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The effects of pre-salting methods on salt and water distribution of heavily salted cod, as analyzed by ^{1}H and ^{23}Na MRI, ^{23}Na NMR, low-field NMR and physicochemical analysis

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Report summary

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Titill / Title	**The effects of pre-salting methods on salt and water distribution of heavily salted cod, as analyzed by ^{1}H and ^{23}Na MRI, ^{23}Na NMR, low-field NMR and physicochemical analysis** / Áhrif forsöltunaraðferða á salt- og vatnsdreifingu fullsaltaðra þorsk afurða, greint með ^{1}H og ^{23}Na MRI, ^{23}Na NMR, lágsviðs NMR og eðliseiginleika mælingum		
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Ágrip á íslensku:	Áhrif mismunandi forsöltunaraðferða (sprautusöltun með eða án fosfats, pæklun og pækilsöltun) á vatns- og saltdreifingu í þurrsöltuðum þorskflökum (*Gadus morhua*) var rannsökuð með róteinda og natríum NMR og MRI aðferðum. Auk þessa var salt- og vatnsinnihald metið, sem og vatnsheldni. Niðurstöðurnar bentu til þess að tvísprautun með salti og fosfati gæfi af sér ójafnari vatnsdreifingu í flökunum samanborið við aðrar forsöltunaraðferðir. Aftur á móti voru pækilsöltuð flök með minnstu einsleitnina hvað varðar saltdreifingu. Flök frá öllum sýnahópum höfðu bletti með ómettuðum pækli, en slíkir blettir geta aukið hættuna á örveruskemmdum í flökunum við geymslu. Áhrif forsöltunaraðferðanna helst í gegnum allan vinnsluferilinn á bæði fullsöltuðum og þurkuðum afurðum. Þar sem einsleit vatns- og saltdreifing náðist ekki með þeim forsöltunaraðferðum sem voru rannsakaðar, er þörf á frekari rannsóknum á söltunarferlinu.		
Lykilorð á íslensku:	*Fullsaltaður þorskur (bacalao); söltunaraðferðir; MRI; NMR; eðliseiginleikar; dreifing; þurrkun*		
Summary in English:	The effect of different pre-salting methods (brine injection with salt with/without polyphosphates, brining and pickling) on the water and salt distribution in dry salted Atlantic cod (Gadus morhua) fillets was studied with proton and sodium NMR and MRI methods, supported by physicochemical analysis of salt and water content as well as water holding capacity. The study indicated that double head brine injection with salt and phosphates lead to the least heterogeneous water distribution, while pickle salting had the least heterogeneous salt distribution. Fillets from all treatments contained spots with unsaturated brine, increasing the risk of microbial denaturation of the fillets during storage. Effects from the pre-salting treatments remained throughout the processing line to both dry salted and dried products. Since a homogeneous water and salt distribution was not achieved with the studied pre-salting methods, further optimizations of the salting process, including the pre-salting and dry salting steps, must be made in the future.		
English keywords:	*Dry salted cod (bacalao); salting methods, MRI; NMR; physicochemical properties; diffusion; drying*		

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1 Introduction

The distribution of water and salt is a determining factor in the characterization of salted, brined and dried products. During the production of dry salted cod the choice of salting method has a great influence on the quality of the final product, partly due to their effect on heterogeneity of the salt and water distribution in the muscle (Erikson et al., 2004; Thorarinsdottir et al., 2010; Gudjónsdóttir et al., 2011). When full saturation of the salt in the muscle solution is not reached, an increased risk for microbial, yeast and mould spoilage activity may rise, leading to loss of quality and shorter storage time of dry salted products (ICMSF, 1980). Optimization of salting methods with regards to optimal salt and water content, as well as uniformity of these in the muscle are therefore of crucial importance.

Proton (^{1}H) and sodium (^{23}Na) Nuclear Magnetic Resonance (NMR) spectroscopy and Magnetic Resonance Imaging (MRI) are excellent noninvasive methods for the analysis of the distribution, mobility, diffusion and heterogeneity of water and salt in muscle based food and can be used for optimization of industrial salting techniques (Erikson et al., 2004, 2012; Veliyulin and Aursand, 2007). Relaxation time (T_1 and T_2) and diffusion coefficient (D) studies can be used to obtain information about the relative water and sodium distribution and behavior in food (Foucat et al., 1995; Renou et al., 1994; Veliyulin and Aursand, 2007). Several studies have shown significant relationships between proton relaxation times of water in muscle based food products to known quality attributes, such as moisture content of cod (Andersen and Rinnan, 2002; Gudjónsdóttir et al., 2011), water holding capacity in fish and meat (Jepsen et al. 1999; Bertram et al., 2007, Gudjónsdóttir et al., 2011a, 2011b, 2011c, Aursand et al., 2009), muscle pH (Bertram et al. 2000; Gudjónsdóttier et al., 2011), and how these are affected by different raw material or choice of processing methods (Erikson et al., 2012). Moreover, ^{1}H and ^{23}Na MR imaging methods can give additional information about the structure, anatomy as well as water and sodium dynamics in the intact muscle (Erikson et al., 2012; Foucat et al., 2004; Mathiassen et al., 2011).

The studies of Thorarinsdóttir et al. (2010; 2011) and Gudjónsdóttir et al. (2011) indicated that the pre-salting methods had an effect on the water distribution and water binding ability of the muscle at all processing stages of dry salted Atlantic cod. The objective of this study was to investigate further whether these differences could be explained by different levels of homogeneity in salt and water distribution and characteristics in the muscle, as affected by different salting methods, by means of proton and sodium NMR and MRI, supported by physicochemical analytical results.

2 Material and methods

2.1 Experimental design

The salting experiment was initiated on August 27th 2012 at a dry salting fish factory in Iceland. The raw material used was fillets from Atlantic cod (*Gadus morhua*) with skin, caught by long-line in the North Atlantic Ocean, west of Iceland in August 2012. The fish was bled and gutted on board the ship and stored on flake ice until processed further at the factory 3 days post-catch. The fish were divided into four groups, each containing 5 fish, or 10 fillets. Each individual fillet was identified with a numbered plastic tag. The average fillet weight was 866 ± 148 g, and no significant weight differences were observed in the raw material between the groups.

2.1.1 Salting treatments

Two of the groups were pre-salted by injecting the fillets with salt (referred to as ***Inj(S)***) and a combination of salt and phosphates (referred to as ***Inj(S+P)***). The injections for the ***Inj(S)*** group were performed in a FOMACO FGM20 80F (FOMACO Food Machinery Company A/S, Køge, Denmark) injection machine with a single head injection system (4×20 needles, each 1.6 mm in diameter), using a pressure of 0.8 bar and a salt concentration of 18.4 ± 0.1 % NaCl. The fillets were then immersed in brine with a 12.4 ± 0.1 % NaCl concentration for 2 days. Injections for the ***Inj(S+P)*** group were performed in a FOMACO FGM 64/256F injection machine with a double head injection system (2×64 quatro needles), using a pressure of 0.1 bar, salt concentration of 12.3 ± 0.1 % NaCl and phosphate concentration of 2.7 ± 0.1 %. After brine injection the fish in the Inj(S+P) group were immersed in brine with a salt concentration of 14.4 ± 0.1 % for 2 days. Fillets from the third group were pre-salted by immersion in salt brine (12.4 ± 0.1 % NaCl) for 2 days (referred to as ***brined***). All brining treatments during pre-salting were performed at a 1:1 fish-to-brine ratio. The fillets of the final group were pre-salted by the pickle salting method (referred to as ***pickled***), where the fillets were stacked in alternating layers of fish and salt in closed tubs for 2 days. This lead to the formation of saturated brine around the fish fillets when water extracts from the muscle due to concentration triggered diffusion during the pre-salting period (van Klaveren and Legrende, 1965). After pre-salting all fillets were dry salted by stacking them in alternating layers of fish and salt in open tubs at 3 - 5°C for 3 weeks. The fillets were then stored in styrofoam boxes at 0-4°C until analyzed.

The salt used for all experiments was commercial coarse salt from Tunisia. A mixture of sodium- and potassium pyrophosphates and sodium and potassium tripolyphosphates (Carnal 2110, CFB Budenheim, Budenheim, Germany) was used in the salt and phosphate injected (Inj(S+P) group.

2.1.2 Sampling

The left and right fillets of each fish were registered and numbered with a plastic number tag during the pre-salting step. The left and right fillets of each fish were assumed identical and therefore the right fillet of each fish was used for proton (^{1}H) and sodium (^{23}Na) Magnetic Resonance Imaging (MRI) and high field sodium Nuclear Magnetic Resonance (HF ^{23}Na NMR) analysis, while the left fillet was used for analysis with low-field NMR (LF-NMR) and physicochemical analyses.

Based on the observations of Wold et al. (2001), it was assumed that an uneven salt or water distribution was most likely to appear in the loin part of the fillet. Therefore an emphasis was laid on analyses of the loin part of each fillet (sampling places A, B, D and E). LF-NMR analysis was in addition performed on the tail/loin (area C) of the left fillet of each fish (Figure 1).

Two small samples (approx. 0.5 g) from each sampling area respectively were then cut for LF-NMR analysis before the rest of the left fillet of each fish was sent to the chemical laboratory for determination of water and salt content and WHC.

^{1}H and ^{23}Na MRI analysis were performed on the right fillets in areas D and E (Figure 1). After the MRI analysis small samples were cut from areas D and E and placed into 10 mm NMR tubes and total sodium concentration and distribution were analyzed with HF ^{23}Na NMR. The settings for these measurements are described in more detail in chapter 2.2 and 2.3.

2.2 Magnetic Resonance Imaging (MRI) analysis

All MRI measurements were performed on a 4.7 T Bruker Biospec 47/40 instrument interfaced to an Avance III console (Bruker BioSpin MRI GmbH, D-76275 Ettlingen, Germany), with an in-house built double-tuned 72-mm diameter ^{1}H/^{23}Na coil, inserted into the 40 cm clear bore along with a surrounding Bruker BGA-12 gradient coil. All acquisitions were performed using the ParaVision 5.0 software (Bruker BioSpin MRI GmbH, D-76275 Ettlingen, Germany). Samples from areas D and E (Figure 1) were cut from the fillets and packed in vacuum bags (50 % vacuum) to prevent water dripping into the magnet. The slices were placed parallel with the magnetic field and acquisitions were performed in the sagittal direction. This was chosen since pre-analysis indicated less heterogeneity in the magnetic field B_0 in the sagittal direction than in the axial direction. Optimal placement of the sample in the magnet was assessed with the standard Bruker TRIPILOT protocol. The imaging methods used in this study included:

- A **TurboRARE** (Rapid Acquisition with Refocused Echoes) analysis to obtain high resolution ^{1}H T_2 weighted contrast anatomical proton image of the fillets. TurboRARE acquisitions were

performed using TE=11 ms, effective echo time 33 ms, rare factor 8, TR=3000 ms, 16 averages, 1 repetition, and with fat suppression. Analyzing time = 25 min and 36 s.

