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Effects of freezing/thawing on the microstructure and the texture of smoked Atlantic salmon (*Salmo salar*)

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Abstract

The changes in microstructure and texture during smoking of fresh and frozen/thawed Atlantic salmon was studied in fish from three different origins; ocean-ranched Atlantic salmon *(Salmo salar)* from Iceland and two groups of farmed Atlantic salmon from northern and western Norway. The muscle fibers from the frozen and thawed fish shrank, and the extracellular space increased compared to the fresh muscle. The muscle fibers from salmon fillets with smaller fiber diameter shrank to a less extent than fibers from salmon material with a larger fiber diameter. After smoking the space between the fibers and the fiber shrinkage increased to a higher extent in the muscle from the salmon that were frozen prior to smoking than muscle smoked from fresh salmon. The initial cross-sectional area of the fibers was not found to be related to the yield during smoking. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Salmon; Freezing; Smoking microstructure; Shear force

1. Introduction

The traditional cold smoking process of Atlantic salmon (*Salmo salar*) includes salting, drying and smoking at low temperatures which results in a lightly preserved product with salt content ranging between 2 and 4%, water content depending on the fat content but usually ranging between 60 and 70% and pH between 5.8 and 6.3 (Hansen, Gill & Huss, 1995; Hansen, Gill, Rontved & Huss, 1996; Hansen, Rontved and Huss, 1998; Sigurgisladottir, Ingvarsdottir, Sigurdardottir, Torrissen & Hafsteinsson, 2000b).

Freezing of the fresh raw material or the smoked product is commonly done in order to control the supply for smoked salmon in relation to rapid and large changes in demand, and to take benefits from periods with surplus of fresh salmon in the market. Frozen muscle inevitably loses some of the special quality attributes of fresh fish, usually observed as a loss in juiciness and increase in toughness (Mackie, 1993). Particularly under sub-optimal freezing and storage conditions. Ice crystals are formed in the tissue during freezing, and their size, shape and extra- or intracellular location, depend on the freezing conditions (Howgate, 1979).

Numerous papers have been published on the freezing and the biochemistry of fish muscle proteins and textural properties in general (Gill, Keith & Smith-Lall, 1979; Hurling & McArthur, 1996; Jarenback & Liljemark, 1975a,b; Kreuger & Fennema, 1989; Love, Aref, Élerian, Ironside, Mackeay & Varela, 1965; Montero & Borderias, 1989, 1992; Nilson, 1994; Refsgaard, Brockhoff & Jensen, 1999; Sikorski, Olley & Kotsuch 1976; Talesara & Kiran, 1984) and freezing in relation to the structure of the fish muscle (Bello, Luft & Pigott, 1981; Fletcher, Hallet, Jerrett & Holland, 1997; Jarenback & Liljemark, 1975a, b; Lampila, 1990; Love et al., 1965). It has been stated that the muscle structure of whole fresh muscle, during freezing and frozen storage, changes due to shrinking of muscle fiber resulting from movement of water into extracellular spaces on freezing

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(Bello et al., 1981; Hurling & McArthur, 1996; Jarenback and Liljemark). In addition to the fiber shrinking, decrease in the extractability of the proteins and protein denaturation during frozen storage lead to decreased swelling and increased toughening of the fish flesh (Brown, 1986; Howgate, 1979; Kreuger & Fennema, 1989; Lampila, 1979; Love et al., 1965; Mackie, 1993). Myosin and actin, the main contractile proteins, are largely responsible for the functional properties of muscle. During frozen storage, myosin in particular, undergoes aggregation reactions which lead to toughening of the muscle and a loss in water-holding capacity (Gill, et al., 1992; Howgate, 1979; Jarenback and Liljemark, 1975a; Lampila, 1990; Mackie, 1993). The combination of the fiber shrinking and the protein cross-linking may reflect an impaired ability to reabsorb water during thawing (Lampila, 1990).

Howgate (1979) and Mackie (1993) stated that freezing per se causes little damage in cod muscle tissue thawed immediately after freezing or after storage under optimal conditions. The fibers resorb melt water, completely or nearly so, regaining their original shapes although fissures are left as evidence of the previous existence of ice crystals. However, according to Hurling and McArthur (1996), refrozen samples were much less able to retain water on thawing and centrifugation. They concluded that thawing, refreezing and subsequent storage may, therefore, reduce mechanical resistance, rendering fish muscle more susceptible to deterioration during processing.

The effect of freezing temperature applied and freezing rate on structure of muscle has been studied to some extent (Bello, Luft & Pigott, 1982; Chen & Pan, 1995, 1997; Grujic, Petrovic, Pikula & Arnidzic, 1993). Muscle structural damage during slow freezing is greater than at fast freezing. The higher freezing rate results in smaller and more uniform extracellular spacing immediately after freezing, but prolonged storage results in reduced ultrastructural differences (Bello et al., 1982; Grujic et al., 1993; Chen and Pan, 1995, 1997).

