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Water distribution in commercial Icelandic heavily salted Atlantic cod

María Guðjónsdóttir
Ásbjörn Jónsson
Magnea G. Karlsdóttir
Sigurjón Arason
Amidou Traoré

Vinnsla, virðisaukning og eldi

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Report summary

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Titill / Title	**Water distribution in commercial Icelandic heavily salted Atlantic cod (*Gadus morhua*) /** Vatnsdreifing í fullsöltuðum þorsk		
Höfundar / Authors	María Guðjónsdóttir[1,2,3], Ásbjörn Jónsson[1], Magnea G. Karlsdóttir[1], Sigurjón Arason[1,4], Amidou Traoré[2]. Affiliation: [1]Matís, [2]INRA, [3]DTU, [4]University of Iceland		
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Ágrip á íslensku:	Vatnsdreifing í margskonar afurðum af íslenskum fullsöltuðum þorski var greind með prótón segulkjarnómunar aðferðum. Afurðirnar voru bæði flattar og flakaðar, auk þess sem þær voru breytilegar m.t.t. veiðiaðferða, vinnslu fyrir eða eftir dauðstirðnun, forsöltunaraðferða (sprautusöltun með/án fosfati, pæklun og pækilsöltun) sem og vali á sprautunarvélum. Allar afurðirnar höfðu jafna vatnsdreifingu, en einsleitnin var háð vinnsluaðferðum. Tvöföld sprautusöltun, sem og einföld sprautun í vöðva fyrir dauðastirðnun, leiddi til nálarfara í vöðvanum, sem voru jafnvel greinanleg eftir „kench“ söltun. Greiningar á slökunartíma gáfu til kynna að pækilsöltun leiddi til mikillar próteinafmyndunar í vöðvanum samanborið við aðrar forsöltunaraðferðir. Sprautusöltun leiddi til salt-hvetjandi þenslu (*e.* swelling) í vöðvanum, og héldust þau áhrif einnig eftir „kench“ söltunarskrefið. Fjölþáttagreining á öllum breytum sýndi að MR aðferðirnar eru öflugar aðferðir til þess að leggja mat á vinnslueiginleika afurða, sem og til að hámarka vinnsluaðferðir.		
Lykilorð á íslensku:	*Fullsaltaður þorskur (bacalao); söltunaraðferðir; pre-rigor; post-rigor; MRI; NMR; eðliseiginleikar; dreifing*		
Summary in English:	The water distribution of various commercially available Icelandic heavily salted Atlantic cod) products were analyzed with proton magnetic resonance methods. The products varied in choice of catching method, in pre- or post-rigor processing, flattening or filleting cut, and pre-salting technique (brine injection with salt with/without polyphosphates, brining and pickling) and choice of brine injection instruments. All products had a heterogeneous water distribution, but the level of heterogeneity was dependent on the handling during processing. Double brine injection and brine injection into pre-rigor muscle lead to needle traces in the muscle, even after kench salting. Relaxation time analysis indicated that pickle salting lead to the highest degree of protein denaturation in the muscle of the analysed pre-salting methods. Brine injection lead to salt-induced swelling, which effect remained after the kench salting step. The multi-parametric analysis performed indicated how powerful the MR methods are for process and product characterisation and optimization.		
English keywords:	*Dry salted cod (bacalao); salting methods; pre rigor; post rigor; MRI; NMR; physicochemical properties; diffusion*		

Contents

1 Introduction

During the production of dry salted cod several factors along the production line can influence the quality of the final products. Optimized control of water content, it´s distribution and homogeneity within the product is one of the major parameters that are important to maintain a high quality and stable product. Optimization of those processing steps that influence the water behavior in the salted muscle are therefore of crucial importance.

Proton (^{1}H) Nuclear Magnetic Resonance (NMR) spectroscopy and Magnetic Resonance Imaging (MRI) are noninvasive methods, which can be used to analyze the distribution, mobility, diffusion and heterogeneity of water in fish (Erikson, Veliyulin, Singstad & Aursand, 2004; Erikson, Standal, Aursand, Veliyulin & Aursand 2012; Veliyulin & Aursand, 2007). Several studies have shown connections between relaxation times (T_1 and T_2) and diffusion coefficient (D) to water distribution and behavior of various muscle based foods (Foucat, Benderbous, Bielicki, Zanca & Renou, 1995; Renou, Benderbous, Bielicki, Foucat & Donnat, 1994; Veliyulin & Aursand, 2007), including the water content of cod (Andersen & Rinnan, 2002; Gudjónsdóttir, Arason & Rustad, 2011), the water holding capacity in fish and meat (Jepsen, Pedersen & Engelsen, 1999; Bertram & Andersen, 2007, Gudjónsdóttir et al., 2011; Aursand, Veliyulin, Böcker, Ofstad & Erikson, 2009), muscle pH (Bertram, Andersen & Karlsson, 2000; Gudjónsdóttir et al., 2011), and how these are affected by different raw material or choice of processing methods (Erikson et al., 2012). Moreover, ^{1}H MR imaging methods can give additional information about the structure, anatomy as well as water dynamics in the intact muscle (Erikson et al., 2012; Foucat, Taylor, Labas & Renou, 2004; Mathiassen, Misimi, Bondø, Veliyulin & Østvik, 2011).

The objective of the present study was to investigate the differences in commercially available heavily salted Atlantic cod products, which differ in catching method, pre- and post-rigor processing, choice of pre-salting methods and drying on the water distribution and homogeneity, by means of proton NMR and MRI,

supported by physicochemical analytical results. Increased insight in the effects of these parameters was used for further process and quality optimization.

2 Materials and methods

2.1 Experimental design

Nine dry salted fish products from five Icelandic producers of heavily salted Atlantic cod (*Gadus morhua*) were collected and analyzed in November 2012. Two commercial products from Brazil (BRA) and Spain (ESP) were analyzed as well for comparison to the Icelandic products. The products varied in catching method used (line, long line or net), in cutting processing (filleted or flattened fish), pre-salting pre rigor or post rigor, the pre-salting methods (brine injections, brining, pickle salting) used and whether the products were dried or not. However, all products went through a similar kench salting step after the pre-salting. The producers' descriptions of the processing steps of each product can be seen in Table 1.