- A **Multi-Slice-Multi-Echo (MSME)** analysis to obtain ^{1}H T_2 relaxation and proton density contrast maps of the fillets. Settings from the MSME T_2 analysis were 6 echo times (TE) ranging from 12 to 72 ms in 12 ms steps, number of averages (NA) 8, number of repetitions (NR) 1, 180° refocusing flip angle, two 2-mm thick slices with 6 mm slice distance, a FOV of 80x60 mm, 256x256 matrix size, resulting in the analyzing (acquisition) time of 51 min and 12s. The T_2 and ρ (proton density taken as $S_{0,}$ the signal intensity extrapolated at TE=0) maps of each slice were then generated by fitting the echo signal decay (S) to $S=S_0*Exp(-TE/T2)$ pixel wise, using a non-negative least square algorithm.
- A **^{1}H FLASH** (Fast Low Angle Shot) acquisition to analyze susceptibility differences due to differences of texture in muscle, bones, connective tissue etc. using the following settings: TE=5.224 ms, TR=350 ms, 2 averages, 1 repetition, α=40°. Analyzing time = 2 min, 14 s and 400 ms.
- **Sodium ^{23}Na FLASH** analysis to see the sodium distribution in the fillets. Settings for the sodium images were TE=4.094 ms, TR=250 ms, 200 averages, 1 repetition, α=60°, excitation pulse length 2.724 ms, bandwidth 1000 Hz, 10 mm slice thickness, 2 slices with 10 mm slice distance. Analyzing time = 1 h, 46 min and 40 s.
- An **Apparent Diffusion Coefficient (ADC)** mapping analysis for water protons, using a Pulsed-Gradient Spin Echo sequence with parameters: TE=27 ms, TR=2000 ms, 2 averages, 14 ms diffusion time duration (Δ), 7 ms duration of the diffusion gradient pulse (δ). Four diffusion gradient strengths (G) were used per experiment resulting in b values ($b=\gamma^2G^2\delta^2*(\Delta-\delta/3)$), of 2.75 s/mm^2, 100 s/mm^2, 500 s/mm^2 and 1000 s/mm^2. The total acquisition time of each experiment was 42 min and 40 s. The ADC maps were generated by using the linear regression to fit pixel wise for each slice, the diffusion signal (S) to the expression $Ln(S/S_0)= -bD$, S_0 being the signal intensity for G=0. This method was only applied to the Inj(S+P) and pickled samples.

The slice thickness for all ^{1}H MRI acquisitions were 2 mm, with a 6 mm slice distance and 2 slices, while for the sodium FLASH acquisitions a slice thickness of 10 mm and a 10 mm slice distance was used. This was done to compensate for a low sodium signal in the slices. However, choosing a higher slice thickness for the sodium measurements lead to loss in details in the images instead. They do though indicate the general salt distribution throughout the muscle. The same field of view (FOV 80x60 mm) was used for all acquisitions and the matrix size was 128x128 for all sequences, except

for the MSME sequence where a matrix size of 256x256 was used. Two tubes with solutions of 15 % and 20 % w/w NaCl concentration respectively were placed next to the sample for reference.

2.3 High field NMR measurements

The high field NMR experiments were performed on an Oxford Instruments 9.4 T magnet, using a double tuned $^{1}H/^{23}Na$ NMR probe at a 105.8 MHz sodium resonance frequency.

Single quantum (SQ) measurements on total sodium concentration in were performed estimate total salt concentrations in the same samples as used for the MRI analysis. Two samples were cut out from each sampling place D and E respectively and analyzed in the high field instrument. The average sample weight was 1.2±0.2 g. The obtained peak areas were integrated using the PeakFit software (Version 4.12, SeaSolve Software Inc., San Jose, CA 95110 USA), which was then compared to the integrated area of a calibrated reference NaCl solution. All results were normalized based on sample weight.

Transversal relaxation times of sodium ions were determined with the Carr-Purcell-Meiboom-Gill (CPMG) (Carr and Purcell, 1954; Meiboom and Gill, 1958) pulse sequence, using 2048 echoes, 64 scans, 100 ms recycle delay, and a fixed echo time of 100 µs. The on-resonance π/2 pulse was 17.5 µs and the receiver gain was 181 dB for all experiments. Matlab 1212b (The Mathworks Inc, Natric, MA) was used for multi-exponential fitting of the obtained relaxation curves.

2.4 Low field NMR measurements

The instrument used for low field NMR analysis was a Bruker mq 20 benchtop NMR analyzer (Bruker Optics GmbH, Rheinstetten, Germany) with a 20 MHz magnetic field frequency. Two samples (approximate sample weight: 0.5 g) were cut from each sampling position A, B and C respectively and placed in 10 mm sampling tubes. These were analyzed with a CPMG pulse sequence with an echo time of 250 µs and 8100 collected echoes. The receiver gain (RG) was set to 70 dB, the recycle delay (RD) to 10 s, and 16 scans and no dummy shots were used. All measurements were performed at an ambient temperature of 20 ± 1 °C. The obtained relaxation data was maximum-normalized by setting the maximum echo to a value of 100 while other echoes were scaled successively. The Low-field NMR toolbox for Matlab (The Mathworks Inc., Natick, Mass., U.S.A.), as described by Pedersen et al. (2002) was used to fit the relaxation data to a multi-exponential curve.

2.5 Physicochemical reference measurements

The water content of the fillets was analyzed by comparing the weight of 5 g of raw minced muscle to the weight of the sample after drying in a ceramic bowl for 4 h at 103 ± 2 °C. The water holding capacity (WHC) was determined with a centrifugal method as described by Eide et al. (1982) while

the salt content (on a dry basis) was analyzed with the Volhard Titrino method (AOAC, 2000). The salt concentration on a wet basis (Z^{NaCl}-value) was calculated according to the equation:

$$Z^{NaCl} = \frac{X_{salt}}{X_{salt}+X_{water}} \cdot 100 \qquad (1)$$

where X_{salt} and X_{water} were the mass fractions of salt and water respectively.

2.6 Data handling and analysis

All physicochemical and LF-NMR results are presented as averages from the analysis of 3 fillets for each pre-salting treatment, while MRI and HF-NMR results are averages from 2 fillets from each treatment. Statistical analysis and figure plotting were performed in Microsoft Excel 2007 (Microsoft Corporation, U.S.). A two tale t-test assuming unequal variances was used to assess statistical differences between the treatments.

3 Results and Discussion

3.1 Physicochemical reference measurements

Results from physicochemical analysis of water and salt content, Z^{NaCl}-value and WHC of the fillets after dry salting as affected by different pre-salting methods can be seen in Figure 2.

The water content in the fillets after dry salting was in the range from 57.1 to 59.7 % (w/w) which are similar to the values obtained by Thorarinsdottir et al. (2010). As in the Thorarinsdottir et al. (2010) study significantly lower water content was observed in the brined and pickled samples compared to the injection treated samples (p=0.03). However, no significant difference was seen in the water content between the two injection treatments. This indicated that brine injection increased the water uptake of the muscle compared to pre-salting by brining or pickling, as suggested by Thorarinsdottir et al. (2010).

The salt content of the dry salted fillets ranged from 20.7 to 21.6 % on a total sample weight basis, but a significantly lower salt content was observed in the pickled fillets than the fillets from other treatments according to a 1-way ANOVA analysis (p=0.01). However, the Z^{NaCl}-value (salt concentration on a wet basis) indicated that the salt solutions in the muscle were saturated for all treatments on average.

A higher WHC was observed in the brined and pickled fillets compared to the injection treated fillets (p=0.0001). It is possible that the injection treatment is here leading to more drip due to puncturing of cells, as speculated by an earlier study (Gudjónsdóttir et al., 2011). No significant difference

between the WHC in the different injection treated fillets, in agreement with no significant differences observed in water or salt content of these fillets.

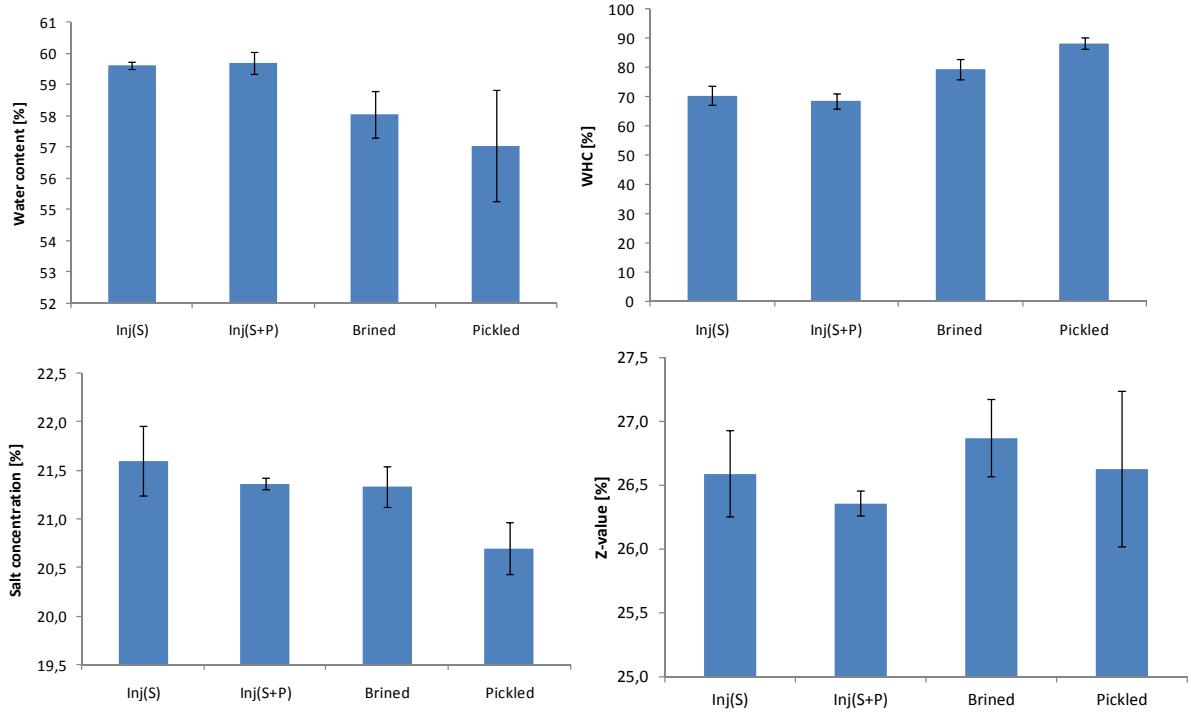

Figure 1: Physicochemical analysis results for water (top left), salt (bottom left), WHC (top right) and Z-value (bottom right) in dry salted cod treated with different pre-salting methods.

3.2 Low field NMR measurements

Multi-exponential fitting of the obtained low field CPMG data indicated the presence of two water populations, with a dominant faster relaxing component ranging from 26.0 to 37.1 ms (T_{21}) and a slower relaxation component ranging from 288 to 459 ms (T_{22}), in agreement with earlier studies (Andersen and Rinnan, 2002; Gudjónsdóttir et al., 2011; Erikson et al., 2004). The faster relaxation component is believed to correspond to myofibrillar water and water within the protein structure, while the slower relaxing component corresponds to extra-myofibrillar water (Bertram et al., 2001, Erikson et al., 2004; Gudjónsdóttir et al., 2011). These findings are in agreement with the results obtained in an earlier study of the same research team (Gudjónsdóttir et al., 2011), where significantly longer relaxation times in the injected fillets were correlated to the effect of salt induced muscle swelling during the pre-salting step. This was linked to less protein denaturation during dry salting of the brine injected fillets. In both studies, the shortest relaxation times were observed in the pickled fillets, indicating the highest degree of protein denaturation. A small but still significant

difference was observed between the two brine injected groups, where the phosphate addition led to slightly longer relaxation times.