The ionic strength and pH are the important factors that influence water binding of muscle proteins (Acton, Henna & Satterless, 1981; Gill, Chan, Phonchareon & Paulson, 1992; Hamm, 1986; Offer & Trinick, 1983). Low content of salt (1–5%) is known to improve the yield and liquid holding capacity of fish muscle (Duerr & Dyer, 1952; Regenstein, Jauregui & Baker, 1984; Shomer, Weinberg & Vasiliver, 1987). Fennema (1990) has pointed out that because freezing causes major changes in muscle pH and concentration of salts, freezing would be expected to profoundly influence swelling of myofibrils and the fraction of muscle water present in extracellular spaces. Deng (1977), found that freezing and frozen storage had a major effect on the initial salt penetration rate into mullet when they were dipped in 25% brine solution. Immediately following freezing the initial salt penetration rate was increased and on subsequent frozen storage the initial salt penetration rate was decreased.

No information has been published in the literature on the effects of freezing and thawing on the microstructure and texture of smoked salmon fillets. The objective of this work was to investigate the effects of freezing and thawing of whole salmon prior to smoking on the microstructure and texture of smoked salmon fillets in comparison to smoking of fresh fish material. We also wanted to investigate interactions with raw material characteristics. Three types of salmon material were, therefore, used in order to examine the discriminatory nature of freezing and thawing. The fish origins were ocean-ranched fish from Iceland and farmed fish from Norway slaughtered in both autumn and spring.

2. Materials and methods

2.1. Fish samples

Three different origin groups of fish were used with a total of 60 salmon in each origin group, they are the same material as previously reported (Sigurgisladottir et al., in press, b). The fish were harvested by netting, and bled by cutting the gill arches on one side. The fish was allowed to bleed in cold seawater. The dead fish were gutted, cleaned and each individual fish was weighed and tagged. All the fish were transported by courier to Institut Francais de Recherche pour I Éxploitation de la Mer (IFREMER) in Nantes, France. During the transport the fish were stored on ice in sealed boxes.

2.2. Freezing and frozen storage

Whole salmon was frozen using cryogenic freezing with CO_2 at $-60^{\circ}C$ at day 5 after harvest at IFREMER. The freezing was executed in a cabinet with a capacity of 60 kg of fish. For 2–3 kg salmon to reach $-20^{\circ}C$ it takes 30 min but for 4–5 kg salmon it takes 45 min. The salmon was stored for 1 month at $-20^{\circ}C$. Thawing of whole salmon was executed in a cold room at $4^{\circ}C$ for 24 h.

2.3. Salting and smoking process

At IFREMER the fish was filleted and trimmed. The trimming removed the rib bones and visual adipose tissue. Yield was calculated for each step in the process. The filleting was done at day 6 after slaughter for the fresh fish, the frozen had been stored fresh for 6 days and frozen for 1 month. The right fillet was utilized for the smoking experiment and the left fillet was used as samples of the raw material. The two processing methods (15 salmon in each) used were based on the dry salting method, and smoking at two different temperatures,

20 and 30°C. These processing methods were used both for the fresh and the frozen/thawed fish material.

2.3.1. Dry salting

Pure refined dry salt was used for salting at 12°C, for 6 h. Salting was carried out on grilles of a trolley. Then the fillets were shortly rinsed with fresh water after salting, and kept in a cold room at 2°C for 12 h. Weight of each fillet was recorded, just before smoking.

2.3.2. Traditional smoking

The smoking process started with drying in the smoking oven for 30 min at 20 or 30°C. Smoking was carried out at 20 or 30°C at humidity of 65% and air velocity 2 m/s for 5 h. Wood chips of beech were used for smoking using smoke autocombustion generator and pyrolysis of 450/500°C. After smoking the trolleys were stored at 2°C until packing the next day. The weight of each fillet was recorded just before packaging.

2.4. Preparation of samples

All fillets were individually tagged with identical numbers for the left and right fillet. Fresh left fillets (15 fillets) were compared to salted and smoked right fillets (15 fillets) of the same fish individual. Fifteen fish individuals were in each treatment (four groups). All samples were collected from the same location on each of the fillets. Samples were collected below the dorsal fin from the white muscle and from the same location on each fillet (Sigurgisladottir, Ingvarsdottir, Sigurdardottir, Torrissen & Hafsteinsson, in press, a). Two samples were collected for the microstructure study using a cork knife and two samples for textural measurements. The samples for microstructure were embedded in plastic tubes containing O.C.T. compound (embedding medium, Tissue Tek, USA) and frozen in liquid nitrogen. Freezing (below −80°C) occurred in approximately 40 s. The frozen specimens were stored at −80°C until sectioning.

