diploid fish (L-2n-7, S-2n-7) (Fig. 4). The distribution in cross-sectional area in fresh muscle from both triploid and diploid fish shows that there is a higher number of muscle fibres with smaller cross-sectional area in diploid than in triploid fish (Fig. 8). However, a cross-sectional area of triploid muscle fibres was found to be significantly larger than the diploid muscle fibres in both fresh and smoked fillets from the same individual fish for the land-based groups L-3n-7 and L-2n-7 (Fig. 4). The observed shrinkage of the muscle fibres was therefore independent of the cross-sectional area of the muscle fibres.

Shear force of the fresh fillets was not significantly different between groups of triploid and diploid fish (Fig. 6). The difference in the cross-sectional area between triploid and diploid muscle fibres could be detected by the shear force measurement in this study on either fresh or smoked fillets. Hurling *et al.* (1996) observed a correlation between sensory firmness and the area of cooked muscle fibre cross-sections from different fish species, but they did not show this correlation within fish species on raw fish material.

Stressing during slaughter was undertaken to simulate the conditions during commercial harvest-

ing of salmon. Mild stress during slaughter did not affect the cross-sectional area of the fresh muscle fibres (Fig. 5). However, the cross-sectional area of muscle fibres from smoked fish that were stressed during slaughtering was significantly larger than muscle fibres from smoked fish that were not stressed during slaughter (Fig. 5). A difference in shear force was detected between the stressed fish (S-2n-7-Str) and the fish that had not been stressed (S-2n-7) for both fresh and smoked fillets. Fillets from the stressed fish showed higher shear force, although the difference was not significant (Fig. 7). However, Sigholt *et al.* (1997) observed that handling stress resulted in softer fillets with lower breaking strength. One reason for the more limited effect of stress on both structure and shear force obtained in this study compared with Sigholt, Erikson, Rustad, Johansen, Nordtvedt & Seland (1997) is that in this study the fish were stressed only during slaughter. In the referred study, stressing was carried out during handling in nets prior to slaughter. Stressing fish during slaughter did not show effects on chemical composition (M. C. Gomez-Guillen, personal communication).

Starvation time prior to harvest (0, 7 or 30 days) did not affect the size of the cross-sectional area of the fresh muscle fibres (Fig. 5). However, smoked fish from groups which had not been starved prior to slaughter had smaller fibres than the smoked fish starved for 7 (significant at $P < 0.05$) and 30 days. Shear force was observed to be significantly higher for the fresh fillets starved for 30 days compared with fillets starved for 7 days and those that were

Table 2 Yield of salmon fillets after salting and smoking

Group	Yield % after trimming, compared with gutted fish	Yield % after salting, compared with trimmed fillet (right)	Yield % after smoking, compared with salted fillet (right)	Yield % after trimming, salting and smoking compared with gutted fish
1 L-3n-7	71.6	95.0	96.5	65.6
2 L-2n-7	71.9	94.8	96.6	65.9
3 S-3n-7	72.1	95.0	96.7	66.2
4 S-2n-7	72.2	94.8	95.9	65.6
5 S-2n-7-Str	72.0	94.8	96.1	65.6
6 S-2n-0	72.5	94.5	96.2	66.0
7 S-2n-30	72.0	94.8	96.6	66.0

See Table 1 for sampling groups.

Data are mean of 15 fish fillets.

not starved (Fig. 7). This is in accordance with results from Einen & Thomassen (1998) where instrumental texture analysis indicated that long-term starvation (86 days) can increase the hardness of raw fillets after starvation. However, the difference obtained in the cross-sectional area of triploid and diploid muscle fibres, and from fish either stressed or starved before slaughtering, could not be detected by shear force measurement of smoked fillets.

The cross-sectional area of muscle fibres from fresh fillets farmed in land-based tanks was smaller than from fresh fillets farmed in sea cages both for triploid and diploid fish, although the difference was not significant (Fig. 4). Differences were also detected in shear force measurements. Shear force was higher for fresh fillets from land-based tanks than from sea cages where there was significant difference for triploid fish (Fig. 6). The main difference between the conditions in tanks and sea cages is water flow. The water flow in tanks is higher and the fish experience more exercise.

In spite of the difference in cross-sectional area of muscle fibres between the groups, the yield through the smoking process was not different between the groups (Table 2). The average yield was approximately 96% as compared with fresh trimmed fillets. The yield was therefore independent of the different biological characters of the raw fish material. The same yield was obtained for triploid and diploid fish through the smoking process. The yield was higher than that reported by Howgate (1979). This is probably due to a higher fat level in the fish produced in aquaculture today than for the fish investigated by Howgate (1979) about 20 years ago.

Sarcomere lengths

Sarcomere lengths of the salmon muscle fibres did not change significantly during the smoking process. Longitudinally sectioned muscles of fresh fillets were analysed using light microscopy and LT-SEM techniques (Fig. 9). The sarcomere lengths both before and after processing were approximately 2.2 μm measured using light microscopy and 2.1 μm using LT-SEM; hence no significant difference. Wilding *et al.* (1986) reported that fibre length or sarcomere length in meat muscle were not affected by low salt concentration. Sarcomere lengths in fish muscle have been reported by Shindo *et al.* (1986) to be 1.7 μm in rainbow trout.

