Water and Salt Distribution in Atlantic Salmon (*Salmo salar*) Studied by Low-Field ^{1}H NMR, ^{1}H and [23]Na MRI and Light Microscopy: Effects of Raw Material Quality and Brine Salting

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The effect of different Atlantic salmon raw materials (prerigor, postrigor and frozen/thawed) on water mobility and salt uptake after brine salting was investigated by using LF ^{1}H NMR T_2 relaxation, ^{1}H and [23]Na MRI and light microscopy. Distributed exponential analysis of the T_2 relaxation data revealed two main water pools in all raw materials, T_{21} and T_{22}, with relaxation times in the range of 20−100 ms and 100−300 ms, respectively. Raw material differences were reflected in the T_2 relaxation data. Light microscopy demonstrated structural differences between unsalted and salted raw materials. For prerigor fillets, salting induced a decrease in T_{21} population coupled with a more open microstructure compared to unsalted fillets, whereas for frozen/thawed fillets, an increase in T_{21} population coupled with salt-induced swelling of myofibers was observed. The result implies that the T_{21} population was directly affected by the density of the muscle myofiber lattice. MR imaging revealed significant differences in salt uptake between raw materials, prerigor salted fillets gained least salt (1.3−1.6% NaCl), whereas the frozen/thawed fillets gained most salt (2.7−2.9% NaCl), and obtained the most even salt distribution due to the more open microstructure. This study demonstrates the advantage of LF NMR T_2 relaxation and ^{1}H and [23]Na MRI as effective tools for understanding of the relationship between the microstructure of fish muscle, its water mobility and its salt uptake.

KEYWORDS: NMR; T_2 relaxation; MRI; Atlantic salmon; prerigor; postrigor; frozen/thawed; brine salting; water mobility; microstructure

INTRODUCTION

Filleting and further processing of Atlantic salmon are traditionally performed postrigor. New slaughter methods, reducing preslaughter stress and chilling prior to death, allow industrial prerigor filleting (*1, 2*). Prerigor fillets have several properties that differ from the postrigor ones (*3−5*). Most of these differences have been found favorable with regard to raw material quality, i.e. to reduce gaping, and better maintenance of texture and color in fresh fillets (*2, 5, 6*). Therefore, prerigor filleting immediately followed by further processing is a goal for many Norwegian salmon processors.

The salting process is influenced by raw material characteristics including rigor status (*3, 7*), fat or water content (*8*) and frozen storage (*9*). Furthermore, the high salt intake among

Europeans is recognized as an important public health concern, and food manufacturers meet new challenges in the development of low salt products and processes. This requires a better understanding of how water, salt and proteins interact in muscle foods. Traditionally, salt content in foods has been measured by chemical methods which may be tedious and time-consuming. The rapid and nondestructive magnetic resonance imaging (MRI) is a technique that offers a unique opportunity to produce cross-section images of intact muscle. As an NMR signal is generally sensitive to water mobility and its interaction with other molecules, the ^{1}H MRI technique is suitable to study macroscopic flesh structure. Furthermore, [23]Na MRI can visualize salt distribution in foods, and might be a useful tool for optimizing salting processes. Earlier [23]Na MRI studies on salted lean cod and fatty salmon fillets (skin on) revealed that salt diffusion took place only from the flesh side of the salmon fillets, whereas the cod fillets were salted from both sides (*8*); subcutaneous fat in salmon probably served as a diffusion barrier. Another [23]Na MRI study found that varying fat content

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along the salmon fillet influenced the salt uptake. The anterior part of the fillet gained more salt than the posterior part (*10*).

The content of water and its distribution within the muscle are one of the most important fish flesh quality parameters, and it makes up for approximately 65−70% of fresh salmon flesh. The distribution of the water in the muscle 3D network is generally believed to be affected by the physical and biochemical changes in the muscle which occur during e.g. rigor mortis and by freezing/thawing. Water properties in muscle tissue have traditionally been assessed by measuring e.g. changes in total water content, water holding capacity (WHC), drip loss, and water activity. However, low-field (LF) ^{1}H NMR T_2 relaxation has been shown to be a valuable tool for increasing the understanding of the state of water in muscle tissue (*11−13*). LF NMR is a rapid and noninvasive method that can be used to investigate water and fat mobility within the fish muscle. The technique has earlier been used to study changes in water distribution occurring in foods during storage and processing (*14*). Several pools of water were defined in fresh (*15, 16*), frozen, thawed, chilled and processed (*17−22*) fish muscle and mince. The technique has also been used to determine total water and fat content in herring (*23*) as well as WHC in cod (*24*), and for mapping the raw material properties of herring in relation to geographical location and seasonal variations (*25*). Nevertheless, only a few LF NMR studies (*26−28*) have been performed on salting of fish, and as far as the authors know, no previous LF NMR studies have been performed on prerigor salmon muscle.

The aim of the present study was to investigate how LF ^{1}H NMR T_2 relaxation, ^{1}H and ^{23}Na MRI, and light microscopy, and thereby water and salt distribution, were affected by different salmon raw material qualities (prerigor, postrigor and frozen/thawed).