2.4.1. Cryosectioning

The specimens were sectioned (10 μm) frozen at −27°C in a cryostat (Leica CM1800, Heidelberg, Germany) for transverse cuts.

2.4.2. Orange G and methyl blue staining method

Cryosections were mounted on slides. The sections were stained for *5* min in Orange G (0.5 g of Orange G, 1 ml acetic acid dissolved in 99 ml distilled water and filtered). The sections were washed with distilled water and stained for 5 min in methyl blue solution (0.07 g Methyl blue, 1 ml acetic acid dissolved in 99 ml distilled water and filtered). The stained samples were washed for 5 min with distilled water before mounting with Mountex (Histolab Products AB). Using this staining method the muscle proteins stain yellow and collagen blue (Sigurgisladottir et al., in press, a).

2.5. Viewing and image processing by light microscope

The samples were examined in a microscope (Leica DML) at l00×, 200×, 400× magnification. TV camera and LEICA Q500MC image processing analysis software (Cambridge, UK) were used for calculations of diameter, cross-sectional area and number of fibers in the images. Three pictures including 60–100 fibers each were processed and used for calculation.

2.6. Textural measurements

The TA.XT2 texture analyzer was used (Stable Micro System, Surrey, UK) with a load cell of 25 kg. 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, Hafsteinsson, Jonsson and Torrissen (1999). The blade approach was applied by pressing the blade through the muscle vertical to the muscle fibers. Duplicate measurements were performed on each sample.

2.7. Salt content

Quantitative determination of chloride from water-soluble chlorides, expressed in NaCl. Chlorides are solubilised in water and titrated by a chloride analyser 926 Corning (Corning Medical and Scientific, Halstead, England).

2.8. Statistic

Data sets were compared by multiple comparison ANOVA using all pair wise comparison by Sigmastat 2.03 (Jandel Scientific Software, Ontario, Canada). The difference was found to be significant at $P < 0.05$.

3. Results and discussion

3.1. Effects of freezing, frozen storage and thawing on fresh muscle structure

The ocean-ranched fish were leaner (9.4% fat content) than the farmed fish from Norway harvested both in October 1998 (24.0% fat content) and in April 1999 (20.2% fat content). Previously obtained results (Sigurgisladottir et al., in press, b) show that the cross-sectional area of muscle fibers from the fresh ocean-ranched salmon fillets were significantly ($P < 0.05$) smaller than the muscle fibers from the two groups of farmed salmon. The cross-sectional area of muscle fibers from salmon harvested in October 1998 were