Table 1: Producers' descriptions of the processing methods of the analyzed dry salted Atlantic cod products, including catching method, pre-salting method, rigor state during processing, dry salting and drying.

Product	Catching method	Product	Pre-/post rigor processing	Pre-salting method	Injection device	Dry salting method	Dried
A	Trawler	Flattened	Post	Pickle salted for 2 days	-	Kench	No
B1	Line	Fillets	Post	Brine injected + brining	RAF	Kench	No
B2	Line	Flattened	Post	Brine injected + brining	TRAUST	Kench	No
C	Line	Fillets	Post	Brine injected (salt + phosphates) + brining	FOMACO	Kench	No
D1	Net	Flattened	Pre	Brine injected + brining	RAF	Kench	No
D2	Net	Flattened	Post	Brine injected + brining	RAF	Kench	No
D3	Line	Flattened	Pre	Brine injected + brining	RAF	Kench	No
E1	Line	Flattened	Post	Brine injected + brining	TRAUST	Kench	No
E2	Line	Flattened	Post	Brining	-	Kench	No
BRA	Line	Flattened	Post	Pickle salted	-	Kench	No
ESP	Line	Flattened	Post	Pickle salted	-	Kench	Yes

Three different injection devices were used for the brine injected products. The corresponding characteristics and settings of these injection instruments were: RAF S900 injection device (RAF ehf., Akureyri, Iceland) with a single head injection system, using 924 needles, each 1.6 mm wide and the brine was injected with a pressure of 0.6-0.8 bar; TRAUST TR-580 XLT (TRAUST know how Ltd. Borgarnes, Iceland) using 294 needles, each 3 mm wide, and a pressure of 0.8-1.0 bar, and finally a FOMACO FGM 64/256F (FOMACO Food Machinery Company A/S, Køge, Denmark) injection machine with a double head injection system (2×64 quatro needles), using a pressure of 0.1 bar.

Three fish from each product were sampled for analysis of water, salt and polyphosphate content, water holding capacity, as well as water distribution by low field Nuclear Magnetic Resonance (LF-NMR) and magnetic resonance imaging (MRI). The individual analyzing methods are described in detail in the

following chapters. Flattened fish was sawn in half along the backbone. The left half/fillet of each fish was sent to INRA in Saint-Génes-Champanelle, France for MRI analysis, while the right half/fillet of each fish was used for all other earlier mentioned analysis, performed at Matís in Reykjavík, Iceland. Each flattened fish and fillet was marked with a numbered plastic tag to allow process and analysis tracking.

2.2 Physicochemical reference measurements

Reference measurements of water, salt and polyphosphate content, along with water holding capacity were performed at the chemical laboratory at Matís, Reykjavík Iceland. The water content of the fillets was assessed by comparing the weight of 5 g of raw minced muscle prior to and after drying of the sample 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, Børresen & Ström (1982). Salt content (on a dry basis) was analyzed with the Volhard Titrino method (AOAC, 2000) and 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. The phosphate content was determined by spectrophotometric absorbance analysis of the phosphorus content by means of the vanadomolybdophosphoric acid complex at wavelength 420 nm (Hanson 1950; Sutton & Ogilvie 1967; AOAC 969.31 1990) with a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan), as described by Nguyen, Jonsson, Thorkelsson, Arason, Gudmundsdottir & Thorarinsdottir (2012). The total phosphate of the fish muscle was expressed as mg of P_2O_5 per sample calculated according to the equation:

$$P_2O_5 = (PO_4 \times 0.747) + (P_2O_7 \times 0.816) + (P_3O_{10} \times 0.842) \qquad (2)$$

2.3 Low field NMR measurements

Water distribution analysis was performed using a Bruker mq 20 benchtop low field NMR analyzer (Bruker Optics GmbH, Rheinstetten, Germany), using a 20 MHz magnetic field frequency. Samples were taken from

three locations along the fish at 5-7 cm (location A), 10-12 cm (location B) and approximately 18-20 cm (location C) from the head cut. Two samples replicates (approximate sample weight: 0.5 g) were cut from each of the three sampling locations in each fish and placed individually in 10 mm sampling tubes. Transversal relaxation times were analyzed with a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958) with an echo time of 250 µs, 8100 collected echoes, a receiver gain (RG) of 70 dB, a recycle delay (RD) of 10 s, and 16 scans. 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 and the relaxation data was fit to a multi-exponential curve using the Low-field NMR toolbox for Matlab (The Mathworks Inc., Natick, Mass., U.S.A.), as described by Pedersen, Bro & Engelsen (2002).

2.4 Magnetic Resonance Imaging (MRI) analysis

For all MRI measurements a 4.7 T Bruker Biospec 47/40 magnet interfaced to an Avance III console (Bruker BioSpin MRI GmbH, D-76275 Ettlingen, Germany) was used. The coil was 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). Cross sectional samples were cut from the loin part of each fish, approximately 10 cm from the head cut. Each sample was approximately 3 cm wide. Each sample was then packed and sealed in a vacuum bag (50 % vacuum) to prevent water dripping into the magnet. Large samples were rolled up, with a plastic sheet between muscle parts for clear separation in the images. The standard Bruker TRIPILOT protocol was used to assess the optimal placement of the sample in the magnet. The samples were analyzed with two imaging methods: Firstly, a **Multi-Slice-Multi-Echo (MSME)** protocol was used to obtain ^{1}H T_2 relaxation and proton density contrast maps of samples from all products. Six echo times (TE), ranging from 12 to 72 ms in 12 ms steps were used along with 8 averages (NA), one repetition, a 180° refocusing flip angle, two 2-mm thick slices with 6 mm slice distance, a FOV of 80x80 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. Six echo images were used, which is not enough to produce a multicomponent decay. Therefore mono-exponential T_2 values were 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. Secondly, an **Apparent Diffusion Coefficient (ADC)** mapping analysis was performed 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 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 products A, B1 and B2.