MATERIALS AND METHODS

Fish. Nine farmed Atlantic salmon (*Salmo salar*) were used as raw material. To obtain the wanted raw material differences (frozen/thawed, postrigor and prerigor) in the following salting experiment, fish had to be sampled at three different points in time. All fish were randomly selected from the same commercial fish farm in February and March 2005, and handled in the same way. The fish were killed, gutted and brought to the laboratory on ice. All fish arrived 2−3 h post mortem with a mean core temperature of 2−3 °C. To obtain the frozen/thawed raw material, the first fish were received 3 weeks before the salting experiment started. These fish were frozen at −20 °C immediately after arrival, and stored for 3 weeks before thawing in air in a cold room (4 °C) overnight. This group, the frozen/thawed group (FT), had a gutted weight of 4.7 ± 0.8 kg (*n* = 3). To obtain the postrigor raw material, a second group was received 3 days before the experiment started. The fish were stored in ice in the cold room for 3 days for letting them pass through rigor mortis. This group, postrigor (PoR), had a gutted weight of 4.5 ± 1.0 kg (*n* = 3). The prerigor raw material was sent from the fish farm 3 h before the experiment started. The average gutted weight was 3.8 ± 0.3 kg (*n* = 3). All fish were filleted, cleaned and tagged before the left fillets were immersed in brine. The right fillets were used for analysis of unsalted raw material, samples were taken as described in **Figure 1**.

Salting and Sampling. The fish were salted in a brine solution of 16% NaCl (w/w) at a ratio 1:7 (fish:brine) in closed plastic tanks for 4 h at 4 ± 1 °C. Ordinary commercial refined salt (Jozo salt, Akzo Nobel Salt, Göteborg, Sweden) and distilled water were used. After salting, the fish were removed from the brine and stored on a grid for another 14 h at 4 °C to drain off excess brine, and allow further salt distribution in the fillets. Thereafter, samples for different analyses were taken as shown in **Figure 1**.

Physicochemical Analyses. The pH was measured directly in the white muscle before and after salting. A shielded glass electrode (WTW
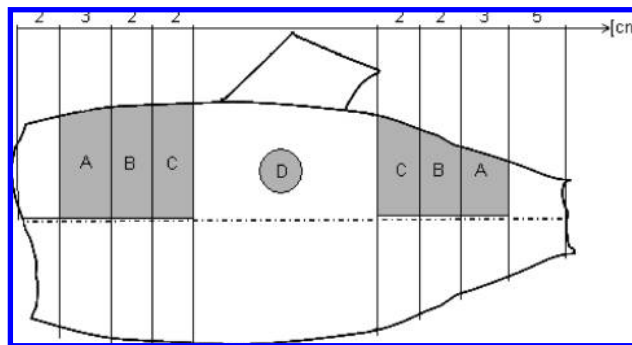

Figure 1. Sampling locations for various analyses of the fillets. (**A**) ^{1}H and ^{23}Na MRI, NaCl, fat and water contents, (**B**) Light microscopy, (**C**) LF ^{1}H NMR, (**D**) pH. For details, see text.

SenTix 41) connected to a portable pH meter (model WTW 315i; WTW, Weilheim, Germany) was used. During the measurements, the instrument was frequently calibrated using pH 4.01 and pH 7.00 buffers. The fillet fat content in unsalted fillets was determined in two replicates using the Bligh and Dyer method (*29*). The water content before and after salting was calculated after drying duplicates of approximately 2 g of muscle at 105 °C for 24 h. The weight difference of the homogenates before and after drying was considered equal to the total water content of the sample. The salt content in salted fillets was determined using the Volhard method (*30*). 2−5 g was weighed accurately into a conical flask, and distilled water (200 mL) was added before the flask was placed in an electric shaker for 45 min. The supernatant (20 mL) was pipetted into Erlenmeyer flasks, and the chloride ions precipitated by adding $AgNO_3$ (0.1 M; 5 to 10 mL). The $AgNO_3$ excess was backtitrated with a NH_4SCN (0.1 M) solution. A ferric indicator ($FeNH_4(SO_4)_2$)·$12H_2O$ in diluted HNO_3) was added for the determination of the end point.

Light Microscopy. Light microscopy was performed before and after salting on all fillets. Samples were cut out of the fillet in two locations as shown in **Figure 1**. Muscle blocks (0.2 × 0.2 × 0.4 cm^3) were chemically fixed in 2.5% glutaraldehyde with 0.1 M cacodylate buffer (pH 7.3), and embedded in Technovit 7100 (Heraeus Kulzer, Werheim, Germany). For all samples, 3 μm plastic sections were cut perpendicular to the myofibers using a Leica RM2165 microtome (Leica Microsystems Wetzlar, Germany). In order to carry out optical light microscopy, the sections were stained with 1 g/100 mL Toluidine Blue solution (Sigma Aldrich Norway AS, Oslo, Norway) to elucidate the general structure of the muscle samples. Three blocks each from both head and tail were excised. The sections were examined with a Leica DM 6000B microscope (Leica Microsystems Wetzlar GmbH, Wetzlar, Germany), and images were acquired at 100× magnification with an Evolution MP 5.0 CCD camera (MEDIA CYBERNETICS The Imaging Experts, Silver Springs, MD). Two hundred fibers per sample were used to determine the fiber diameter, using Image-Pro Plus 4.5 (MEDIA CYBERNETICS The Imaging Experts, Silver Springs, MD).

^{1}H and ^{23}Na MRI. ^{1}H and ^{23}Na MRI measurements were done after the salt distribution period on one fillet from each group (prerigor, postrigor and frozen/thawed). Samples were excised as shown in **Figure 1**, and transported on ice to the MR laboratory. Within 2 h, the head- and tail-parts were scanned together with three reference solutions (2, 4, 6 w/w % NaCl). All MRI studies were carried out at 20 °C using a Bruker Avance DBX 100 Biospec (Bruker Biospin GmbH, Rheinstetten, Germany) with a magnetic field strength of 2.35 T (26 MHz resonance frequency for ^{23}Na) and a horizontal bore opening.

The proton imaging was performed using a 72 mm commercial probe and a spin−echo imaging protocol. The imaging parameters were the following; echo time = 9 ms, recovery time = 400 ms, number of averages = 8, field of view = 6.4 cm, matrix size = 256 × 256, number of scans = 20, slice thickness = 1.5 mm and a total acquisition time of 13 min.