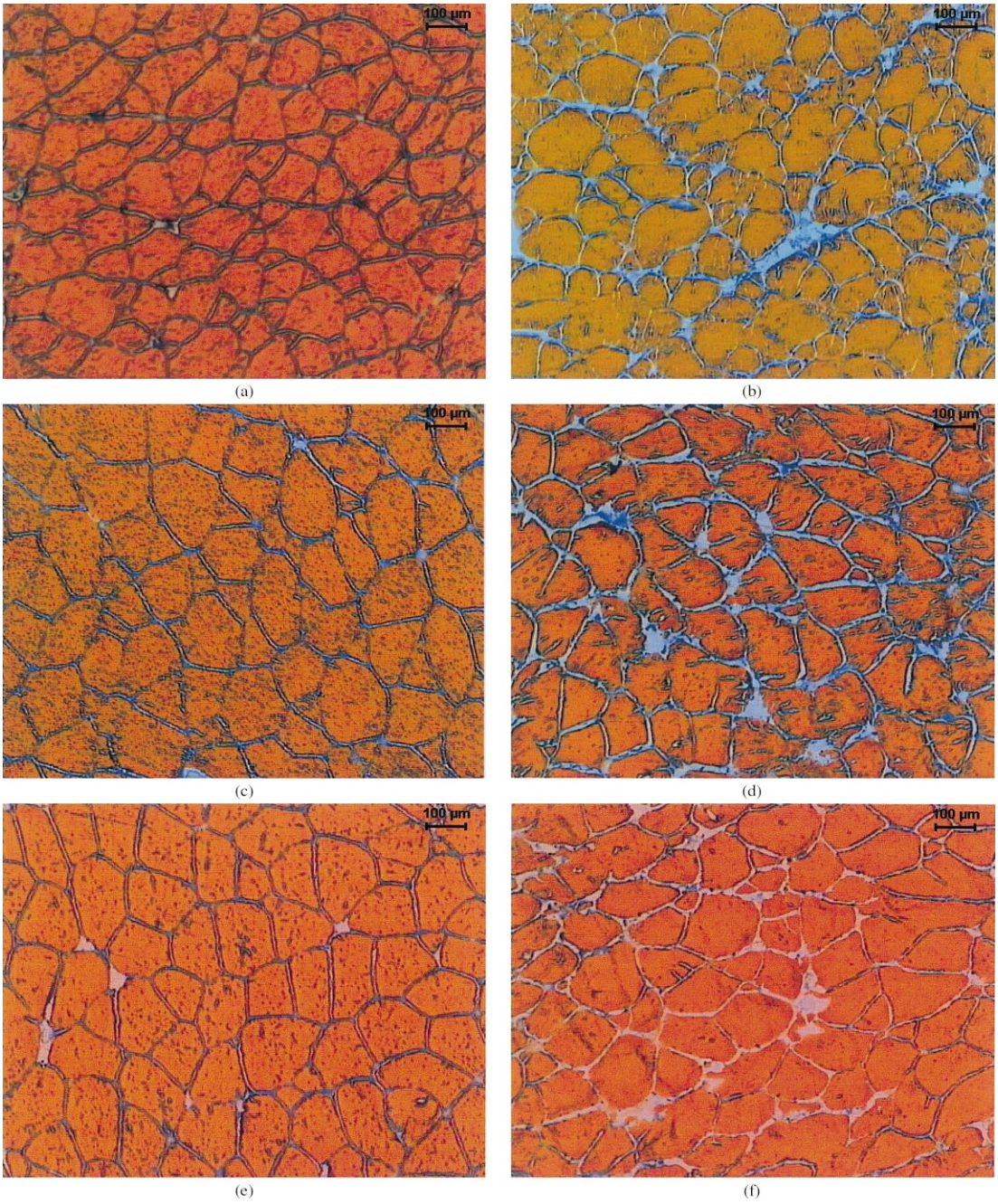

Fig. 1. Transverse sections of muscle from fresh (7 days post slaughter) and frozen/thawed (frozen at day 5 post slaughtered) salmon fillets: (a) fresh, ocean-ranched; (b) frozen/thawed, ocean-ranched; (c) fresh, farmed October 1998; (d) frozen/thawed, farmed October 1998; (e) fresh, farmed April 1999; (f) frozen/thawed, farmed April 1999. The samples were stained by Orange G and Methyl blue.

significantly ($P < 0.05$) smaller than from salmon harvested in April 1999 (Sigurgisladottir et al., in press, b).

Fig. 1(a–f) shows transverse sections of muscle fibers from both fresh muscle and from muscle that had been frozen/thawed from the three origin groups of salmon. The fibers were affected by the freezing, frozen storage and/or thawing in all the three groups. The effects of the freezing treatment on the transverse microstructure of