Andersen and Rinnan (2002) observed variations within the two water populations along the fillets in fresh cod, having a dominating shorter relaxing population near the head, while the longer relaxation time was observed closer to the tail. This was explained by that smaller muscle cells and fibers were found in the tail, which in turn influenced the water distribution in the muscle. No such differences were observed in the relaxation times or their representative populations according to sampling placement along the fillet in this study (data from individual sampling spots not shown), indicating that the protein denaturation obtained during dry salting may minimize the differences in water distribution due to different cell sizes within the fillets.

When the LF-NMR results were compared to the physicochemical parameters on an individual fish only the faster relaxation time T_{21} showed significant correlations to the reference parameters (Figure 4). In agreement with earlier studies (Gudjónsdóttir et al., 2011; Andersen and Rinnan, 2002) the T_{21} parameter increased with a higher water content of the fillets (R^2=0.6074) and salt content (R^2=0.5082) with medium high, but still significant correlations. WHC of the fillets was negatively correlated to the T_{21} relaxation time (R^2=0.877). Since protein denaturation in the muscle can be associated with shorter T_{21} relaxation times, these results indicates that more protein denaturation leads to higher WHC in the muscle after dry salting. This confirms the observations of Thorarinsdottir et al. (2010), who speculated that additional water obtained by brine injection during the pre-salting step was more loosely bound than the water in the more denatured brined and pickled muscles. This muscle swelling effect of the brine injected fillets remains after the dry salting and has a protective effect on the denaturation of proteins due to the salting out effect during the dry salting step. Since the proteins in the pickled and brined fillets are more denatured, they also lose more water during this step. However, the water which remains in the muscle is then more strongly bound, as indicated by a higher WHC.

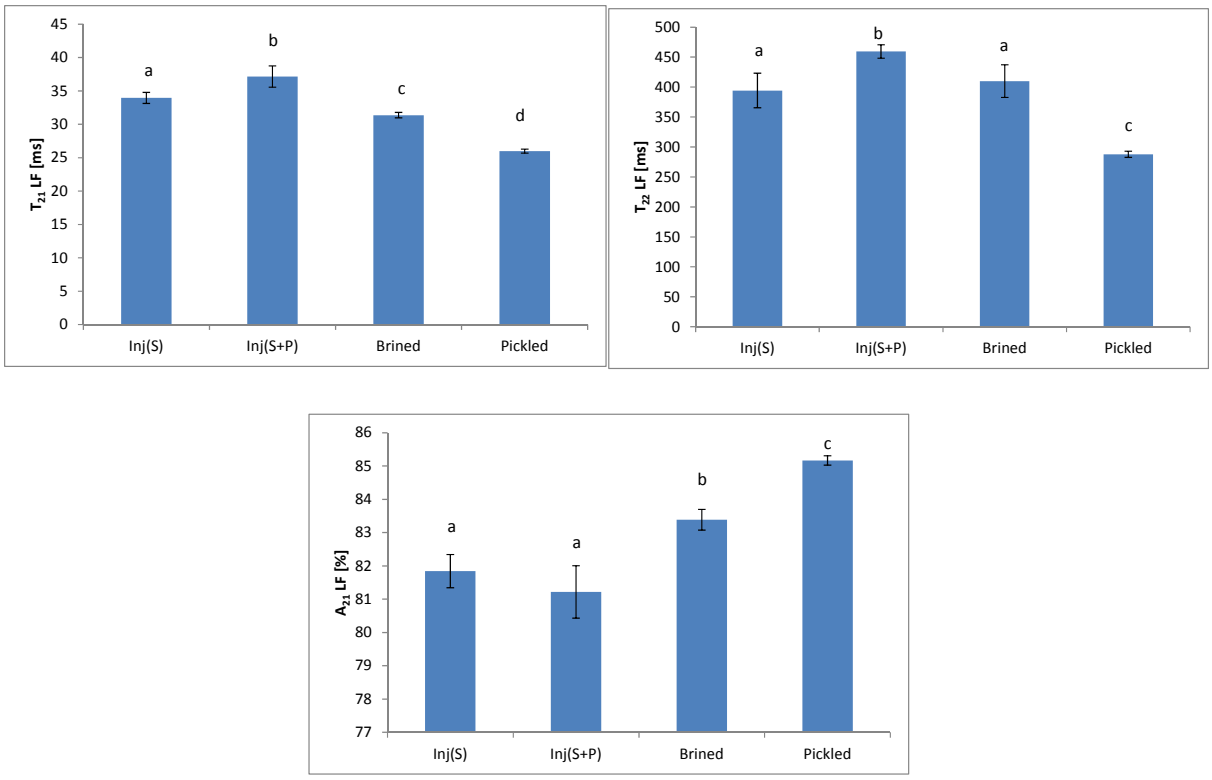

Figure 2: Low field ^{1}H NMR transverse relaxation time results in dry salted cod fillets.

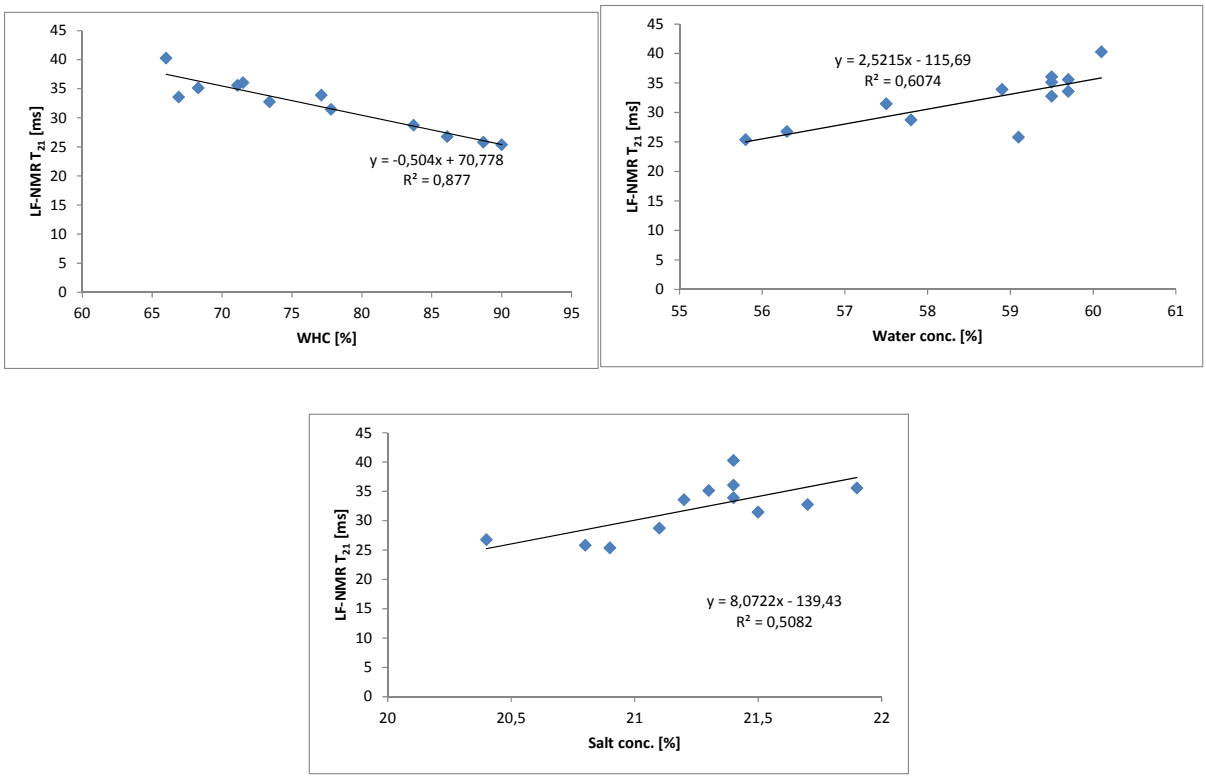

Figure 3: Partial Least Square Regression models of LF-NMR T_{21} relaxation time to WHC, water and salt content respectively.

3.3 MRI measurements

Characteristic MR Images of the dry salted fillets obtained with the different MRI pulse sequences are presented in Figures 2 and 3.

T_2 weighted images obtained with the ^1H TurboRARE sequence showed the muscle structure in great detail, distinguishing well between muscle tissue, skin, connective tissue between muscle "flakes" etc (Figure 2). Water in the muscle were observed as high signal intensity areas, while bones and needle holes after injection treatments were mostly seen as dark spots. The bones were usually found close to the belly flap, on the left side of the images.

Proton density MSME images of fillets from all treatments were obtained (Figure 2) and T_2 relaxation times were calculated from the T_2 distribution maps at three regions of interest (ROIs); the first region close to the fillet surface (ROI 1), the second in the middle of the fillet (ROI 2) and the third close to the skin (ROI 3) in each slice (Table 1). Six echo images were used in this study, which is not enough to produce a multicomponent decay. T_2 values were therefore obtained by fitting the obtained decays with a mono-exponential fit, resulting in pixel T_2 values which represent a weighted average of the water populations in the dry salted cod muscle. The analysis indicated that the Inj(S+P) pre-salting fillets had the least spatial water heterogeneity (smallest range in obtained T_2 relaxation times) in the dry salted product, followed by the brine pre-salted fillets. No significant difference was seen in the water heterogeneity between the Inj(S) and the pickled fillets.

When the water distribution for each pre-salting method was studied individually significantly longer T_2 values were obtained in the middle and closer to the skin in the Inj(S) than close to the fillet surface. In the Inj(S+P) and the brined fillets longer T_2 values were also obtained close to the skin, but in these groups no significant difference was seen in the T_2 values close to the fillet surface or in the middle of the muscle. However, in pickled fillets the longest T_2 relaxation times were observed close to the surface of the fillets. This indicated that the different pre-salting methods led to different inhomogeneity patterns in the water distribution in the dry salted fillets. When comparing the T_2 relaxation times at each ROI longer relaxation times were observed in the injected samples at ROI 2 and 3 indicating that the injection aided water migration into these regions. According to Gallart-Jornet et al. (2007) subcutaneous fat in Atlantic salmon was an effective salt migration barrier during brining, while the skin of Atlantic cod did not serve this purpose. In the brine injected fillets a brining step followed the initial brine injections, possibly explaining the similarities in the T_2 values close to the skin (ROI 3) for these three groups.

^{1}H FLASH images are sensitive to susceptibility differences in the muscle and can therefore be used to distinguish well between matters with large differences in density, such as muscle tissue and water as well as between muscle and bones or solid salt crystals on the fillet surface. The muscle tissue gave a higher signal, while injection holes, bones and salt crystals appeared as black spots (Figure 3). These images revealed clear marks of injection in the Inj(S+P) fillets, indicating a rougher injection treatment in these fillets than in the Inj(S) fillets.