An in-house made 72 mm ^{23}Na probe was used for sodium imaging. A spin−echo ^{23}Na imaging protocol with the following measurement parameters was used: echo time = 2.7 ms, recovery time = 230 ms,

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number of averages = 1024, field of view = 6.4 cm, matrix size = 64 × 64, slice thickness = 15 mm and a total acquisition time of 4 h 11 min.

LF ^{1}H NMR. LF ^{1}H NMR measurements were done before salting and after the salt distribution period on all fillets. One cube was excised from the center of all fillets (half-thickness), and it was thereafter divided into three subsamples (1 × 1 × 3 cm, approximately 2 g) and placed in NMR tubes (diameter 10 mm). The tubes were immediately placed in ice for up to 30 min before they were equilibrated to 4 °C in a thermostatted water bath (Julabo labortechnik GmbH, Germany) before analysis. The measurements were performed on a minispec mq 20 (Bruker Optik GmbH, Ettlingen, Germany) with a magnetic field strength of 0.47 T corresponding to a proton resonance frequency of 20 MHz. The instrument was equipped with a 10 mm temperature-variable probe. A built-in heating element was connected to the temperature control unit (BVT3000, Bruker Optik GmbH). The temperature in the probe was regulated to 4 °C by blowing compressed air through the sample holder. Transversal (T_2) relaxation was measured using the Carr−Purcell−Meiboom−Gill pulse sequence (CPMG) (*31, 32*). The T_2 measurements were performed with a time delay between the 90° and 180° pulses (τ) of 150 μs. Data from 4000 echoes were acquired from 16 scan repetitions. The repetition time between two succeeding scans was set to 3 s. All even echoes were sampled.

The NMR transverse relaxation data were analyzed by three different calculation methods. (1) Multivariate data analysis was performed for all raw relaxation (CPMG) curves. These curves were normalized by setting the first sampled echo to a value of 100, and thereafter scaling the rest of the echo-train. The first 800 data points were used for the principal component analysis (PCA) (*33*) using an in-house made program written in Visual Basic. Each row (*n*) represented a single fish sample, and each column (*m*) represented a signal amplitude from an echo in the CMPG echo train. Four principal components (*k*) were used. The input matrix was not mean-centered. (2) A continuous distribution of exponentials related to water and fat protons located in different muscle compartments (*34, 35*) was fitted for all CPMG curves by use of the CONTIN algorithm (*36*) after normalizing the raw data by setting the first sampled echo to a value of 1 and scaling the rest of the echo-train thereafter. This analysis gave a plot of the continuous T_2 distribution. (3) Biexponential fitting analysis of T_2 relaxation data was performed by fitting the absolute value of the CPMG as shown in eq 1, using the SigmaPlot (version 9.0, Systat Software, Inc., 2004), as reported by Lambelet et al. and Erikson et al. (*22, 26*).

$$\text{signal} = A_{21}\,e^{-t/T_{21}} + A_{22}\,e^{-t/T_{22}} \qquad (1)$$

where T_{21} and T_{22} were the relaxation components, and A_{21} and A_{22} were the corresponding amplitudes. Since the absolute relaxation amplitudes are proportional to the amount of sample (or water and fat) present, the relative amplitudes within samples were used. T_{21} populations were calculated as $A_{21}/(A_{21} + A_{22})$. For the biexponential fitting, the T_{21} population and the T_{22} population sum up to 100%; therefore, only T_{21} population values are given. Three parallel samples from each fish were averaged at both sampling points.

General calculations. The total changes in T_{21} population between unsalted and salted samples were calculated as shown in eq 2:

$$\Delta(m) = \frac{(m)_S - (m)_0}{(m)_0} \qquad (2)$$

where $(m)_0$ is the value before salting, $(m)_S$ is the value after salting and $\Delta(m)$ describes the total changes in each parameter.

Statistical Analysis. Means and standard deviations (SD) are generally shown. The differences in relaxation time between the raw materials were analyzed by use of ANOVA at a significance level of 95% (Minitab version). The differences in relaxation time between unsalted and salted samples were analyzed by use of *t* test, ($P \leq 0.05$).

RESULTS AND DISCUSSION

Physicochemical Properties. **Table 1** and **Table 2** give a summary of the physicochemical characteristics of the raw materials.

Table 1. Gutted Weight, Fish Length, Fillet Weight, and pH before and after Salting [Mean(SD) ($n = 3$)]$^{a-c}$

	gutted wt$_{(0)}$ (kg)	fish length$_{(0)}$ (cm)	fillet wt$_{(0)}$ (kg)	fillet wt$_{(S)}$ (kg)	pH$_{(0)}$	pH$_{(S)}$
prerigor	3.8(0.4)	63(5)	1.19(0.09)	1.17(0.07)	7.1(0.1)^A	6.6(0.0)^{A,*}
postrigor	4.5(1.0)	69(6)	1.42(0.33)	1.45(0.35)	6.2(0.0)^B	6.2(0.1)^{A,*}
frozen/thawed	4.7(0.8)	65(4)	1.40(0.27)	1.41(0.26)	6.4(0.1)^C	6.1(0.0)^{B,*}

^{a} Subscripts (0) and (S) refer to unsalted and salted samples, respectively. ^{b} Unequal letter (A, B or C) indicates significant difference ($P \leq 0.05$) between prerigor, postrigor or frozen/thawed fillets (ANOVA). ^{c} Asterisk indicates significant difference ($P \leq 0.05$) between unsalted fillets and salted fillets (*t* test).