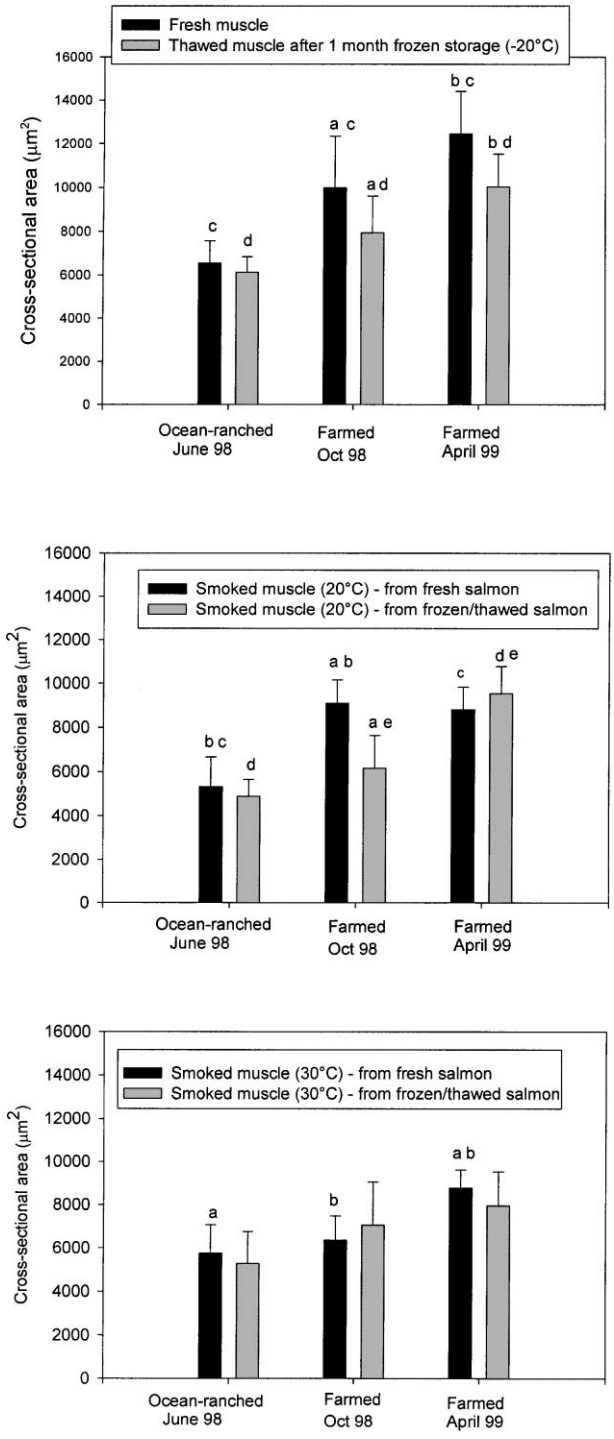

Fig. 2. Average cross-sectional area of muscle fibers from Atlantic salmon fillets. Salmon groups were: ocean-ranched salmon slaughtered in July 1998, farmed salmon slaughtered in October 1998 and farmed salmon slaughtered in April 1999. (a) Fresh fillets (7 days post-slaughtered) and frozen/thawed fillets (frozen at day 5 post slaughtered). (b) Smoked fillets at 20°C from fresh salmon and from frozen/thawed salmon. (c) Smoked fillets at 20°C from fresh salmon and from frozen/thawed salmon. Data are mean and standard deviation of five samples. Columns indicated by the same letter are significantly different ($P < 0.05$).

the salmon fillets in the three groups are mainly seen as increased extracellular space between the fibers that had been frozen. In addition some disrupted and shrunken cells can be seen in the frozen/thawed fish, while the cells from the fresh fish seem to have no fissures. These results are in agreement with earlier results with respect to increase in extracellular space with cellular and myofibrillar compression between ice crystals during freezing and frozen storage of fish muscle (Bello et al., 1981; Chen & Pan, 1997; Hurling & McArthur, 1996; Jarenback & Liljemark, 1975b; Nilson, 1994).

The cross-sectional area of muscle fibers was calculated both for muscle from fresh raw salmon material and frozen/thawed raw material (Fig. 2a). However, it was found difficult to calculate the area for frozen/thawed fish as part of the fibers were disrupted. The muscle fibers shrank more in the farmed salmon than in the ocean-ranched fish. The fibers in the farmed salmon groups were significantly ($P < 0.05$) smaller in the frozen/thawed fillets than the fresh fillets (Fig. 2a). The farmed group (farmed April 1999) that had initially bigger cross-sectional area fibers, shrank to a higher extent than the fibers from the other groups (ocean ranched June 1998 and farmed October 1998) with initially smaller cross-sectional area. According to results from this study the effect of the freezing and frozen storage is dependent on the fiber cross-sectional area of the initial raw fish material, i.e. the salmon group with the initial bigger cross-sectional area shrank to a higher extent than fish that had smaller fiber cross-sectional area initially. Fig. 3 shows that there is a high curvilinear correlation ($r^2 = 0.999$) between the initial diameter of the muscle fibers and the extent of shrinkage during

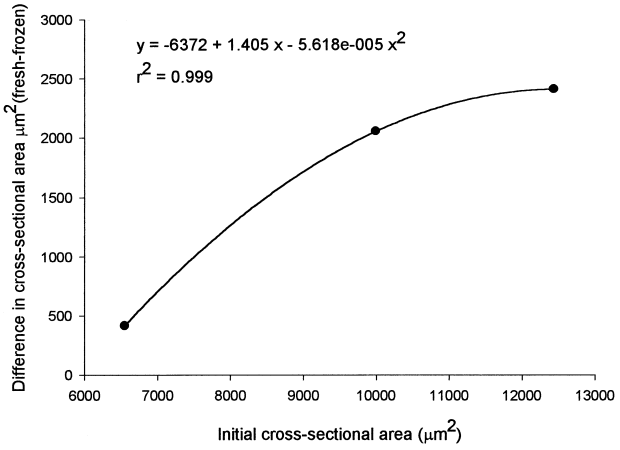

Fig. 3. Difference in fiber cross-sectional area of muscle fibers from fresh (7 days post slaughtered) and from muscle that has been frozen (frozen at day 5 post slaughtered) plotted versus the initial fiber cross-sectional area of muscle fibers from Atlantic salmon of different origin: ocean-ranched salmon slaughtered in July 1998, farmed salmon slaughtered in October 1998 and farmed salmon slaughtered in April 1999. Data are mean and standard deviation of 5 samples.