All imaging slices were placed parallel with the magnetic field and acquisitions were performed in the sagittal direction.

2.5 Data handling and analysis

All results are presented as averages from the analysis of 3 fish from each product. Statistical analysis, correlation calculations 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 ($p<0.05$). A Principal Component Analysis (PCA) was performed in Matlab R2012b (The MathWorks Inc., US) on all obtained parameters from physicochemical, low field NMR and MRI analysis for the Icelandic products. The parameters were normalized with the inverse of their standard deviation and centred prior to analysis.

3 Results and discussion

3.1 Physicochemical analysis

Some variation was observed in the weight of the fillets and flattened fish (Figure 1), but product B2 (1857 ± 3 g) was significantly lighter than the flattened products A, D1, E1 and E2, and a significant difference was seen between product E2 (2027 ± 147 g) and D1 (3162 ± 989 g). No significant differences were observed between other flattened products. The fillets of group C were larger (835 ± 69 g) than the fillets of product B1 (604 ± 41 g). Unfortunately no weight was recorded for the Brazilian product. The size of the samples can have an important effect on the diffusion of water and salt in and out of the muscle during processing.

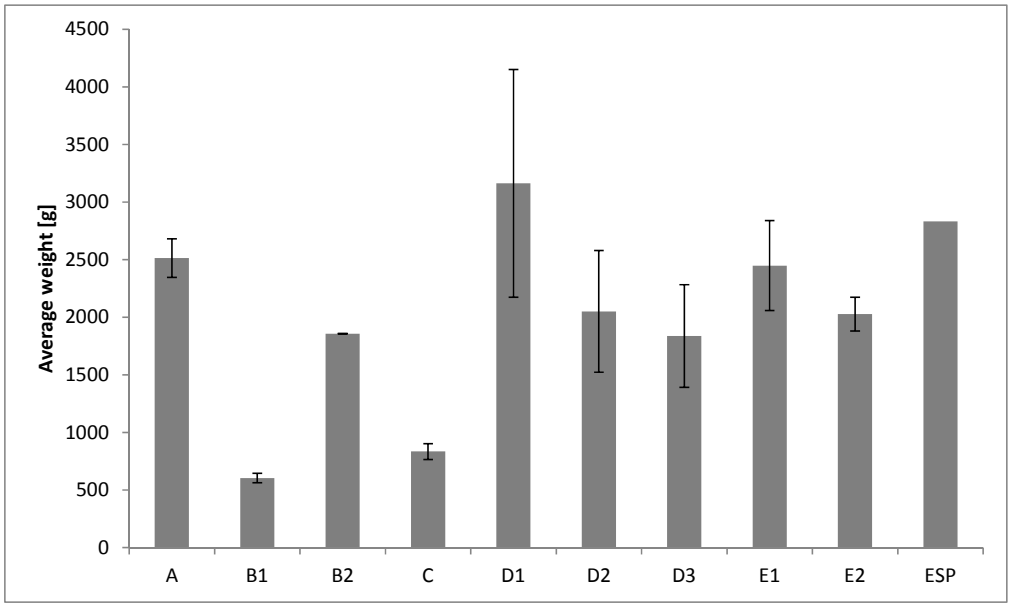

Figure 1: Average weight of fillets (B1 and C) and flattened cod products (A, B2, D1-D3, E1-E2, ESP).

The water content of the products ranged from 51.9 % in the dried Spanish product (ESP) to 58.5 % in the brine injected product C (Figure 2). Fish processed pre rigor (D1 and D3) showed a significantly lower water content than other brine injected products (B1, B2, C), with the exception of product E1, where the difference was not significant. This is in agreement with the observations of Gudjonsdottir et al. (2010), where limited salt-induced muscle swelling was observed in both farmed and wild Atlantic cod when pre rigor fillets were brine injected, while post rigor wild cod was subjected to significant salt-induced swelling during the same brine injection treatment. Larsen, Olsen, Kristoffersen & Elvevoll (2008) showed that brining of pre rigor farmed cod fillets led to an increased muscle contraction during rigor, which in turn is likely to expel some of the injected brine from the muscle during the rigor process. Brine injected fish caught by net (D1 and D2) also showed a significantly lower water content than the line caught brine injected products (B1, B2 and E1). Pickle pre salting resulted in a lower water content than in brine injected products B2 and C in agreement with earlier studies (Gudjónsdóttir et al., 2011; Thorarinsdottir, Arason, Sigurgisladottir, Valsdottir & Tornberg, 2011).

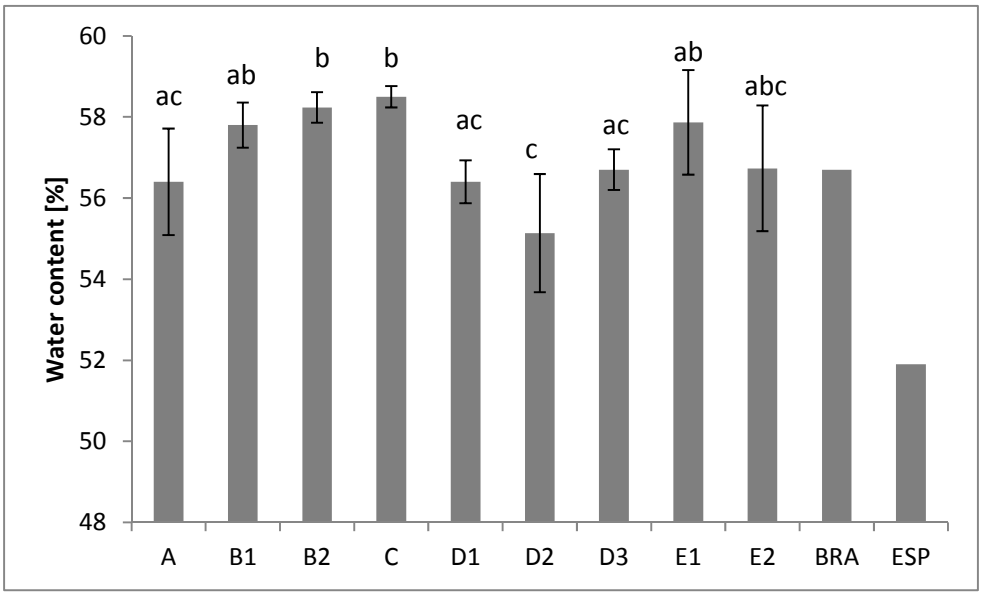

Figure 2: Water content in dry salted Atlantic cod products.