All ^{1}H imaging methods showed clear marks of injection in the Inj(S+P) treated fillets, while these injection marks were not as evident for the Inj(S) fillets. This effect was especially apparent in the proton FLASH images, due to their sensitivity to susceptibility differences. The clear injection marks in the Inj(S+P) fillets, compared to the Inj(S) fillets, were believed to be primarily caused by the different injection settings, including the number of needles, used pressure etc., rather than due to the different compositions of the injected brines. Water accumulation, due to these injection punctuations, mostly appeared between the muscle flakes and close to connective tissue. However, the higher degree of injection punctuation of the Inj(S+P) fillets did not have a significant effect on the WHC of the muscle after dry salting between the injected groups. No differences were observed in the fillet surface appearance between the two injected groups as viewed with bare eyes. It is therefore clear that MRI gives a unique insight into the effect of injection into the muscle. Lower WHC in the brine injection treated fillets compared to brined and pickled fillets supports that brine injection leads to punctuation denaturation of the muscle and thus also a loss in water retaining ability of the muscle. Naturally, no punctuation holes were observed in the brined or pickled samples, since no needles were used for these treatments. However, more heterogeneity in proton signals were observed in these fillets compared to the brine injected fillets.

Sodium MRI images were obtained with 10 mm thick slices (Figure 3). This lead to a decrease in spatial resolution and details in the images, compared to the proton images, which had 2 mm thick slices. They did though give insight into the salt distribution of the fillet. Higher sodium signals were generally observed where the proton signal also was high, which is in agreement with the study of Martinez et al. (2003). This indicated that most of the salt was dissolved in the muscle water. Ishida et al (1991) stated that the ^{23}Na MRI signal intensity was proportional to the sodium ion concentration but also depended strongly on the sodium mobility. This supports that a higher sodium signal would be obtained from less restricted sodium ions, rather than the more restricted/bound ones in the dry salted muscle as well. Earlier studies have also shown that sodium visibility during ^{23}Na MRI analysis is restricted and in correlation with the sodium concentration and that up to 60% of the sodium may be “invisible” at high salt concentrations in the muscle (Veliyulin and Aursand, 2007; Renou et al., 1994). This had to be kept in mind during interpretation of the results. However,

in this study the sodium images were not used to quantify the sodium in the muscle directly, but rather to indicate possible effect of the different salting methods on the homogeneity of the sodium distribution in the muscle.

The images revealed different patterns in heterogeneous sodium distribution in the fillets after dry salting depending on their pre-salting method. Interestingly, the most heterogeneous salt distribution was observed in the brined fillets, while the most homogeneity in salt distribution was observed in the pickle salted fillets. According to Erikson et al. (2004) brining of fresh and frozen thawed Atlantic cod lead to a non-uniform salt distribution and Renou et al. (1994) and Foucat et al. (1995) indicated that brine injection could also lead to an uneven salt and water distribution. Erikson et al. (2004) also reported the highest salt concentrations close to the fillet surface with a gradual decrease towards the skin side. A similar trend was only seen in the Inj(S) samples. This indicated that the dry salting step has a significant effect on the salt distribution as well. However, since a heterogeneous salt and water distribution are obtained with all pre-salting methods one could speculate whether the dry salting period is long enough for optimization of the water and salt distribution.

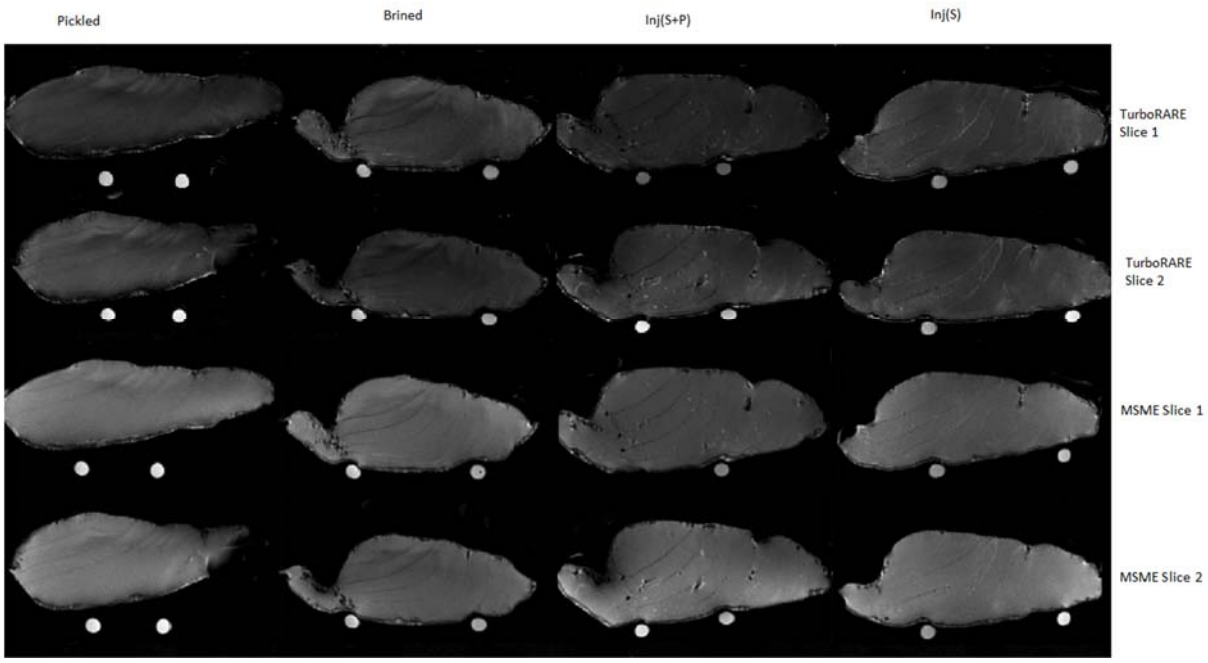

Figure 4: ^{1}H MRI cross-sectional images of dry salted cod fillets using TurboRARE (above) and MSME (below) pulse sequences. Light circles indicate reference salt solutions. Two slices from each treatment are shown.

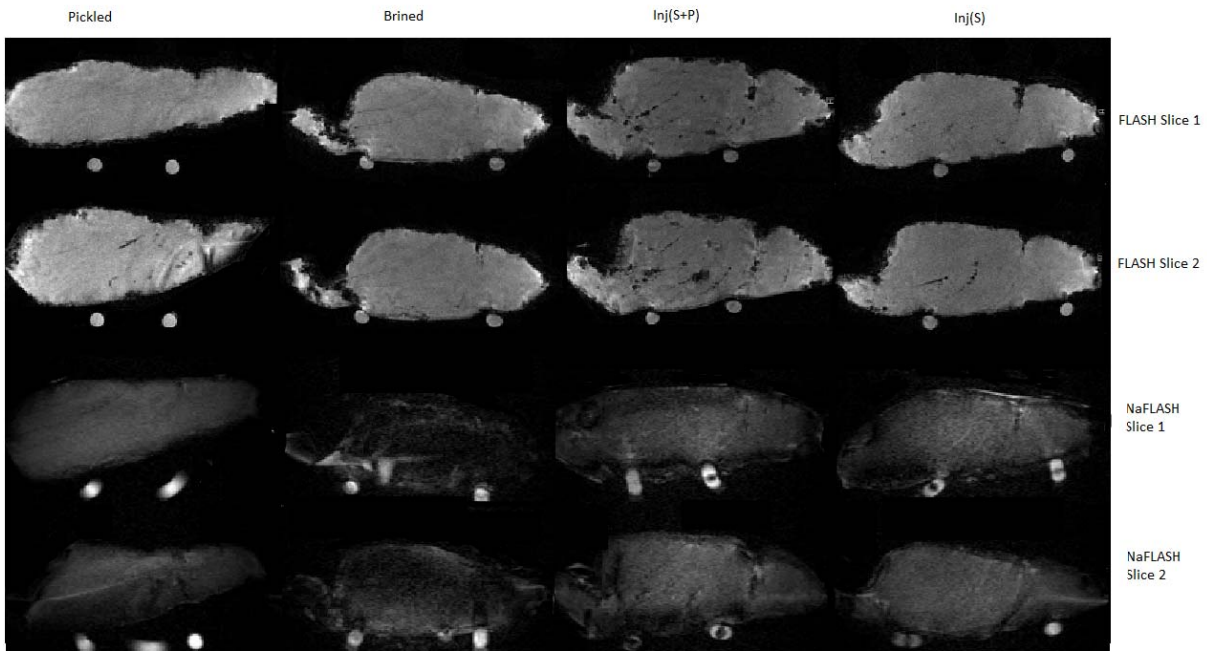

Figure 5: MRI cross-sectional images of dry salted cod using proton (^{1}H) (above) and sodium (^{23}Na) (below) FLASH pulse sequences. Light circles indicate reference salt solutions. Two slices from each treatment are shown.

An additional experiment was performed to see the effects of short time storage on the final products. One fillet of the Inj(S) treatment was left in the MRI magnet for 24 hours and then analysed again with the TurboRARE method (Figure 7). The image revealed substantial amounts of water that had been expelled from the muscle structure into the extracellular space. The water gathered especially between flakes, as well as close to the skin. This indicated that the storage conditions can have a significant effect on the water distribution in the fillets and this emphasized the importance of proper storage and immediate analysis of the fillets.

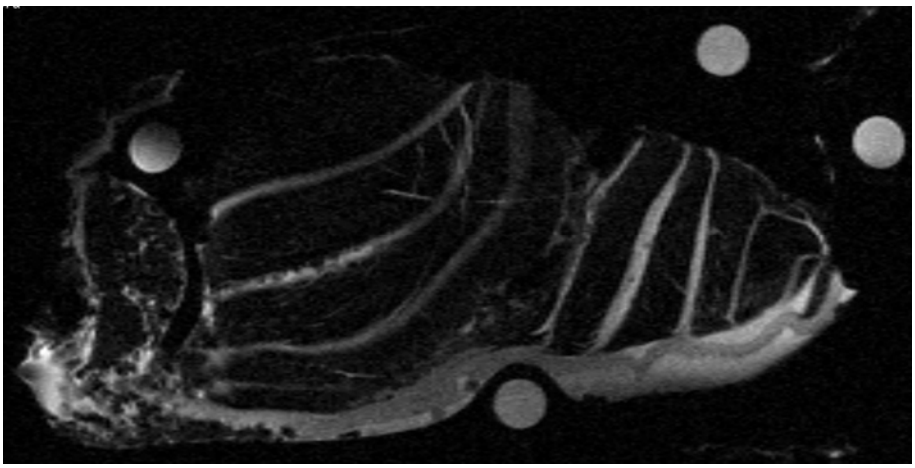

Figure 7: TurboRARE image of brine injected (Inj(S)) fillet after 24 h in the magnet. Light circles indicate reference salt solutions.

3.4 Diffusion analysis

Proton diffusion was analyzed in the Inj(S+P) and pickled fillets by generating Apparent Diffusion Coefficient (ADC) maps in the MRI instrument. According to Foucat et al. (1995) the diffusion coefficient (D) is more sensitive to changes in muscle structure than the transversal relaxation time T_2, due to its connection to the translational motion of water, while T_2 is more related to the rotational motion of water. The proton diffusion images are shown in Figure 8. Assessment of the diffusion constants at the three regions of interest (ROIs) showed heterogeneous water diffusion behavior in the muscle (Figure 9). Generally, a higher apparent diffusion coefficient D was observed in the Inj(S+P) fillets compared to the pickled fillets. This is in agreement with the higher water content and higher water mobility in the Inj(S+P) treated fillets (R^2=0.615). This is in agreement with the observations of Veliyulin and Aursand (2007), which showed a positive connection between the longitudinal relaxation time T_1 and apparent diffusion coefficients in brined cod and salmon muscle salted at various brine concentrations and linked lower diffusion constants with muscle shrinking and denaturation at high salt concentrations. A slightly lower D was observed close to the fillet surface (ROI 1) in the Inj(S+P) fillets compared to the diffusion coefficients obtained in regions deeper into the muscle (ROI 2) or close to the skin (ROI 3). This indicated more muscle shrinkage and denaturation at the surface, while the brine injection deeper into the muscle seems to have a slight protecting effect against the denaturation caused by the dry salting treatment. Diffusion coefficients in the pickle salted fillets were lower than in the Inj(S+P) fillets but also more uniformly distributed throughout the muscle, indicating an overall higher degree of muscle shrinkage and denaturation in these fillets.