Table 2. Fillet Fat, Water, and Salt Contents as Well as Fiber Diameter for the Head and Tail Region of the Different Raw Materials [Mean(SD)]$^{a-c}$

	% fat$_{(0)}$	% H$_2$O$_{(0)}$	% H$_2$O$_{(S)}$	% NaCl$_{(S)}$	fiber diam$_{(0)}$ (μm)	fiber diam$_{(S)}$ (μm)
prerigor head	13.1(4.1)	67.3(2.1)	68.7(4.3)	1.6(0.2)^{A,*}	87.1(4.8)	95.2(2.8)
prerigor tail	6.0(1.3)	72.2(0.5)	69.1(0.2)	1.3(0.2)^{A,*}	87.8(11.9)	78.9(3.3)
postrigor head	11.0(1.0)	69.4(0.6)	68.4(1.0)	1.8(0.3)^{A,*}	87.7(3.8)	101.6(6.9)^c
postrigor tail	7.5(1.5)	71.4(1.1)	72.1(1.4)	2.3(0.2)^{AB,*}	86.3(6.4)	89.9(10.0)
frozen/thawed head	11.5(3.1)	68.8(2.2)	67.4(1.1)	2.9(0.2)^{B,*}	88.4(1.8)	95.9(5.6)
frozen/thawed tail	7.8(2.4)	71.4(0.2)	71.1(0.4)	2.7(0.3)^{B,*}	79.9(5.9)	89.6(12.2)

^{a} Subscripts (0) and (S) refer to unsalted and salted samples, respectively. ^{b} Unequal letter (A or B) indicates significant difference ($P \leq 0.05$) between prerigor, postrigor or frozen/thawed fillets for head or tail region separately (ANOVA). ^{c} Asterisk indicates significant difference ($P \leq 0.05$) between unsalted fillets and salted fillets (*t* test).

Both post mortem muscle changes and addition of NaCl are known to influence the pH in muscle foods. The mean fillet pH in the prerigor group was 7.1 ± 0.1, which confirms that the fillets still were in a prerigor state, since in salmon, rigor onset occurs at about pH 6.6−6.7 (*37*). The mean fillet pH in the postrigor group was 6.2 ± 0.1 shortly before brine-salting (3 days post mortem), and the mean pH value for the FT group was 6.4 ± 0.1 before salting (1 day post thawing), confirming that the two latter groups were in a postrigor state (*37*). After brining and the salt distribution period, the pH of the prerigor brined fillets dropped by 0.5 unit. The pH in the PoR fillets did not change after salting, while the pH in the FT fillets dropped by 0.3 unit after brining, possibly because of the higher salt uptake compared to postrigor fillets.

The fillet fat content of 6−13% exhibited typical values for the head and tail region of salmon of this size (*38*). Individual head parts had significantly higher fat content than the tail parts on the same fillet. However, the differences were not significant within each group.

Before salting, the water content in the flesh was 64−72% (**Table 2**), and exhibited an inverse relationship with the fat content, as expected (*38*). The water content was generally somewhat higher in the tail part than in the head part, as previously reported (*38*). There were no significant changes in water content after brining.

In general, the salt content of the fillets was in the lower range of what is typical for Norwegian smoked salmon, and it might therefore be denoted as a low salt product. The salt uptake in the prerigor fillets (1.3−1.6%) was significantly lower than in the frozen/thawed (2.7−2.9%) fillets both in the head region and in the tail region. In the tail region, the postrigor fillets revealed higher salt content (1.8−2.3%) than the prerigor fillets, while no significant differences in salt content were observed in the head region between the postrigor and the prerigor groups
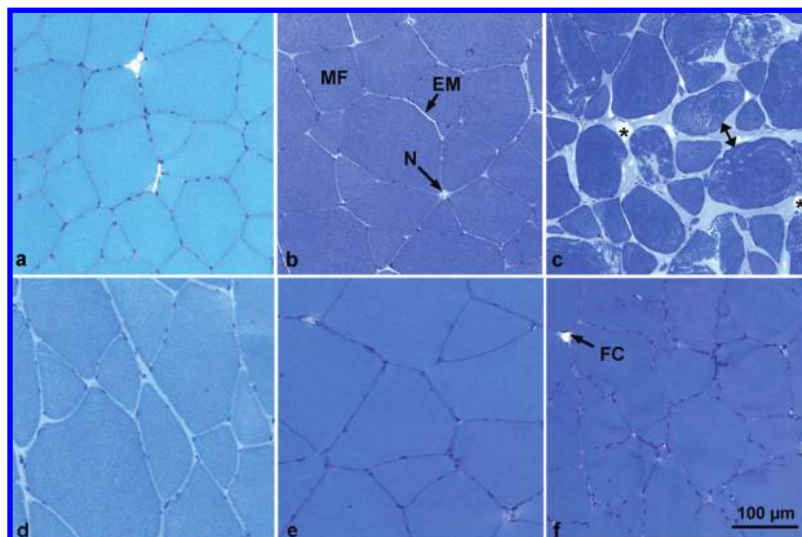
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Figure 2. Transverse sections of plastic-embedded salmon muscle stained with Toluidine Blue. The images in the upper panel (**a**−**c**) show samples from the tail part of the fish before salting, the lower panel (**d**−**f**) from the tail part after salting. (**a**) and (**d**) derive from prerigor, (**b**) and (**e**) from postrigor, and (**c**) and (**f**) from frozen/thawed salmon samples. Myofibers (MF), endomysium (EM), and myocommata (MC) and nuclei (N) stain blue and empty fat cells (FC) appear unstained. Widening of the extracellular matrix in frozen/thawed muscle is indicated by the double-headed arrow, and freezing damages are indicated by an asterisk.

(**Table 2**). Considering each group separately, there were no significant differences in salt content between the head and the tail region.

The fillet weight did not change significantly after salting (**Table 1**). This is in agreement with earlier salting experiment on Atlantic salmon (*8*).