The salt content in the products ranged from 18 to 21 % on average (Figure 3). The lowest salt content was observed in the dried Spanish product (ESP), followed by the pickle salted product A, and the net caught products D1 and D2. The highest salt content was observed in the brine injected products B1, B2 and C, although B2 was not significantly different from the other brine injected or brined products. When the salt content on a wet basis (Z-value) was analysed, results revealed that the salt solution in the muscle was not saturated in all products (Figure 3). The Z-value of the pickle salted product A and the net caught products D1 and D2 and the dried product ESP had averages below 26% (Figure 4). However, some individuals within groups D1 and D2 did showed saturated salt solutions in their muscle, but these treatments seemed to lead to a wider variation in salt distribution, based on the large standard deviation observed in these groups.

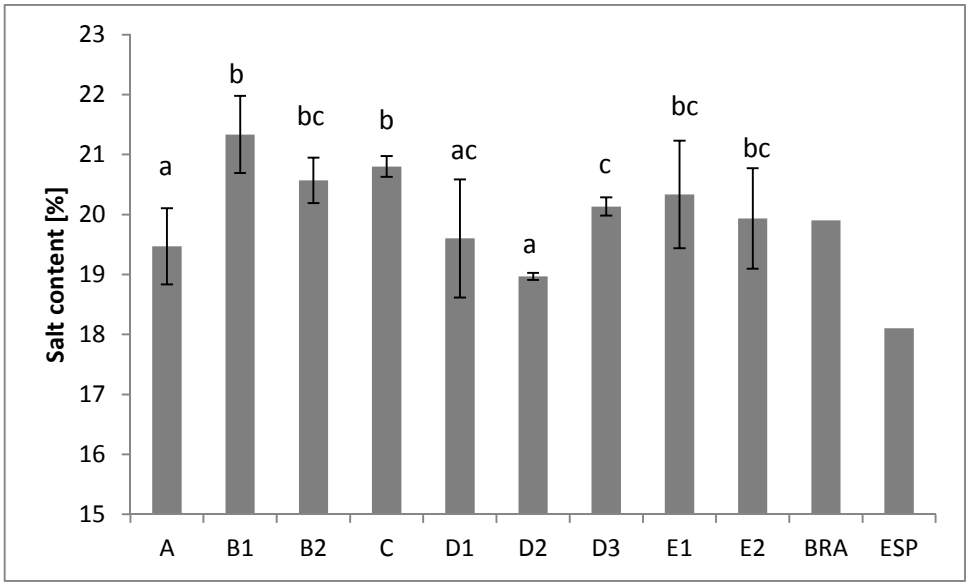

Figure 3: Salt content in dry salted Atlantic cod products.

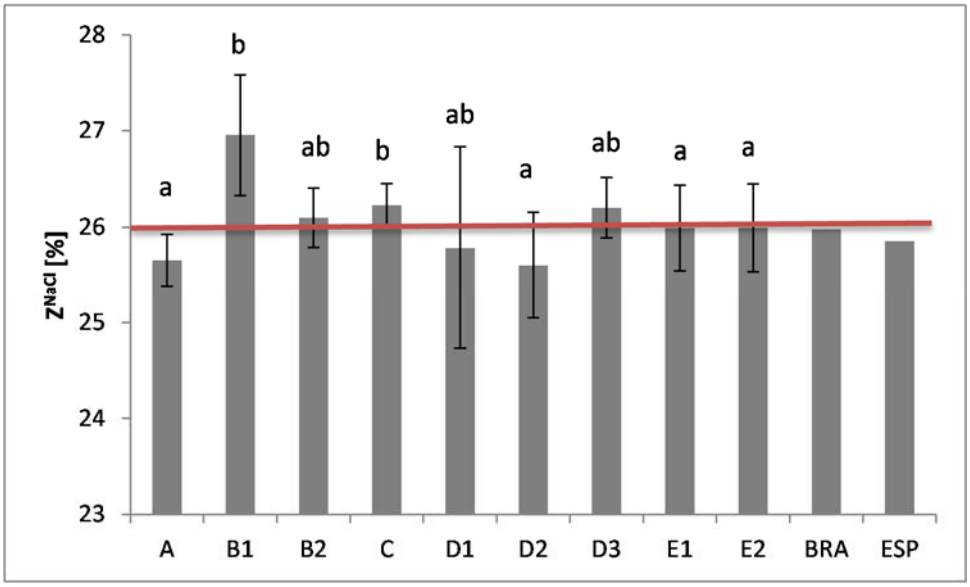

Figure 4: Salt content on wet basis (Z-value) in dry salted Atlantic cod products.

The main gain of using phosphates during salt fish production is to retain the quality and light appearance of the products during processing, storing and rehydration (Nguyen et al., 2012). The phosphate analysis showed that only orthophosphates (PO_4) were present in the dry salted products, indicating that polyphosphates added to some of the products were broken down to mono-phosphates during processing. Naturally occurring phosphates in seafood range from 0.11 to 4.8 %, depending on the species, regional origin, composition, differences between individuals and so on (Thorarinsdottir, Bjørkevoll & Arason, 2010). The quantification of phosphates does not differentiate between added and naturally occurring orthophosphates or other phosphorous compounds, such as phospholipids. This makes the detection of added phosphates during processing challenging. According to EU legislation a maximum phosphate level of 0.5 g/ 100g (or 5 mg P_2O_5/g) is allowed in the final fish product (Goncalves & Ribeiro, 2008). The measured phosphate contents in the studied products were all well below this limit. However, two products stood out in phosphate values in the present study, but the elevated phosphate content of the brine injected products C and E1 indicated that polyphosphates had been used during processing of these products. It cannot be ruled out that polyphosphates have been added

to the other products during processing based on the obtained results, but they have in that case been used at such a low level or had been drained away during the dry salting step that the final phosphate levels are not distinguishable from the naturally occurring phosphates in the muscle.