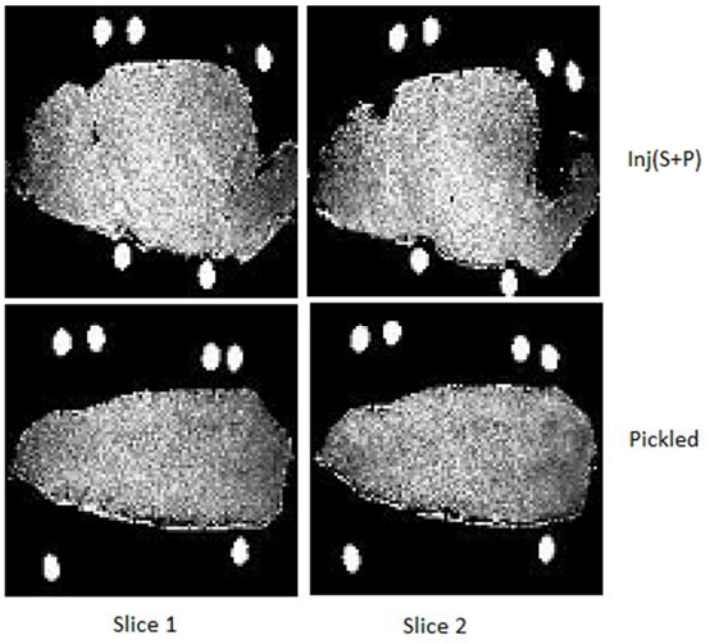

Figure 8: Apparent Diffusion Coefficient (ADC) maps of dry salted cod fillets pre-salted with sodium and polyphosphate injections (Inj(S+P)) or with pickle salting.

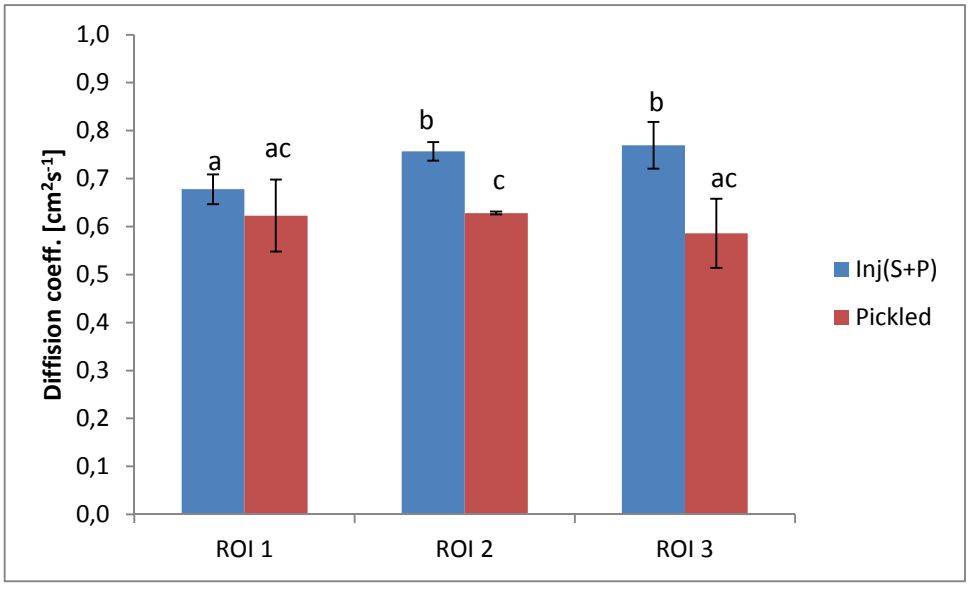

Figure 9: Apparent Diffusion Coefficient (ADC) of dry salted cod fillets pre-salted with sodium and polyphosphate injections (Inj(S+P)) or with pickle salting in the three regions of interest (ROI 1, close to the surface, ROI 2 in the middle of the muscle and ROI 3 close to the skin).

3.5 HF ^{23}Na NMR measurements

Mouaddab et al. (2007) described a technique for absolute quantification of bound and free ^{23}Na nuclei using a double-quantum filtered (DQF) NMR method. This method was successfully adapted to fit investigation of the salt distribution in model cheeses (Gobet et al., 2010; Andriot et al., 2011; Boisard et al., 2013, Boisard et al., 2014) and French baguette breads (Gudjónsdóttir et al., 2013). This method was tried in this study as well. However, since the dry salted cod fillets had a salt content of a magnitude up to approximately 25 times higher than in the earlier mentioned studies, and some dry salted samples showed more sodium relaxing components than assumed by in the DQF method (one bound and one free component), this method could not be used for absolute quantification of bound and free sodium in the current study. Thus ^{23}Na relaxation time analysis was performed on the samples instead to give a relative sodium distribution in the dry salted fillets, coupled with an absolute quantification of the overall salt concentrations in the muscle using the single quantum method (SQ).

3.5.1 Total salt concentration (SQ-method)

Total sodium analysis with the SQ NMR method was performed on samples cut from the middle of the muscle from sampling point D and E from each fillet. The salt concentration (NaCl) of the muscle was calculated from the obtained sodium peak areas in comparison to the concentration of a calibrated reference peak. No significant differences were observed in the total salt concentrations when analyzing the averages of the different treatments (salt concentration 17.8±0.7 %). However when the salt concentrations in individual fish were analyzed an uneven salt distributions along the length of the fillets could be observed. A general trend of higher salt concentrations closer to the head (sampling area D) was observed, although this difference was not significant in all fish (Figure 10).

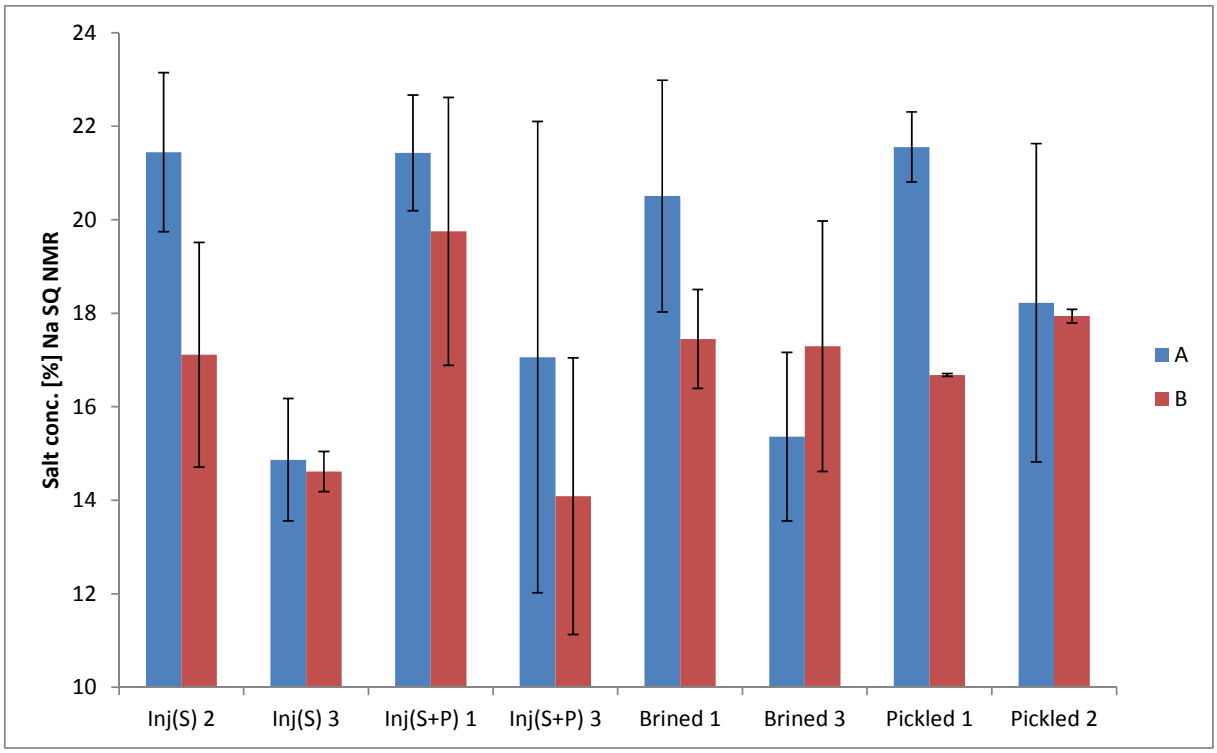

Figure 10. Salt concentrations in the middle of the fillets (ROI 2) from sampling D (close to head) and E (closer to tail) as obtained with the SQ ^{23}Na NMR method. Concentrations are based on an overall sample weight basis.

When the salt concentrations obtained with the SQ NMR method was compared to the chemically obtained results one could see that the SQ results were on average 15.5% lower than the chemical salt concentration results. This can possibly be explained by the sampling method, but sampling for the SQ method was taken from the middle of the fillet (relating to ROI 2 in MRI analysis) and did not include any of the surface crystalline salt. This sampling was chosen for two reasons: i) the middle of the fillet is more likely to include unsaturated salt solution than muscle closer to the surface and ii) crystalline salt cannot be detected with this NMR method.

Furthermore, the analysis indicated that the salt solutions in the middle of the muscle were only fully saturated (Z^{NaCl}-value > 26%, figure 11) in some fish and on average in none of the treatments. The samples that reached salt saturation were all sampled in position D, closer to the head, except one sample taken at position E in the Inj(S+P) group. It is though important to note, that this method does not distinguish between sodium from salt (NaCl) or from other sources, such as sodium polyphosphates. The salt concentrations of the fillets treated with sodium polyphosphates (Inj(S+P) and Brined) may therefore be overestimated. However, the spatial salt distribution obtained with the ^{23}Na NMR method indicated that a risk for microbial growth, due to unsaturated salt solution in the muscle, is present in the dry salted fillets irrelevant to the pre-salting methods analyzed. The analysis indicated furthermore that the risk for desalted spots was higher in the loin part further down from the head (sampling place E) rather than closer to the head (sampling place

D). The spatial salt distribution obtained with the ^{23}Na NMR method therefore indicates that this risk is present in all salting methods analyzed, and that the risk is higher for desalted spots in the loin further down from the head (sampling place E).