Table 1
Yield after salting and smoking of trimmed fillets compared to unprocessed fillets and salt content in the smoked fillets[a]

Process	Ocean-ranched June 1998		Farmed October 1998		Farmed April 1999	
	Yield %	Salt content %	Yield %	Salt content %	Yield %	Salt content %
Frozen/thawed temp. 20°C	86.3±0.8	3.7±0.3	90.9±0.8	2.7±0.4	91.1±1.1	3.0±0.4
Frozen/thawed temp. 30°C	88.8±1.5	3.9±0.4	93.4±0.7	2.8±0.4	92.8±1.4	2.8±0.2
Fresh temp. 20°C	88.8±1.3	3.3±0.4	92.2±0.7	2.4±0.3	91.1±0.8	3.1±0.3
Fresh temp. 30°C	89.2±0.8	3.4±0.4	92.7±0.4	2.3±0.2	93.1±0.9	2.7±0.3

[a] Additional loss (1.3%) was during thawing of frozen/thawed samples. Data are mean±standard deviation of 15 fillets.

freezing, frozen storage and/or thawing. This leads to there being less difference in the cross-sectional area of muscle fibers between the three different groups of salmon after freezing/thawing than observed for fresh salmon fillets. The ocean-ranched fish with the smallest initial muscle fibers cross-sectional area shrank to a less extent than the other two groups (Fig. 2a).

3.2. Effects of freezing, frozen storage and thawing on smoked muscle structure

The salt content in fillets smoked from fresh material and those smoked from frozen/thawed material in this study was not found to be different ($P > 0.05$). Salt content was found equal between the groups (Table 1). However, according to Deng (1977) salt migration in the fish can be affected by frozen storage, to increase within short frozen storage up to 5–9 weeks of storage at −18°C then the salt penetration started to decrease. In this study no difference was obtained in yield between the groups that had been frozen prior to smoking and groups smoked from fresh material (Table 1). However, the loss during the thawing process was 1.3% and that decreases the total yield using a prefrozen raw material compared to fresh material. The yield was different for the ocean-ranched group as compared to two farmed groups which were similar (Table 1). The initial cross-sectional area of the fibers was not found to be related to the yield during smoking.

The effects of freezing/thawing of the raw fish material on the microstructure of the smoked salmon muscle fiber transverse section is demonstrated in Fig. 4(a–f). The fibers from the smoked fillets from frozen/thawed fish compared to smoked muscle from fresh fillets had more space between the fibers and looked more shrunken on the pictures from the transverse cut from the muscles. The smoking process is, therefore, found to increase the effects of the freezing and not to reduce it. Although the effects of freezing leads to shrunken fibers the yield during smoking was not affected. The extracellular fluids did not drain from the muscle during salting and smoking. Following salting there is an increase in ionic strength influencing water binding of the muscle proteins. This can lead to improved waterbinding to the same extent in both the cellular/extracellular locations.

The cross-sectional areas of all smoked muscle samples were smaller than for the fresh material (Fig. 2a, b, c). The difference in fiber cross-sectional area was found to be less after smoking between the different origin groups of the fillets that had been frozen/thawed and fillets that were smoked from fresh fillets (Fig. 2 b, c). The average fiber cross-sectional area was found to even out during freezing/thawing treatment. The pictures (Fig. 4) from the transverse cut from the muscle fibers from the smoked fillets showed the effects clearly and even more pronounced effect was demonstrated than for the calculated average cross-sectional area for the smoked fillets. The pictures can be used to identify the fish material that has once been frozen.

3.3. Shear force of fresh and smoked muscle

Forces required to shear fresh salmon fillets were compared to shear force for fillets that had been frozen as whole fish, stored for 1 month (−20°C) and thawed prior to the measurement. The shear force required for the frozen/thawed fillets in the group from April 1999 was significantly ($P < 0.05$) lower than for the fresh fillets (Fig. 5a). The same trend was seen for the other groups but was not significant. However, earlier reports have shown that protein denaturation, water loss and toughening of fish flesh are associated with frozen storage (Gill et al., 1979; Howgate, 1979; Kreuger & Fennema, 1989; Mackie, 1993; Nilson, 1994). This has mainly been demonstrated using fish species like cod, haddock, hake, Alaska pollock, and tilapia but to a lesser extent with salmon or trout (Gill et al., 1979; Hurling & McArthur, 1996; Kreuger & Fennema, 1989). Refsgaard et al. (1999) found fresh cooked salmon to be flaky, firm and juicy based on sensory panel evaluation, but frozen storage changed the texture to more firm, less juicy and more fibrous. Similar results were observed by Nilson (1994) who studied the effect of frozen storage on the quality of trout and found that the storage temperature affected the juiciness and firmness as judged by a sensory panel and found that trout