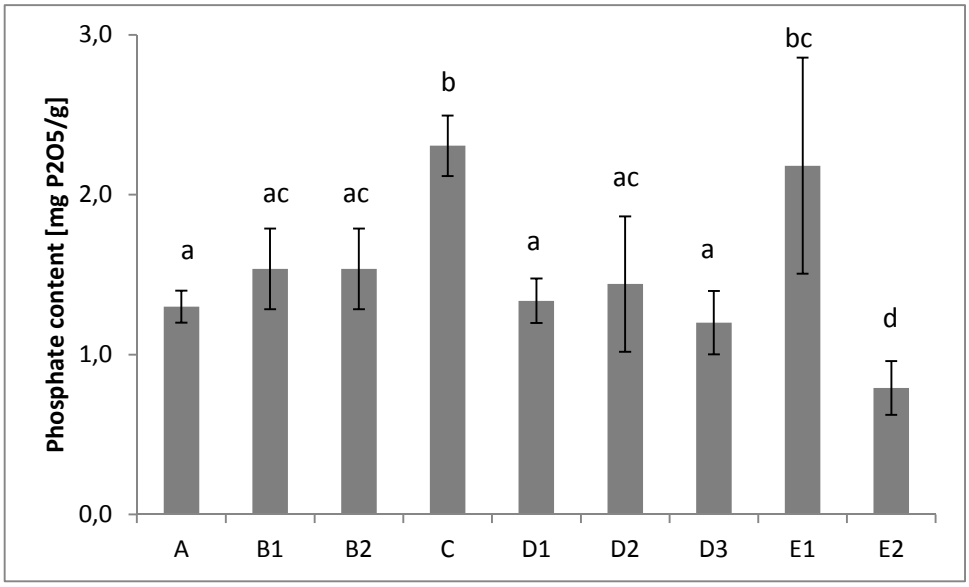

Figure 5: Phosphate content in dry salted Atlantic cod products.

WHC analysis in the muscle showed a significantly higher WHC in the pickle salted product A, while the brine injected products B1, B2 and C had the lowest WHC observed in the muscle. No significant difference in water, salt and WHC was observed between these brine injected product, indicating that the phosphate used in product C did not have any additional effect on the water retention abilities of the muscle. It should also be kept in mind that when salt and phosphates are used together, the solubility of the phosphate in water is reduced and the salt increases the osmotic pressure of the solution. This limits the amount of water that can be absorbed in the muscle (Lampila, 1992). Net catching (D1 and D2) also lead to a relatively high WHC compared to other brine injected line caught products (B1, B2, C). Earlier studies have shown that fish caught by net or by trawl is more stressed than fish caught by line, which in turn affects the overall quality of the fish post mortem, including the WHC (Borderías & Sánchez-Alonso, 2011; Botta, Bonnell & Squires, 1987). This is due to the effect of increased stress on the production of lactic acid in the muscle, leading to lowering of the muscle pH and loss of ATP, which in turn affects the

rigor onset and duration and the cellular structure (Ordóñez-Peneda, 2005). When the brine injected pre-rigor products (D1 and D3) were compared to the post-rigor products treated with the same type of injecting instrument (RAF) (products B1 and D2), a lower WHC was observed in the post rigor fish caught by line (B1) than in the pre rigor fish caught by line (D3), which was correlated with a higher salt content or salt uptake in the post rigor B1 product. Earlier studies have shown that salt uptake in pre-rigor muscle during brining and brine injections are limited (Gudjonsdóttir et al., 2010; Larsen et al., 2008, Wang, Tang & Correia, 2000). According to Larsen et al. (2008) brining of pre rigor filleted farmed cod lead to an increased rigor contraction, expelling the salt from the muscle during the contraction, resulting in an increased salt concentration in the brine. Also according to Wang et al. (2000) the salt uptake in pre-rigor salmon muscle was significantly slower when brined in a 20 % (w/v) salt solution, than in in-rigor or post-rigor muscle. The same study showed that the salt diffusivity was slightly lower in the pre-rigor muscle than in the post-rigor muscle. No significant differences were seen in water and salt content and WHC between the brined product E2 compared to the brine injected product from same material (E1).

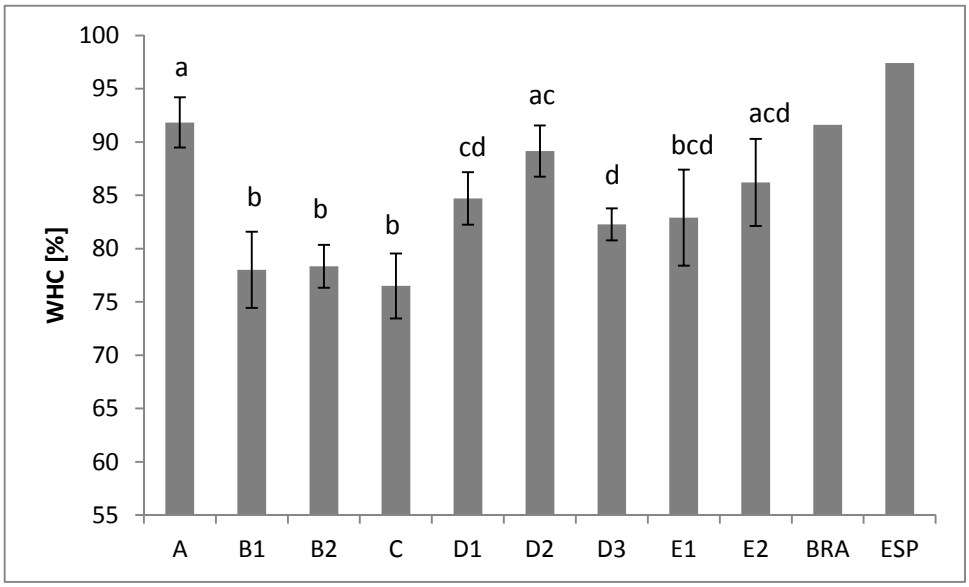

Figure 6: Water holding capacity (WHC) in the dry salted Atlantic cod products.