Microstructure. The fiber diameter increased after salting in the head and the tail region both for postrigor and for frozen/thawed fish, whereas for prerigor samples the fiber diameter either decreased (tail region) or remained approximately unchanged. However, large individual differences were observed. **Figure 2** shows light microscopic images of representative samples from the tail part of prerigor, postrigor and frozen/thawed salmon muscle before and after salting. Before salting, there were obvious differences in microstructure between all raw materials. The prerigor muscle samples displayed a very dense structure making it difficult to differentiate individual myofibers. Samples from postrigor salmon also revealed a quite dense architecture, but the endomysium was better discernible than in prerigor salmon, and the individual myofibers appeared more distinct. An obvious increase in extracellular space could be observed for frozen/thawed salmon muscle. In addition, pale areas within the myofibers became visible. These "holes" in the fibers were probably caused by ice crystal formation in the sarcoplasm, or freeze-damage (*39*). The different raw material muscle structures had a pronounced influence on the salt uptake (**Table 2**). After salting, the prerigor muscle structure was less dense compared to unsalted muscle. Both postrigor and frozen/thawed muscle myofibers underwent a salt-induced swelling (*40, 41*) leading to a more dense muscle structure compared to unsalted samples. This effect was most pronounced for the frozen/thawed samples. The observations made on the light microscopic images represented a useful basis to explain possible differences in the salt diffusion rate between the three different raw material qualities as can be seen in the MR images presented in **Figure 3**. Further microscopic analyses and FT-IR data from the same experiment have been published by Böcker et al. (*42*).

MR Imaging: Salt Distribution. Figure 3 shows the ^{23}Na and ^{1}H MR images of the same slice for prerigor, postrigor and frozen/thawed fillet pieces excised from the head and tail

regions. The MR images showed that the sodium distribution in fillets after brining and the salt distribution period were still inhomogeneous. As expected, the highest salt content was observed near the surface of the fillet with a gradual decrease inward the muscle tissue. As earlier reported, the skin side was an obvious barrier for salt penetration (*8*). It was also evident that the salt had penetrated further into the leaner part of the fillet (tail region) than the more fatty part (head region), as earlier reported (*8, 10*). The ^{23}Na MR images indicate that the salt was more homogeneously distributed in the frozen/thawed fillet pieces, apparently pointing toward a more rapid salt diffusion, which was expected due to the more open muscle architecture (**Figure 2**). Deng et al. (*9*) reported a significantly higher salting rate for frozen/thawed mullet fillets compared to fresh ones, which is in agreement with our results. Our prerigor fillets had the least even salt distribution. This is in accordance to earlier findings of Wang et al. (*7*), who reported that salt distribution in prerigor Atlantic salmon was less uniform than in in-rigor or postrigor fillets. Also, Rørå et al. (*3*) reported that prerigor fillets gained less salt than postrigor fillets. Possible explanations for the slower salt diffusion/migration in prerigor fillets might be a more intact and compact muscle structure (**Figure 2**) that slows down the diffusion (*3*), and that ATP-driven ionic pumps are still active and able to maintain concentration gradients across membranes, increasing resistance to salt distribution (*43*). Also, rigor mortis and freezing/thawing are known to cause cell membrane breakage which makes water leak out of the cells. These changes in muscle structure are known to enhance the salt diffusion into the muscle (*3, 7, 9*). The results indicated that one can expect that salting procedures may have to be changed and optimized if different raw materials are to be used for salting processes.