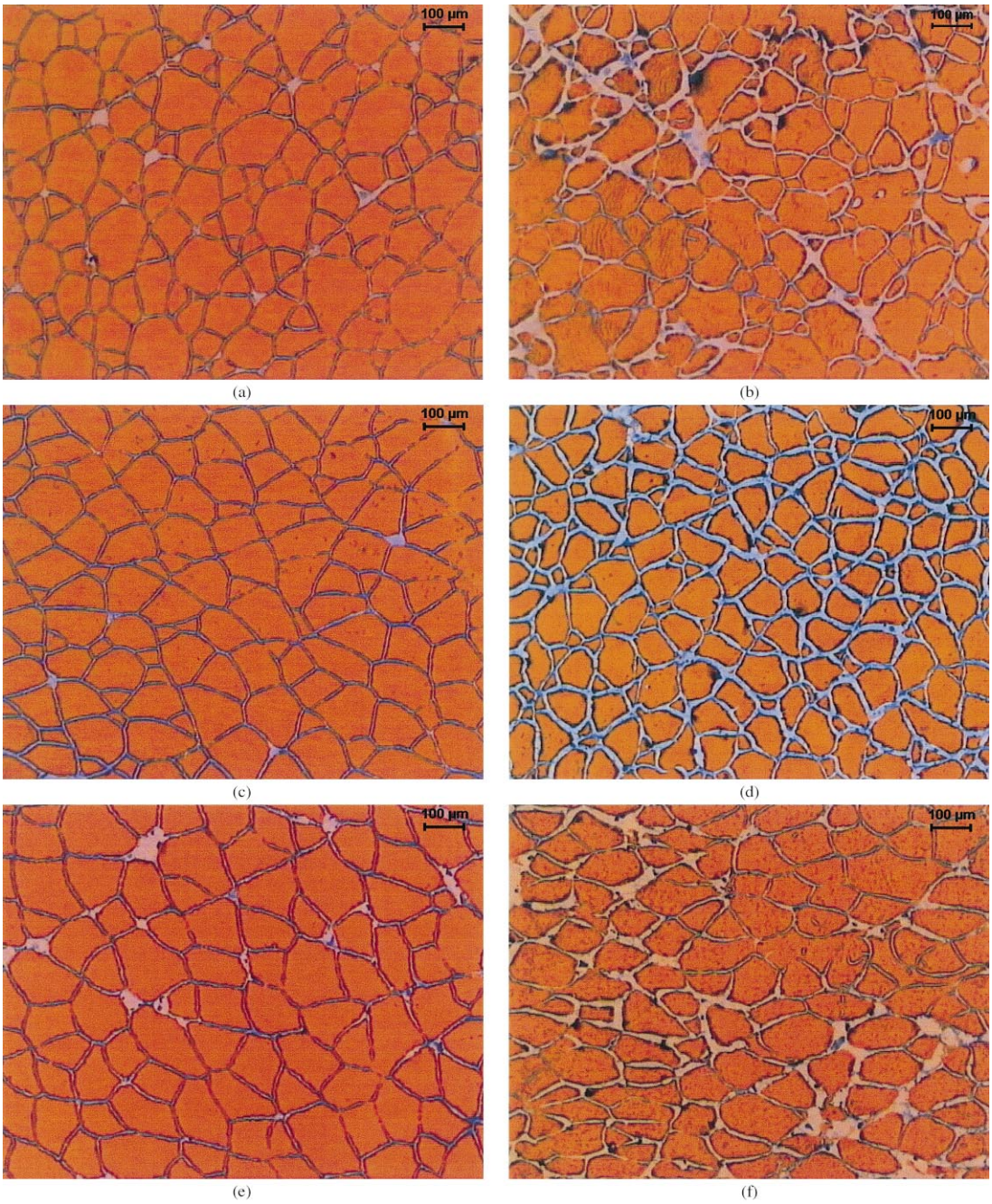

Fig. 4. Transverse sections of smoked muscle from fresh (7 days post slaughtered) and frozen/thawed (frozen at day 5 post slaughtered) salmon fillets: (a) ocean-ranched; (b) pre-frozen, ocean-ranched; (c) farmed October 1998, (d) pre-frozen, farmed October 1998; (e) farmed April 1999; (f) pre-frozen, farmed April 1999. The samples were stained by using Orange G and Methyl blue.

stored at −18°C for 18 months generally scored lower in juiciness and higher in hardness than those stored at −40°C (Nilson). In the present study the whole fish was only stored for 1 month.