3.2 LF-NMR

Low field NMR analysis resulted in the observation of two water populations. The faster relaxing dominant component $T_{21,}$ ranging from 24.6 ms to 30.5 ms, had a 81.4-87.7 % relative water population, while the slower relaxing component ranged from 168 to 342 ms. The faster relaxing component is generally related to myofibrillar water, while the slower relaxing compenent is related to extra-myofibrillar water ((Gudjónsdóttir et al., 2011; Aursand et al., 2009; Bertram & Andersen, 2007; Bertram et al., 2009). Several studies have shown that a decrease in relaxation times may indicate protein denaturation. Evans et al. (1998) correlated protein denaturation and aggregation during freezing of meat to a decrease in the longitudinal relaxation time T_1, while the transverse relaxation parameter (T_2) has been shown to be sensitive to protein denaturation during heating of whey proteins (Lambelet, Berrocal & Ducret, 1989) as well as during cooking of shrimp (Gudjónsdóttir, Jónsson, Bergsson, Arason & Rustad, 2011).

The pickled fish in product A showed a significantly shorter T_{21} than the other treatments, in agreement with earlier observations where shorter relaxation times in dry salted cod muscle were linked to a higher degree of protein denaturation during dry salting of the brine injected fillets (Gudjónsdóttir et al., 2011a). These findings were further supported by differential scanning calorimetry (DSC) and electrophoresis (SDS–PAGE) results, which indicated less protein aggregation, mainly in the heavy myosin chain, in brine injected fillets compared to fillets of other pre-salting treatments (Thorarinsdottir et al., 2011).

The relaxation times, especially T_{21}, of the products where the fish had been caught with a stress inducing method (A, D1 and D2) were generally shorter than for the line-caught products. This was correlated to a lower water and salt uptake in the stressed muscle as well as a higher WHC. Aursand, Erikson & Veliyulin (2010) documented that excessive ante-mortem stress lead to a stronger rigor contraction and a less water within the muscle structure and overall lower water mobility in fresh and brined salmon fillets. The current research also implies that stress prior to catching thus has a significant

effect on protein denaturation and thus affects the water retention properties of the muscle even after heavily salting.

No significant difference was seen in the relaxation times due to pre rigor or post rigor processing of the RAF brine injected cod products (B1, D1-D3). This is in agreement with an earlier study on pre and post rigor light salting of wild and farmed cod muscle (Gudjónsdóttir et al., 2010). A significantly lower relative water population (A_{21}) was observed in the post rigor line-caught product (B1) compared to the pre rigor line-caught product (D3). This was correlated to a significantly lower WHC in the post rigor muscle (B1) compared to the pre rigor product (D3). For the net caught fish this relationship between pre and post rigor processing on the water distribution was inversed, that is a larger restricted water population (A_{21}) was observed in the pre rigor product (D1) than in the post rigor product (D2). This trend in the net-caught products could not be described by differences in water or salt uptake or differences in WHC.

The brine injected products (B2, C and E1) using other injection devices (TRAUST or FOMACO) showed no significant differences in water and salt content, nor in WHC. However, elevated phosphate content in products C and E1 was correlated with a smaller intracellular water population (A_{21}) compared to product B2. Significantly longer relaxation times observed in product C, indicated that the double injection system used with the FOMACO injection device had a significant effect on the water mobility (T_{21} and T_{22}) in the products, rather than from the use of additional phosphate. This was concluded since no significant difference was seen between the relaxation times of products B2 (without phosphate) and E1 (with phosphate).