Low-Field ^{1}H NMR: Water Dynamics. To investigate water mobility and water distribution in the muscle tissue, the LF NMR T_2 relaxation method was used. The overall variation in the NMR relaxation data of the salmon fillet groups (prerigor, postrigor and frozen/thawed) were analyzed by performing a PCA on samples taken before and after salting separately (**Figure 4**). This method allowed an overall investigation of the variation in T_2 data between raw material groups. The relaxation
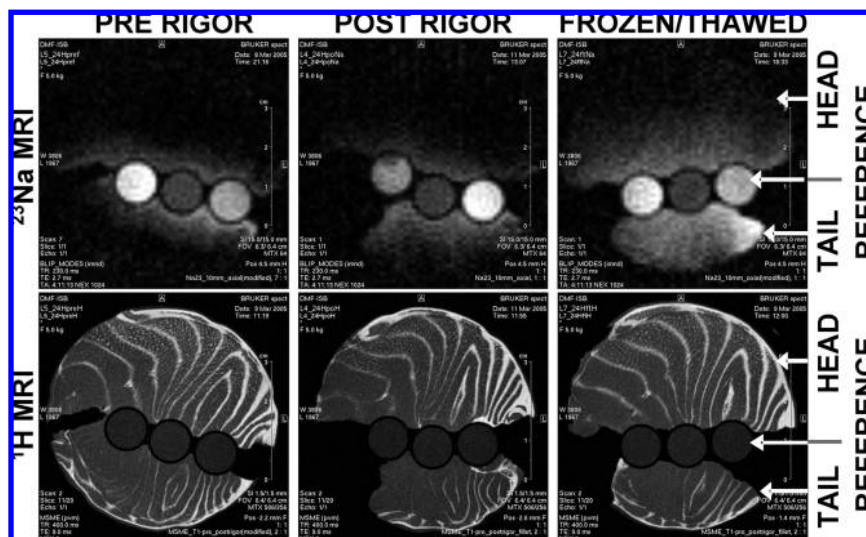
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Figure 3. Sodium (^{23}Na) and proton (^{1}H) magnetic resonance imaging of Atlantic salmon fillets salted prerigor, postrigor and after freezing/thawing. Three reference solutions (2, 4 and 6% NaCl brines) are shown as circles in the middle of each image: The circle with highest light intensity in the ^{23}Na MR images reflects the cylinder containing 6% brine solution, and the circle with lowest light intensity reflects the cylinder containing 2% brine solution.
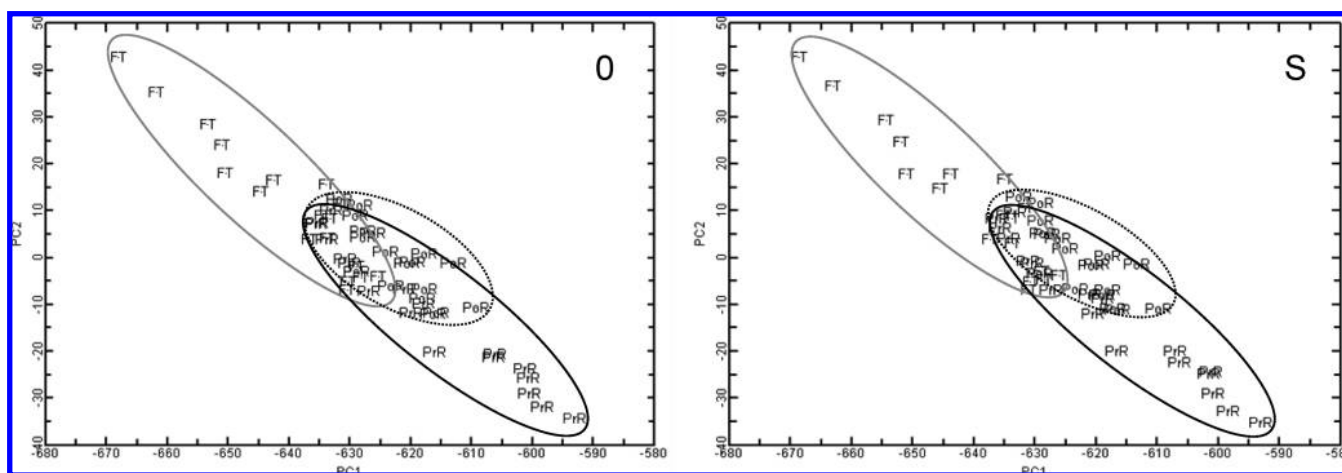
Figure 4. PCA score plots of raw T_2 data measured prerigor (PrR) (3 h post mortem), postrigor (PoR) (72 h post mortem) and after freezing and thawing (FT) (4 days post mortem). Unsalted (0) and salted (S) salmon fillets were plotted separately. For both unsalted and salted fillets, the explained variances were 99.9% and 0.07% by PC1 and PC2, respectively.

curves were normalized against maximum amplitude. In that way, only the differences in water distribution, and not the differences in total water content, influenced the PCA score plot. For the unsalted samples, the first and second principal component clearly separated between prerigor, postrigor and frozen/thawed samples. The postrigor group was placed in between the two other groups, and had the most homogeneous sample distribution. These findings are in agreement with the microstructural analyses. The prerigor and the frozen/thawed groups had a greater variance in water mobility. The spread of the prerigor group may be explained by the fact that the prerigor fillets gradually entered the in-rigor state, accompanied by a muscle contraction, whereas the spread in the frozen/thawed group may be explained by varying degree of freeze-damage as explained in **Figure 2**. After salting, the same separation between the groups was obtained. This indicated that differences in raw material quality still could be detected after addition of small amounts of salt (1−3%).

Figure 5 shows examples of the continuous T_2 distributed curves obtained in the different raw materials before and after salting. This calculation method allowed a determination of the number of T_2 populations in the different raw materials. Two main "peaks" were detected in the range of 20−100 ms (T_{21}), reflecting less mobile water, and 100−400 ms (T_{22}), reflecting more mobile water, respectively. These findings correlate well with other reported T_2 values in Atlantic salmon (*21, 28*). A shorter relaxation component, often referred to as T_{2b}, was observed in the range of 2−10 ms. The component is expressed by only the few first echoes in the raw T_2 relaxation curves, and is not always reproducible in terms of its position and amplitude. Therefore we cannot conclude with confidence what information is contained in this component, and further investigations preferably with shorter echo times need to be done. The first few echoes of CPMG relaxation curves can be prone to instability related to NMR hardware. Thus the T_{2b} component might both reflect bound water or a processing artifact arising in the Contin processing step. In some samples, most pronounced in the head part of the prerigor fish, a T_2 population arises in the range of 300−800 ms. A possible explanation might be that this peak reflects water expelled during muscle contraction (rigor mortis). However, further investigations needs to be done. Earlier studies on intact raw salmon muscle (*16*) revealed three peaks, where the two with the longest relaxation times (50 and 182 ms) correlate well with our results. Studies of other
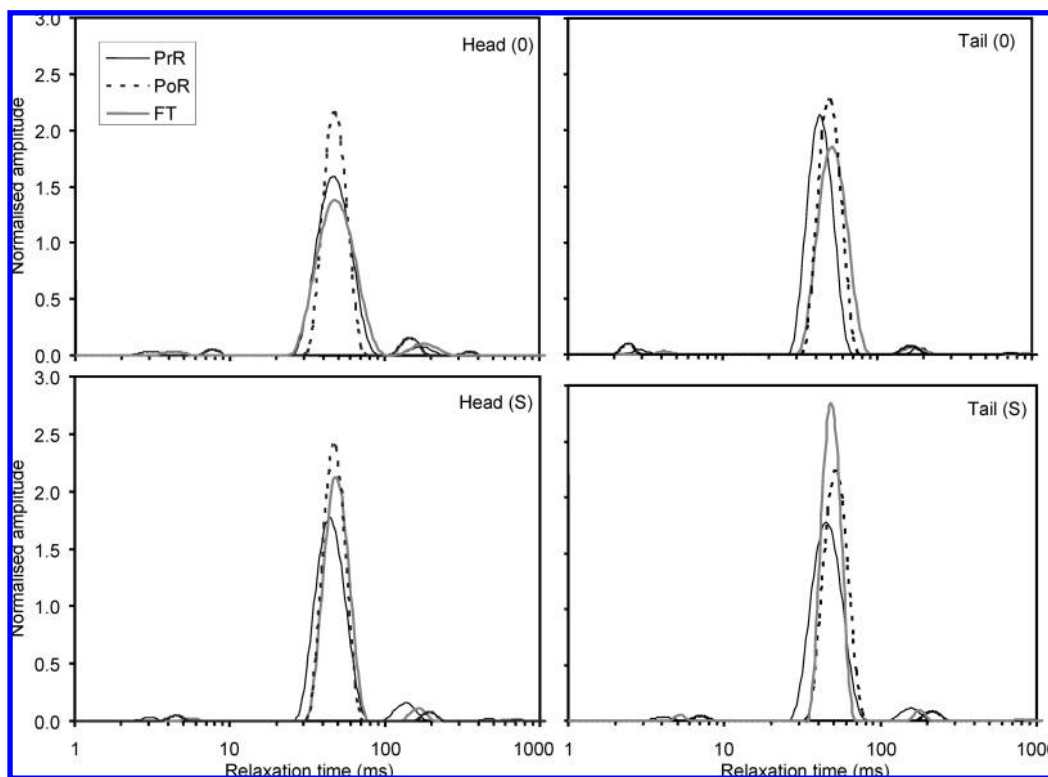
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Figure 5. Typical T_2 relaxation distributions for prerigor (PrR), postrigor (PoR) and frozen/thawed (FT) samples taken from head and tail regions of the fillet before (0) and after (S) salting. Each curve represents an average of three parallel measurements.