Lipid distribution

Figure 10A and B are light micrographs of both fresh and smoked samples from salmon fillets stained with oil red (lipid is red coloured). Lipids within the fresh muscle fibres or between them were visible to a very limited extent. Zhou, Ackman & Morrison (1995) found that the main lipid storage within the Atlantic salmon fillets was in myosepta and not within the muscle fibres, and this is in agreement with our observations. According to Zhou, Ackman & Morrison (1996), the connective tissue and associated adipocytes decreased sharply from the belly flap region to the leaner dorsal white muscle, and lipid droplets occurred in the endomysium around dark muscle fibres, but were only occasionally observed around white muscle fibres. Zhou *et al.* (1996) observed that finely dispersed intracellular lipid droplets were present in dark

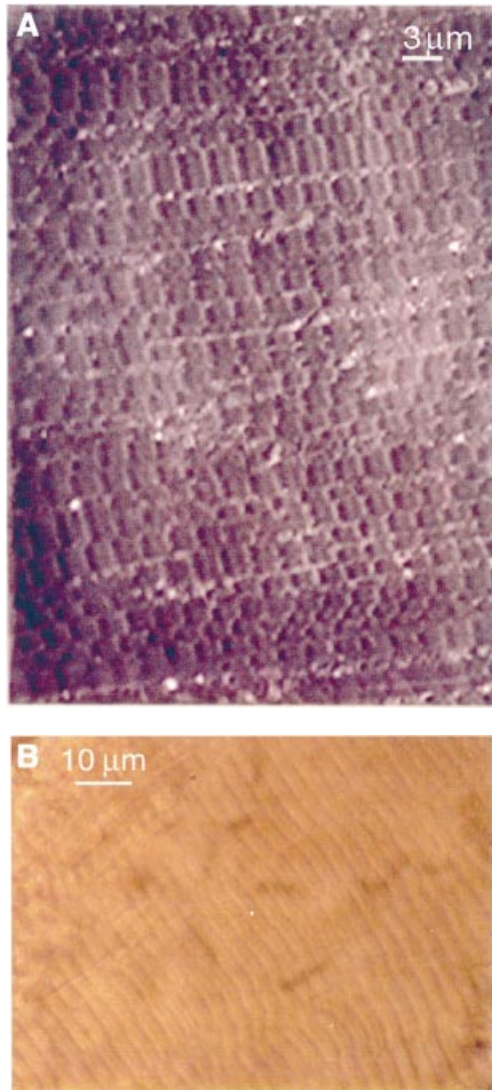


Figure 9 Longitudinal section of muscle from fresh salmon fillet (A) SEM and (B) LM.

muscle cells but were not observed in dorsal white muscle cells. This agrees with Shindo *et al.* (1986), who found lipid droplets within the interspaces of myofibrils in the red muscle of fresh rainbow trout and other fish species.

According to our finding, lipids were clearly visible in the perimysium between and around the fibres in the smoked fillet (Fig. 10). Lipids were presumably released from the fat cells, and droplets were freely floating between the muscle fibres. These results confirm that overall weight loss during the smoking process is due to loss of lipids as well as to dehydration.

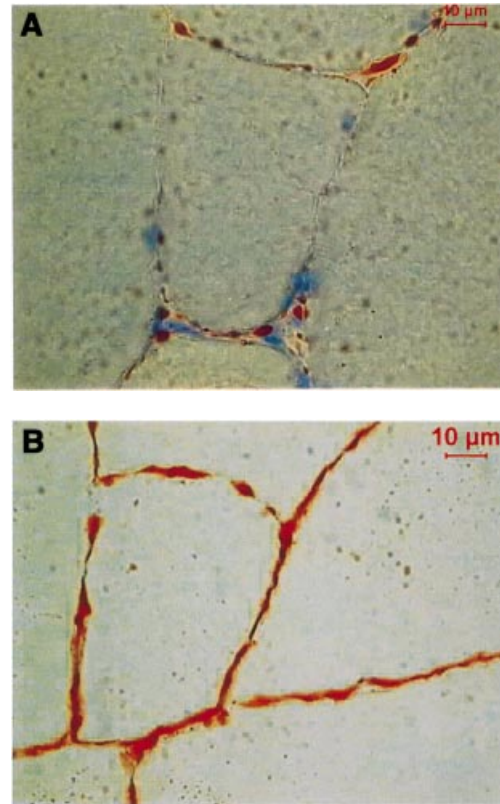


Figure 10 Transverse sections of muscle from fresh (A) and smoked (B) salmon fillets. The samples were stained using oil red. Lipid is red coloured.

Our results suggest that microscopy techniques can be used to study the effects of smoking processes on the structural properties of salmon fillets and possibly explain the effects of various process variables on overall quality. Thus, these techniques will aid in optimizing processes for different raw materials to optimize product quality.

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