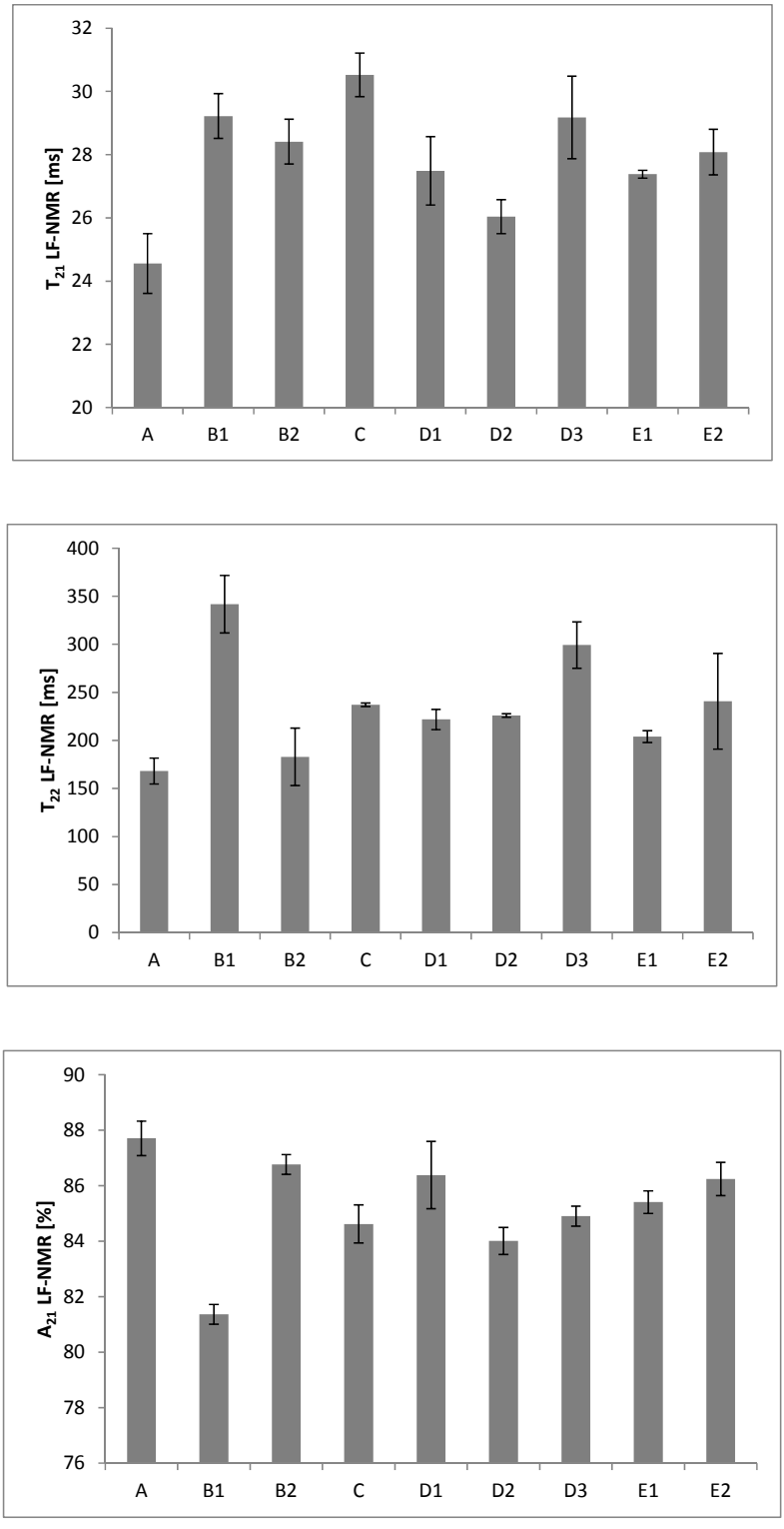

Figure 7: Low field NMR transverse relaxation times (T_{21} and T_{22}) and relative myofibrillar water distribution (A_{21}) in the analysed heavily salted cod products.

3.3 MRI results

3.3.1 MSME T_2 relaxation maps

Magnetic Resonance Imaging using a MSME pulse sequence showed clear anatomical details of the muscle as well as the spatial water distribution within the muscle (Figures 8-11). The presence of bones was evident in most samples, especially in the area connecting the loin part to the belly flap. Evidences of a heterogeneous water distribution were observed in all products, but to different degrees dependent on the treatment of the products.

A clear flake structure of the muscle remained in the pickle salted products A (Figure 8) and the Brazilian product (BRA) (Figure 11), as well as the brined product E2 (Figure 9). This flake structure was not as evident in the brine injected products and had almost disappeared completely in products B1, B2 and E1. It is possible that the low-concentration salt treatment during pre-salting lead to muscle protein gelation (Gudjonsdottir et al., 2011c), which in turn decreases the clarity of the flake muscle structure. When looking at the Spanish (ESP) product a clear flake structure was still apparent even though this product has also been dried after the kench salting. A similar structure was observed in the Spanish product, although the lower water content of this group was indicated with a lower T_2 signal intensity (Figure 11).

Fish caught by net (D1 and D2) showed a significantly higher degree of heterogeneity in the water distribution than fish caught by line (D3 and B1) and pre salted with the same injection instrument (RAF) (Figure 8 and 10). Brine injection marks were most evident in the pre rigor processed fish caught by net (D1), but some traces of injections were also observed in the pre rigor processed cod caught with a line (D3), especially in the mount between the loin and the belly flap area. Few to none injection traces were evident in most post rigor brine injected products (B1, B2, D2 and E1). According to this pre rigor processing increases the risk of muscle puncturing and formation of injection marks in the final product and that increased stress levels prior to catch and the following increased muscle contractions during rigor can

increase this risk even further. The clear injection marks observed in product C are believed to originate from the double injection treatment that this product underwent. It is therefore clear that double injection has a negative effect on the muscle structure and that this effect remains through the whole salting process.