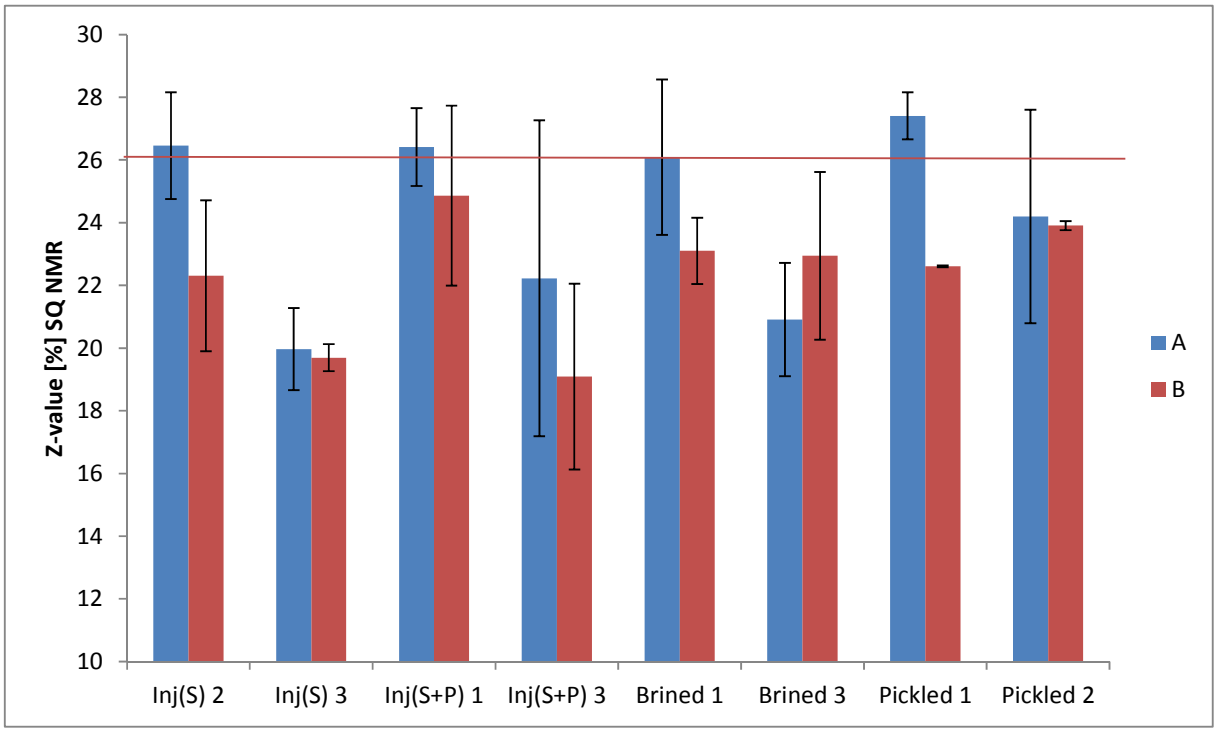

Figure 11: Z-salt value based on SQ ^{23}Na NMR analysis. Red horizontal line indicates fully saturated salt brine (26 %).

3.5.2 Sodium distribution and mobility (CPMG pulse sequence method)

The sodium distribution within the muscle at each sampling position was analyzed with the CPMG pulse sequence. Most samples showed bi-exponential behavior, indicating the presence of two salt populations $A_{21}{}^{23}$Na and $A_{22}{}^{23}$Na with corresponding relaxation times $T_{21}{}^{23}$Na and $T_{22}{}^{23}$Na (Figure 12).

The analysis indicated that there was no significant difference in the salt distribution within the muscle between the different treatments (A_{21} ^{23}Na). However, some differences were observed in the relaxation times of the fillets according to the salting method. The sodium in the pickled fillets had shorter relaxation times (both T_{21} ^{23}Na and T_{22} ^{23}Na) than in the other treatments, indicating a more bound state of these sodium ions. This is likely related to the lower water content measured in the pickled fillets, compared to the other treatments. The faster relaxation time of Inj(S+P) fillets (T_{21} ^{23}Na) was significantly longer than for the other treatments. This can possibly be explained by the sodium polyphosphate present in these samples or by the fact that the muscle was partially damaged/denatured by the injection procedure, leading to less salt binding ability of the muscle.

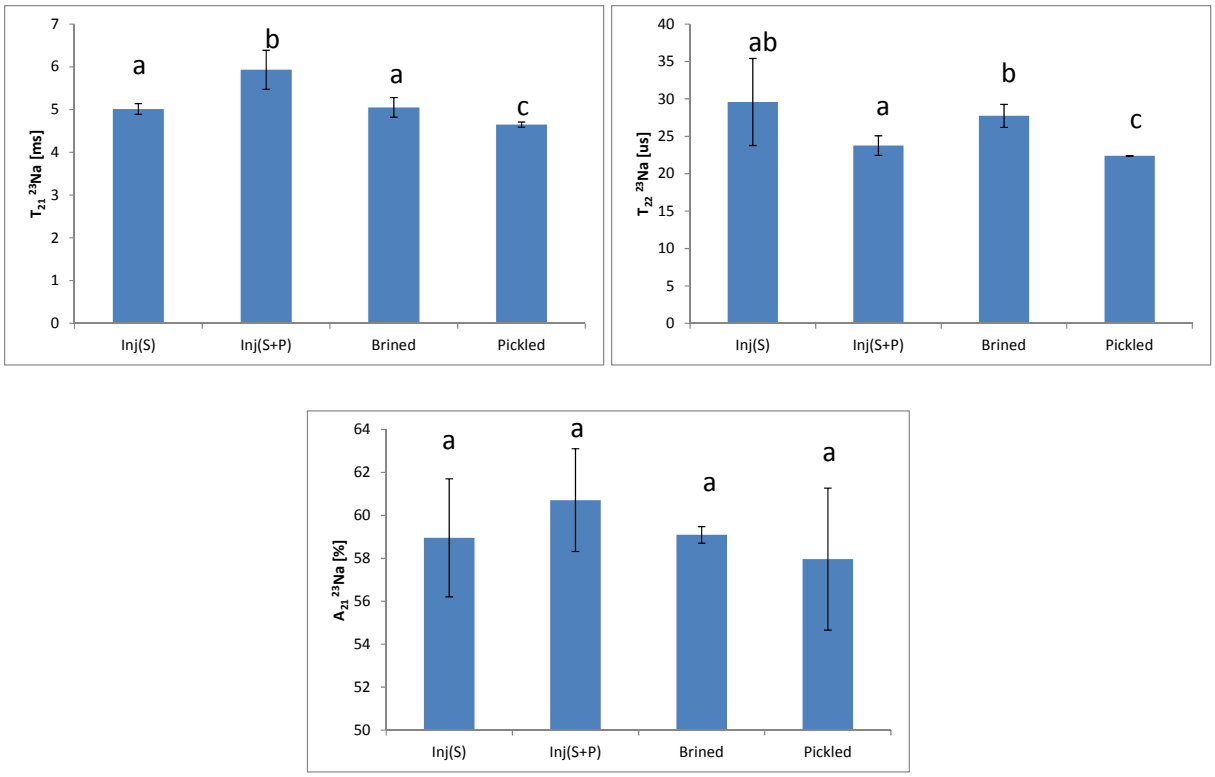

Figure 11: Sodium relaxation times for dry salted cod of different pre-salting methods.

3.6 Multivariate and variable correlation analysis

A multivariate analysis of principal components (Principal component analysis, PCA) was performed on the data (Figure 12), indicating the combined effects of the various quality parameters on the characteristics of the products analyzed. Diffusion coefficients were left out of this analysis since they were not analyzed in all samples. To get a quantitative assessment on the correlations between individual variables a correlation analysis was also performed in Microsoft Excel (Table 1).

The analysis indicated a strong positive correlation between water content and proton relaxation times obtained from both LF-NMR and the MRI MSME techniques, as well as to the relative amount of water in less restricted water population (LF-NMR A_{22}). Higher water content was also connected to a higher salt content, which in turn showed a strong positive correlation to the mobility of the more restricted sodium (HF-NMR T_{22} Na) and the amount of more restricted sodium (HF-NMR A_{21} Na). Interestingly a negative correlation was observed between the salt concentration obtained with traditional chemical analysis and with the SQ method. This can possibly be explained by the fact that the salt concentration range measured was fairly narrow, as well as some of the salt may be non-detectable by the NMR method. This is something that requires further inspection. The fillets injected with salt and phosphates were characterized with a high water content, followed by Inj(s) fillets, Brined fillets and finally Pickled fillets.

A low water content was strongly correlated to a higher WHC, connected to protein denaturation and loss of water retaining ability, especially in the pickle salted fillets. This was also related to a higher relative amount of water in the intracellular space (LF-NMR A_{21}). Strong positive correlations were then found between the LF-NMR proton relaxation times to the HF-NMR sodium relaxation times. The relative amount of intracellular water (LF-NMR A_{21}) was though negatively correlated to the relative sodium amount of the more restricted sodium population (HF-NMR A_{21} Na).

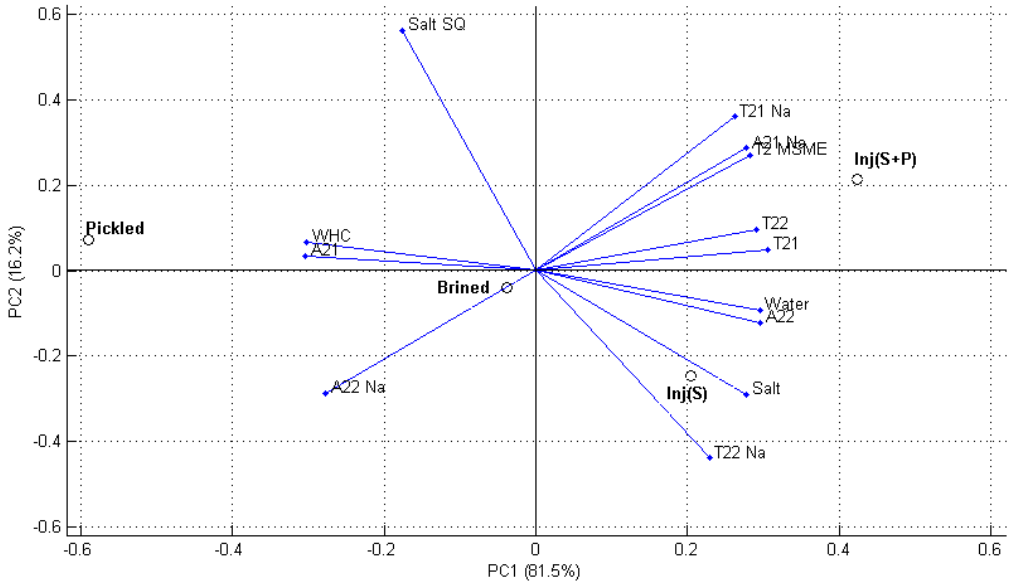

Figure 12: Principal Component Analysis (PCA) of all measured variables, except diffusion coefficients. The first two components described 97.7 % of the variation between samples.

Table 1. Correlation table between analyzed variables generated in Microsoft Excel. Red correlation parameters indicate strong positive correlation, yellow indicate strong negative correlation (Correlation > ±0.6).