Table 3. Transversal Relaxation Times, T_{21} and T_{22} and Corresponding Relative T_{21} Populations Obtained in Unsalted (0) and Salted (S) Salmon Fillets Pieces of Different Raw Materials, Prerigor (PrR), Postrigor (PoR) and Frozen/Thawed (FT) [Mean(SD)][a,b]

	prerigor		postrigor		frozen/thawed	
	head	tail	head	tail	head	tail
$T_{21(0)}$ (ms)	44(0)[A]	44(0)[A]	46(1)[B]	47(1)[B]	47(1)[B]	47(1)[B,*]
$T_{21(S)}$ (ms)	45(1)[AB]	45(1)[A]	47(1)[BC]	48(0)[C]	48(1)[C]	50(1)[C,*]
$T_{22(0)}$ (ms)	134(11)[A]	139(18)[ABC]	159(12)[BC]	160(17)[C]	140(3)[AB]	140(5)[ABC,*]
$T_{22(S)}$ (ms)	140(6)[A]	133(2)[A]	160(8)[BC]	171(15)[C]	150(9)[AB]	169(14)[C,*]
T_{21} population$_{(0)}$ (%)	92(3)[B,*]	94(2)[BC,*]	94(1)[ABC]	95(1)[C]	89(3)[A,*]	93(3)[BC,*]
T_{21} population$_{(S)}$ (%)	88(2)[A,*]	91(2)[B,*]	94(1)[CD]	96(1)[DE]	93(2)[BC,*]	97(1)[E,*]

[a] Unequal letter (A, B, C, D, E) indicates significant difference ($P \leq 0.05$) comparing both raw materials and fillet regions (head/tail) for each T_2 parameter separately (ANOVA). [b] Asterisk indicates significant difference ($P \leq 0.05$) comparing unsalted and salted samples for each T_2 parameter and each raw material separately (*t* test).

fish species reported different number of peaks ranging from two to four depending on species, season and processing (*16, 18, 20, 25*). However, these studies did all report T_2 relaxation components with relaxation times similar to T_{21} and T_{22} observed in the present study. In pork meat, three peaks (T_{2b}, T_{21} and T_{22}) were reported and explained as water tightly associated with macromolecules, water located within highly organized protein structures, and water outside the myofibrils, respectively (*44*).

In **Table 3**, the relaxation times and the corresponding populations found by biexponential fitting are shown. This simple and robust method has traditionally been used to interpret T_2 relaxation data. However, one should remember that this form of data processing forces the curve to fit to two exponents. A triexponential fitting of the data set was also approached, but this fitting seemed to "overfit" the data by splitting T_{21} instead of determining the faster relaxing component, referred to as T_{2b}.

Before salting, 89−94% of the proton relaxation amplitude was reflected as T_{21} population, and the lowest T_{21} population values were obtained for the frozen/thawed head region. Furthermore, the T_{21} relaxation times were significantly shorter ($P \leq 0.05$) for prerigor samples (approximately 44 ms) compared to postrigor (approximately 46−47 ms) and frozen/thawed samples (approximately 47 ms), indicating that the water mobility was lower in the prerigor muscle structure than in the postrigor and the frozen/thawed muscle structure. No significant differences between head and tail regions were found except for in the frozen/thawed fish where the tail part exposed a larger T_{21} population than the head part of the fillet.

After salting, the T_{21} population decreased significantly ($P \leq 0.05$) in prerigor fillets (head region), corresponding to an increase in the more mobile T_{22} population. For frozen/thawed samples, an increase in the T_{21} population was found both for the head and the tail region. In case of the postrigor samples, the T_{21} population remained approximately unchanged. Furthermore, no changes in T_{21} relaxation times were observed, except for the frozen/thawed tail region, which increased significantly. A comparison of the two fillet regions revealed significantly higher values in the tail part of frozen/thawed fish for both the T_{22} relaxation time constant and the T_{21} population. The same difference in T_{21} population was observed for the prerigor salted fillets. An earlier study on cod (*27*) reported two components in fresh fish (45 and 124 ms) and in frozen/thawed fish muscle (50 and 117 ms). After brining, an increase in T_{21} values both for fresh salted and frozen/thawed salted cod, 69 and 68 ms respectively, was reported, whereas T_{22} was not found to change significantly.