Shear force required for fillets smoked from fresh was compared to that required for fillets smoked from frozen/thawed fish material. The shear force for the fillets smoked at 20°C was equal for fillets that were smoked

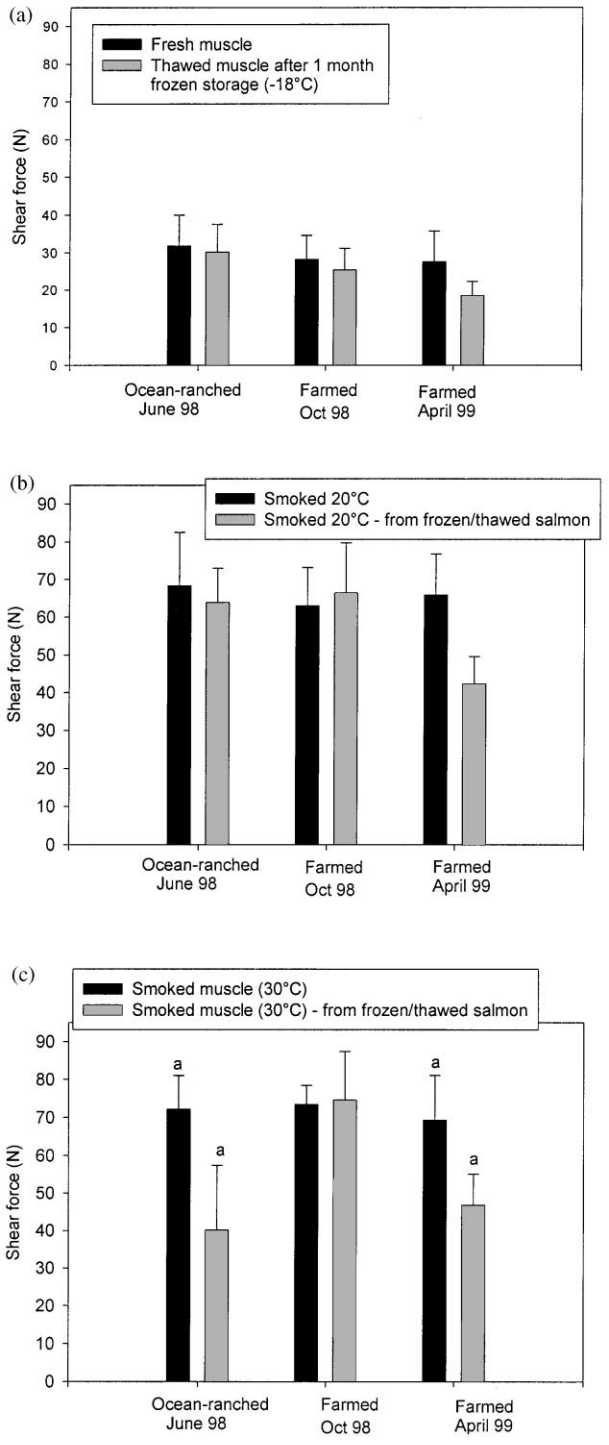

Fig. 5. Shear force of fresh (7 days post slaughtered), frozen/thawed and smoked Atlantic salmon fillets. Salmon groups were: ocean-ranched salmon slaughtered in July 1998, farmed salmon slaughtered in October 1998 and farmed salmon slaughtered in April 1999. (a) fresh fillets and frozen/thawed fillets; (b) smoked fillets at 20°C from fresh salmon and from frozen/thawed salmon; (c) smoked fillets at 20°C from fresh salmon and from frozen/thawed salmon. Data are mean and standard deviation of 15 samples. Columns indicated by the same letter within each group of fish are significantly different ($P < 0.05$).

fresh or smoked after frozen/thawed treatment for the ocean-ranched and the farmed salmon from October 1998 (Fig. 5b). However, for the farmed salmon from April 1999 the shear force was significantly ($P < 0.05$) lower for fillets, smoked from frozen/thawed fish material than fillets smoked from fresh fish material (Fig. 5b). Smoking at 30°C gave similar results except that shear force for smoked fillets of ocean-ranched fish and farmed salmon from April 1999 was significantly ($P < 0.05$) lower for smoked fillets, that had been frozen/thawed than for those smoked from fresh fillets (Fig. 5c). The shear force was equal for fillets that were smoked fresh (30°C) or smoked after freezing/thawed treatment (30°C) for only farmed salmon from October 1998 (Fig. 5c).

4. Conclusion

It can be concluded that freezing does affect the muscle structure of the smoked fillets, the fibers shrank and extracellular space increased which can on the other hand lead to liquid leakage from the smoked fillets. The amount of shrinking caused by freezing and thawing was proportional to the cross-sectional area of the initial raw material. The shrinkage increased further during the smoking process compared to smoked material never been frozen. Although freezing leads to fiber shrinkage, the yield during the smoking process was not affected.

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