	Water	*Salt*	*WHC*	*Z-value*	*MRI T_2 MSME $_{avg}$*	*LF-NMR T_{21} [ms]*	*LF-NMR T_{22} [ms]*	*LF-NMR A_{21} [%]*	*LF-NMR A_{22} [%]*	*HF-NMR Salt SQ [%]*	*HF-NMR T_{21} Na [ms]*	*HF-NMR T_{22} Na [ms]*	*HF-NMR A_{21} Na [%]*	*HF-NMR A_{22} Na [%]*
Water	1													
Salt	0.871	1												
WHC	-0.995	-0.883	1											
Z-value	-0.575	-0.099	0.544	1										
T_2 MSME $_{avg}$	0.815	0.639	-0.860	-0.585	1									
LF-NMR T_{21} [ms]	0.951	0.832	-0.976	-0.540	0.947	1								
LF-NMR T_{22} [ms]	0.819	0.807	-0.873	-0.312	0.943	0.947	1							
LF-NMR A_{21} [%]	-0.987	-0.870	0.998	0.550	-0.891	-0.988	-0.898	1						
LF-NMR A_{22} [%]	0.997	0.904	-0.995	-0.516	0.806	0.952	0.834	-0.987	1					
HF-NMR Salt SQ	-0.641	-0.894	0.627	-0.188	-0.227	-0.507	-0.466	0.591	-0.685	1				
HF-NMR T_{21} Na [ms]	0.742	0.506	-0.787	-0.660	0.986	0.891	0.880	-0.823	0.724	-0.068	1			
HF-NMR T_{22} Na [ms]	0.754	0.974	-0.760	0.093	0.451	0.684	0.674	-0.738	0.797	-0.967	0.299	1		
HF-NMR A_{21} Na [%]	0.783	0.615	-0.833	-0.561	0.998	0.931	0.943	-0.867	0.776	-0.196	0.988	0.426	1	
HF-NMR A_{22} Na [%]	-0.783	-0.615	0.833	0.561	-0.998	-0.931	-0.943	0.867	-0.776	0.196	-0.988	-0.426	-1	1

3.7 Drying

A drying experiment was performed on the dry salted products and the water and salt content was measured in the dried products (Table 2). No significant difference was seen in the water content in the injected and brined fillets. However, the pickle salted fillets showed a significantly lower water content than fillets of the other treatments after drying. No significant differences were observed in the salt content of any of the differently treated fillets.

Table 2: Water and salt content in dried fillets.

	Water [%]	Salt [%]
Inj(S+P)	48.2 ± 4	24.2 ± 3
Inj(S)	47.1 ± 4	23.0 ± 3
Brined	47.1 ± 4	23.7 ± 3
Pickled	41.4 ± 4	22.3 ± 3

To see the effect of the drying on the muscle structure and water distribution an analysis was performed on fillets from the Inj(S+P) and pickle salted fillets with the MRI MSME method. Analysis of relaxation times in the three ROIs indicated an overall decrease in water content during the drying as expected (Figure 13). However, although the variation within the fillets of the same treatment was smaller in the Inj(S+P) fillets before the drying, the drying procedure led to a heterogeneous water distribution in the dried product, but a significantly lower T_2 was observed close to the surface than deeper into the muscle. The opposite processing trend was observed in the pickled fillets, which showed more variation in MSME T_2 relaxation times before the drying, but no significant differences due to ROIs in the dried product.

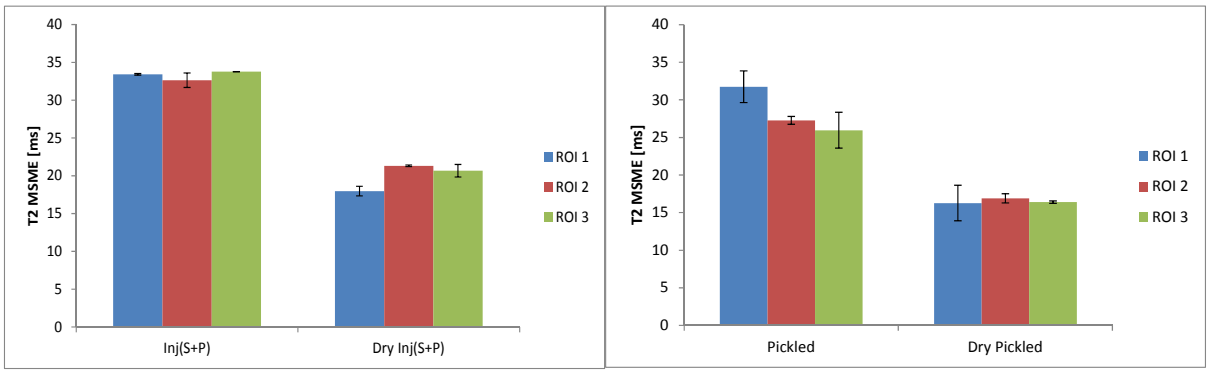

Figure 13: T_2 MSME relaxation times in Inj(S+P) (left) and pickle salted (right) fillets before and after drying.

When the obtained images were viewed structural differences between the products could be seen (Figure 14). Flakes were more visible in the Inj(S+P) fillets than in pickled ones, both prior to and after drying. It is then also noteworthy to point out that injection holes can still be observed after the drying stage in the Inj(S+P) fillets. The pickled fillets showed on the other hand a more

uniform muscle structure, although other analyzing methods indicate that the muscle is more denatured after pickle salting than after the other salting treatments.

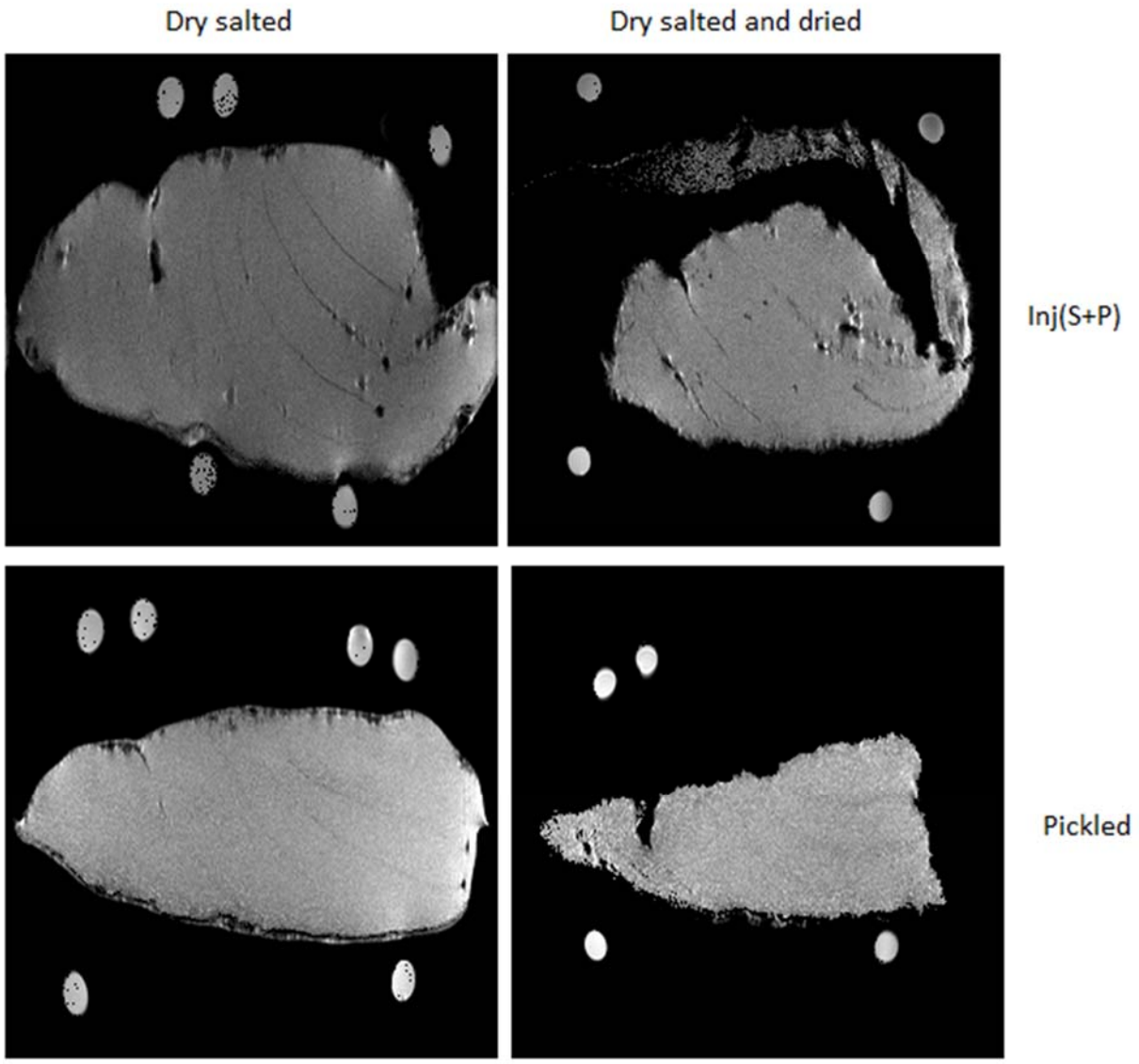

Figure 14: ^{1}H MRI cross-sectional images of dry salted cod fillets using the MSME pulse sequence. Light circles indicate reference salt solutions.

4 Conclusions

The study indicated that the most homogeneous water distribution was obtained with injection of salts and phosphates, while the most homogeneous sodium distribution was seen in pickle salted fillets. However, relaxation time and proton diffusion MRI results indicated the highest degree of protein denaturation in the pickle salted fillets. Harsh injection treatment lead to injection and muscle punctuation marks in the dry salted fillets, indicating that the brine injection settings are of crucial importance when it comes to overall quality of salted fillets. None of the pre-salting

treatment in this study lead to a fully homogeneous water and salt distribution, indicating that further optimization on pre-salting, as well as other processing steps, are necessary to optimize the water and salt distribution of dry salted cod fillets.

The drying experiment indicated that a more homogeneous water distribution can be achieved using pickle salting as a pre-salting method. However, although a more uniform water distribution is achieved with this method, the muscle is more denatured during this process. Analysis of injected fillets indicated that marks of rough injection treatments during pre-salting can be seen after drying of the fillets as well.

Overall a higher risk of heterogeneous water distribution comes with a higher water content in the final product. However, the muscle is denatured to a higher degree if pickle salting is used during pre-salting than if brining or brine injections are used. Brine injection settings are of crucial importance to the quality of the final product, not least with regards to appearance as well as heterogeneity of water and sodium distribution in the muscle.

5 Acknowledgements

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7 References

Andersen, C.M., Rinnan, Å. (2002). Distribution of water in fresh cod. *Lebensmittel Wissenschaft und Technologie* 35, 687–696.

Andriot, I., Boisard, I., Vergoignan, C., Salles, C., Guichard, E. (2011). Sodium ions in model cheeses at molecular and macroscopic levels. In: Renou, J.-P., Belton, P.S. and Webb, G.A. (Eds.) *Magnetic Resonance in food science. An exciting future.* Cambridge (UK): RSC Publishing. Pp. 67-70.

Aursand, I.G., Veliyulin, E., Böcker, U., Ofstad, R., Rustad, T., Erikson, U. (2009). 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. *Journal of Agricultural and Food Chemistry* 57, 46–54.

AOAC 976.18. (2000). Association of Official Analytical Chemists. *Official methods of analysis, 17th edition*. AOAC, Arlington, Va.

Bertram, H.C., Andersen, H.J. (2007). NMR and the water-holding issue of pork. *Journal of Animal Breeding and Genetics* 124, 35–42.

Bertram, H.C., Andersen, H.J., Karlsson, A.H. (2000). Comparative study of low-field NMR relaxation measurements and two traditional methods in the determination of water holding capacity of pork. *Meat Science* 57, 125–132.