Figure 6 shows how changes in T_{21} populations were related to varying fillet pH and salt contents. If all three groups (PrR, PoR and FT) were pooled as one, linear relationships between the relative change in T_{21} population and fillet pH (ΔT_{21} population $= -0.01$ pH $+ 0.7$, $R^2 = 0.78$) as well as salt content
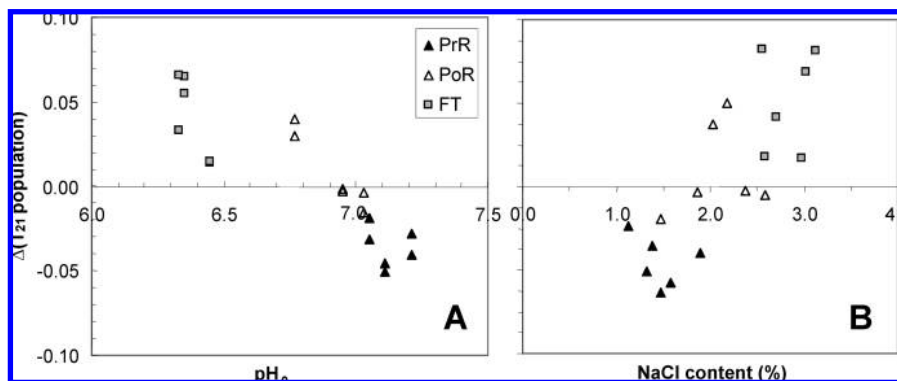
Figure 6. Fillet pH in unsalted raw material (A) and salt content (B) plotted against relative changes in T_{21} populations after salting of prerigor (PrR), postrigor (PoR) and frozen/thawed (FT) salmon fillets. The fillet pH of the salted samples differed between the groups, values are given in Table 1.

(ΔT_{21} population $= 0.05x^{\text{NaCl}} - 0.09$, $R^2 = 0.63$) were found. These relationships strongly indicated that there was a close link between T_2 relaxation data, the fillet pH and the salt uptake in the different raw materials.

A comparison of the applied methods reveals that, before salting, prerigor fillets had the most dense structure and shortest T_{21} relaxation time. Furthermore, the prerigor fillets gained least salt and no salt-induced swelling of fibers was observed, and compared to the unsalted fillets, a significant decrease in T_{21} population was observed. As earlier mentioned, these fillets went through the rigor process during salting and the salt distribution period. As described by Misimi et al. (*45*), the fish become gradually more hard and stiff during the rigor process, and the fillets change their geometrical size and shape as they shrink in length (*45*). Onset of rigor mortis is one of the main factors involved in the shrinkage and/or swelling of myofibrils. Myosin heads bind with actin to form actomyosin. As a result, the filaments spacing is reduced, and water is expelled. pH is also an important factor. During post-mortem glycolysis, the pH decreases. Consequently, the electrostatic repulsion between negatively-charged myofilaments decreases and the myofibrils shrink. These changes induces a movement of water in the muscle structure from intra- to extracellular matrix (*46–48*). In our T_2 data, the mentioned changes were probably reflected as a decrease in the T_{21} population (less mobile water) and a coupled increase in the T_{22} population (more mobile water). However, as salt was introduced into the system, other factors might also have affected the microstructure and hence the water mobility. Another explanation to the increased relative T_{22} population might be the ionic pumps possibly still functioning, trying to maintain the Na^+ concentration inside the cells, i.e., in the early post mortem period, water molecules could pass freely across cell membranes, whereas charged ions could not. Since a fraction of the muscle water will "bind" to the Na^+ ions, a larger amount of water might have been kept in the extracellular space, leading to an increase in T_{22} population. Before salting, the postrigor fillets had a less dense microstructure and significantly longer T_{21} relaxation times than the prerigor fillets. After salting, a salt-induced swelling of fibers was observed as the postrigor fillets gained somewhat more salt than the prerigor fillets; however, no significant changes in T_{21} relaxation values were observed. The frozen/thawed fillets clearly possessed a more open microstructure and had the lowest T_{21} population values, corresponding to a higher amount of water reflected in the T_{22} population, before salting. The frozen/thawed samples gained most salt, and an evident salt-induced swelling was observed. These changes were coupled to an increase in both T_{21} relaxation time and T_{21} population. The observed "holes" in the muscle fibers possibly caused by freeze-damage

presumably lead to a disruption of the muscle structure, and hence a movement of water inside the muscle 3D network before salting. These changes were reflected as a larger T_{22} population. Furthermore, the salt-induced swelling probably led to diffusion of water into the muscle fibers, as indicated by the LF NMR data as a coupled increase in both T_{21} relaxation time and T_{21} population. These findings are in agreement with earlier studies on pork meat, where a salt-induced swelling of myofibrils at salt concentrations of 3, 6, 9 and 13.9% NaCl was coupled to increased T_{21} relaxation times (*49−51*). The results indicate that LF NMR T_2 relaxation is a sensitive tool in the investigation of water mobility and water distribution within the muscle structure in different raw material qualities and during salting.

This study demonstrates the advantage of LF NMR T_2 relaxation and ^1H and ^{23}Na MRI as effective tools for obtaining further understanding of the relationship between the microstructure of fish muscle, its water mobility and its salt uptake. Moreover, it demonstrates the importance of the structural characteristics within muscle tissue for water mobility and salt diffusion.

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