Bertram, H.C., Karlsson, A.H., Rasmussen, M., Pedersen, O.D., Dønstrup, S., Andersen, H.J. (2001). Origin of multiexponential T-2 relaxation in muscle myowater. *Journal of Agricultural and Food Chemistry* 49, 3092–3100.

Boisard, L., Andriot, I., Arnould, C., Achilleos, C., Salles, C., Guichard, E. (2013). Structure and composition of model cheeses influence sodium NMR mobility, kinetics of sodium release and sodium partition coefficients. Food Chemistry 136, 1070-1077.

Boisard, L., Andriot, I., Martin, C., Septier, C., Boissard, V., Salles, C., Guichard, E. (2014). The salt and lipid composition of model cheeses modifies in-mouth flavour release and perception related to the free sodium ion content. Food Chemistry 145, 437-444.

Carr, H.Y., Purcell, E.M. (1954). Effects of diffusion on free precession in nuclear magnetic resonance experiments. *American Journal of Physiology* 94, 630–638.

Eide, O., Børresen, T., Ström, T. (1982). Minced fish production from capelin (Mallotus villosus). A new method for gutting, skinning and removal of fat from small fatty fish species. *Journal of Food Science* 47, 347–354.

Erikson, U., Veliyulin, E., Singstad, T.E., Aursand, M. (2004). Salting and desalting of fresh and frozen-thawed cod (Gadus morhua) fillets: a comparative study using ^{23}Na MRI, low-field ^{1}H NMR, and physicochemical analytical methods. *Journal of Food Science* 69, 107–114.

Erikson, U., Standal, I.B., Aursand, I., Veliyulin, E., Aursand, M. (2012). Use of NMR in fish processing optimization: a review of recent progress. *Magnetic Resonance in Chemistry* 50, 471-480.

Foucat, L., Benderbous, S., Bielicki, G., Zanca, M., Renou, J.-P. (1995). Effet of brine injection on water dynamics in postmortem muscle: study of T_2 and diffusion coefficients by MR microscopy. *Magnetic Resonance Imaging* 13 (2), 259-267.

Foucat, L., Taylor, R.G., Labas, R., Renou, J.-P. (2004). Soft flesh problem in freshwater rainbow trout investigated by magnetic resonance imaging and histology. *Journal of Food Science* 69, C320-327.

Gallart-Jornet, L., Barat, J.M., Rustad, T., Erikson, U., Escriche, I., Fito, P. (2007). A comparative study of brine salting of Atlantic cod (Gadus morhua) and Atlantic salmon (Salmo salar). *Journal of Food Engineering* 79, 261-270.

Gobet, M., Rondeau-Mouro, C., Buchin, S., Le Quéré, J.L., Guichard, E., Foucat, L., Moreau, C. (2010). Distribution and mobility of phosphates and sodium ions in cheese by solid-state ^{31}P and double-quantum filtered ^{23}Na NMR spectroscopy. *Magnetic Resonance in Chemistry* 48 (4), 297-303.

Gudjónsdóttir, M., Arason, S., Rustad, T. (2011). The effects of pre-salting methods on water distribution and protein denaturation of dry salted and rehydated cod – A low-field NMR study. *Journal of Food Engineering 104*, 23-29.

Gudjónsdóttir, M., Traoré, A., Renou, J.-P. (2013). The effect of crystal size and encapsulation of salt on sodium distribution and mobility in bread as studied with ^{23}Na double quantum filtering NMR. In: (van Duynhoven, J., Belton, P.S., Webb, G.A. (Eds.). *Magnetic Resonance in Food Science. Food for Thought.* Cambridge (UK), RSC Publishing. Pp. 35-43.

ICMSF (1980). *Microbial ecology of foods*, Vol.2: Food Commodities. New York and London, Academic Press.

Ishida, N., Toshio, K., Hiromi, K., Nagai, S., Ogawa, H. (1991). ^{23}Na-NMR Imaging of Foods. *Agricultural and Biological Chemistry* 55 (9), 2195-2200.

Jepsen, S.M., Pedersen, H.T., Engelsen, S.B. (1999). Application of chemometrics to low-field ^{1}H NMR relaxation data of intact fish flesh. *Journal of the Science of Food and Agriculture* 79, 1793–1802.

van Klaveren, F.W., Legrende, R. (1965). Salted cod. In: Borgstrom, G. (Ed.), *Fish as Food*, vol. III. Academic Press, New York, pp. 133-160.

Martinez, I., Aursand, M., Erikson, U., Singstad, T.E., Veliyulin, E., van der Zwaag, C. (2003). Destructive and non-destructive analytical techniques for authentication and composition analyses of foodstuffs. *Trends in Food Science & Technology* 14, 489-498.

Mathiassen, J.R., Misimi, E., Bondø, M., Veliyulin, E., Østvik, S.O. (2011). Trends in application of imaging technologies to inspection of fish and fish products. *Trends in Food Science and Technology* 22, 257-275.

Meiboom, S., Gill, D. (1958). Modified spin-echo method for measuring nuclear times. *Review of Scientific Instruments* 29, 688–691.

Mouaddab, M., Foucat, L., Donnat, J.-P., Renou, J.-P., Bonny, J.-M. (2007). Absolute quantification of Na+ bound fraction by double-quantum filtered ^{23}Na NMR spectroscopy. *Journal of Magnetic Resonance* 189 (1), 151-155.

Pedersen, H.T., Bro, R., Engelsen, S.B. (2002). Towards rapid and unique curve resolution of low-field NMR relaxation data: trilinear SLICING versus two dimensional curve fitting. *Journal of Magnetic Resonance* 157, 141–155.

Thorarinsdottir, K.A., Arason, S., Thorkelsson, G., Sigurgisladottir, S., Tornberg, E. (2010). Effects of pre-salting methods from injection to pickling on the yields of heavily salted cod (*Gadus morhua*). *Journal of Food Science* 75 (8), E544–E551.

Thorarinsdottir, K.A., Arason, S., Sigurgisladottir, S., Valsdottir, T., Tornberg, E. (2011). Effects of different pre-salting methods on protein aggregation during heavy salting of cod fillets. *Food Chemistry* 124 (1), 7–14.

Renou, J.-P., Benderbous, S., Bielicki, G., Foucat, L., Donnat, J.-P. (1994). ^{23}Na Magnetic Resonance Imaging:Distribution of brine in muscle. *Magnetic Resonance Imaging* 12, 131-137.

Veliyulin, E., and Aursand, I.G. (2007). ^{1}H and ^{23}Na MRI studies of Atlantic salmon (Salmo salar) and Atlantic cod (Gadus morhua) fillet pieces salted in different brine concentrations. *Journal of the Science of Food and Agriculture*, 87,2676–2683.

Wold, J.P., Arason, S., Erikson, U. (2001). *Hurtig og ikke destruktiv måling av vanninnhold i klippfisk*. Nordic Council of Ministers, Copenhagen, Publication number 2001: 434. Pp. 1 29. (In Norwegian and English)

8 Appendix

Table 3: Analytical results from physicochemical, ^{1}H LF-NMR, ^{23}Na HF-NMR and ^{1}H MRI methods for all salting methods.

	Variable	Inj(S)	Inj(S+P)	Brined	Pickled
Physicochemical analysis	Water [%]	59.6 ± 0.1^{a}	59,7 ± 0.3^{a}	58.1 ± 0.7^{b}	57.1 ± 1.8^{b}
	Salt [%]	21.6 ± 0.4^{a}	21.4 ± 0.1^{a}	21.3 ± 0.2^{a}	20.7 ± 0.3^{b}
	WHC [%]	70.5 ± 3.3^{a}	68.6 ± 2.8^{a}	79.5 ± 3.6^{b}	88.3 ± 2.0^{c}
	Z-value [%]	26.6 ± 0.3^{ab}	26.4 ± 0.1^{b}	26.9 ± 0.3^{a}	26.6 ± 0.6^{ab}
^{1}H LF-NMR	T_{21} [ms]	34.0 ± 0.8^{a}	37.1 ± 1.6^{b}	31.4 ± 0.4^{c}	26.0 ± 0.3^{d}
	T_{22} [ms]	394 ± 29^{a}	459 ± 11^{b}	410 ± 27^{a}	288 ± 5^{c}
	A_{21} [%]	81.8 ± 0.5^{a}	81.2 ± 0.8^{a}	83.4 ± 0.3^{b}	85.2 ± 0.1^{c}
	A_{22} [%]	18.2 ± 0.5^{a}	18.8 ± 0.8^{a}	16.6 ± 0.3^{b}	14.8 ± 0.1^{c}
^{23}Na HF-NMR SQ	Salt SQ ROI 2[%]	17.0 ± 3.2^{a}	18.1 ± 3.5^{a}	17.7 ± 1.9^{a}	18.6 ± 0.7^{a}
	Z-value ROI 2 [%]	22.1 ± 3.1^{a}	23.1 ± 3.2^{a}	23.3 ± 2.1^{a}	24.5 ± 2.0^{a}
^{23}Na HF-NMR	T_{21} Na	5.0 ± 0.5^{a}	5.9 ± 1.3^{b}	5.1 ± 0.4^{a}	4.6 ± 0.5^{c}
	T_{22} Na	37.8 ± 10.8^{ab}	33.9 ± 7.3^{a}	35.3 ± 10.3^{b}	29.1 ± 3.7^{c}
	A_{21} Na	59.0 ± 4.5^{a}	60.5 ± 6.7^{a}	59.1 ± 3.0^{a}	58.0 ± 4.1^{a}
	A_{22} Na	41.0 ± 4.5^{a}	39.5 ± 6.7^{a}	40.9 ± 3.0^{a}	42.0 ± 4.1^{a}
^{1}H MSME MRI	T_2 MSME avg [ms]	30.4 ± 2.9^{a}	33.3 ± 0.6^{b}	30.5 ± 2.4^{a}	28.3 ± 3.0^{a}
	T_2 ROI 1 [ms]	27.9 ± 0.1^{a*}	33.4 ± 0.1^{b*}	30.6 ± 2.6^{c*†}	31.7 ± 2.1^{c*}
	T_2 ROI 2 [ms]	29.8 ± 1.4^{a†}	32.6 ± 1.0^{b*}	28.0 ± 0.6^{c†}	27.3 ± 0.5^{c†}
	T_2 ROI 3 [ms]	33.6 ± 4.1^{a†}	33.77 ± 0.02^{a†}	32.8 ± 1.9^{b*}	26.0 ± 2.4^{c†}
^{1}H Diffusion MRI	D_e avg [cm^2/s]		0.735 ± 0.050^{a}		0.612 ± 0.023^{b}
	D_e ROI 1 [cm^2/s]		0.678 ± 0.031^{a*}		0.623 ± 0.075^{a*}
	D_e ROI 2 [cm^2/s]		0.757 ± 0.019^{a†}		0.628 ± 0.003^{b*}
	D_e ROI 3 [cm^2/s]		0.769 ± 0.049^{a†}		0.586 ± 0.072^{b*}

- **All values are stated as mean ± standard deviation**
- **Different superscripts ^{a},^{b},^{c} indicate significant difference between salting treatments in each MRI variable (horizontal comparison).**
- **Different superscripts *,†indicate significant difference in a MRI variable between sampling places within each salting treatment (vertical comparison)**
- **All significance levels were set to $p<0.05$**