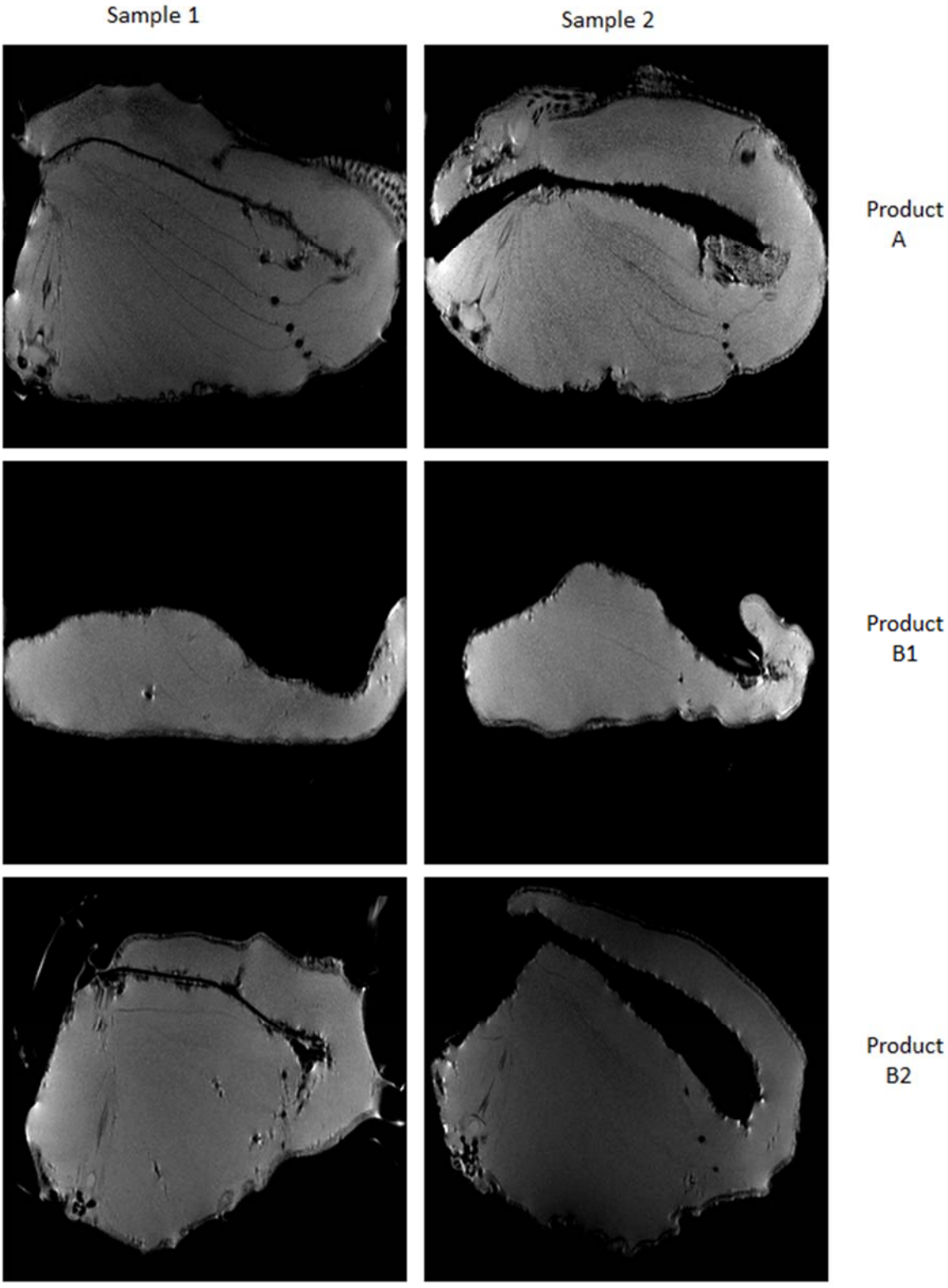

Figure 8: MSME images of products A (post rigor, trawler caught, pickle salted), B1 (post rigor, line caught, RAF injected) and B2 (post rigor, line caught, TRAUST injected). All samples are placed skin-down and with the belly flap folded to the right.

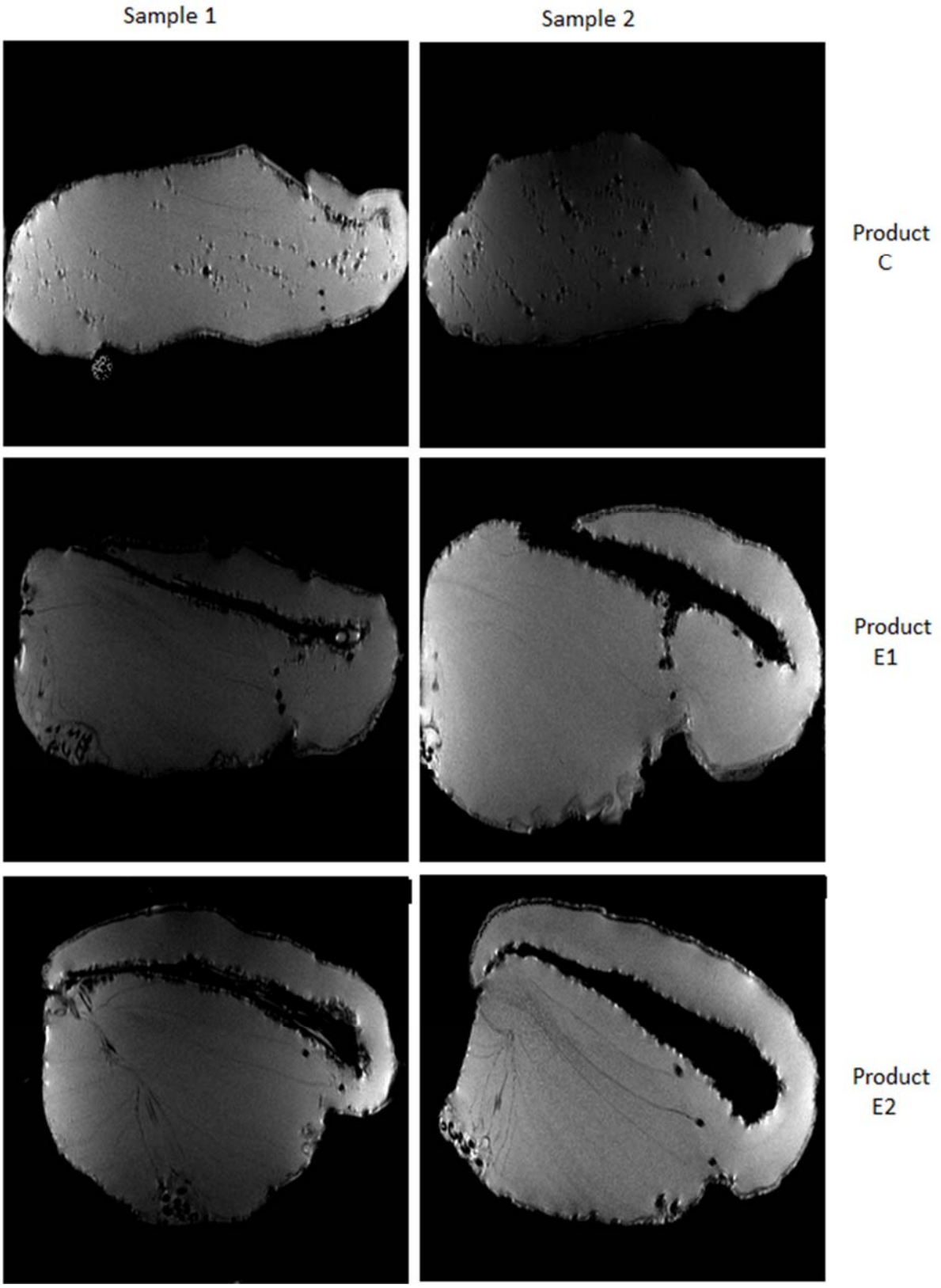

Figure 9: MSME images of products C (post rigor, line caught, FOMACO injected), E1 (post rigor, line caught, TRAUST injected) and E2 (post rigor, line caught, brined). All samples are placed skin-down and with the belly flap folded to the right.

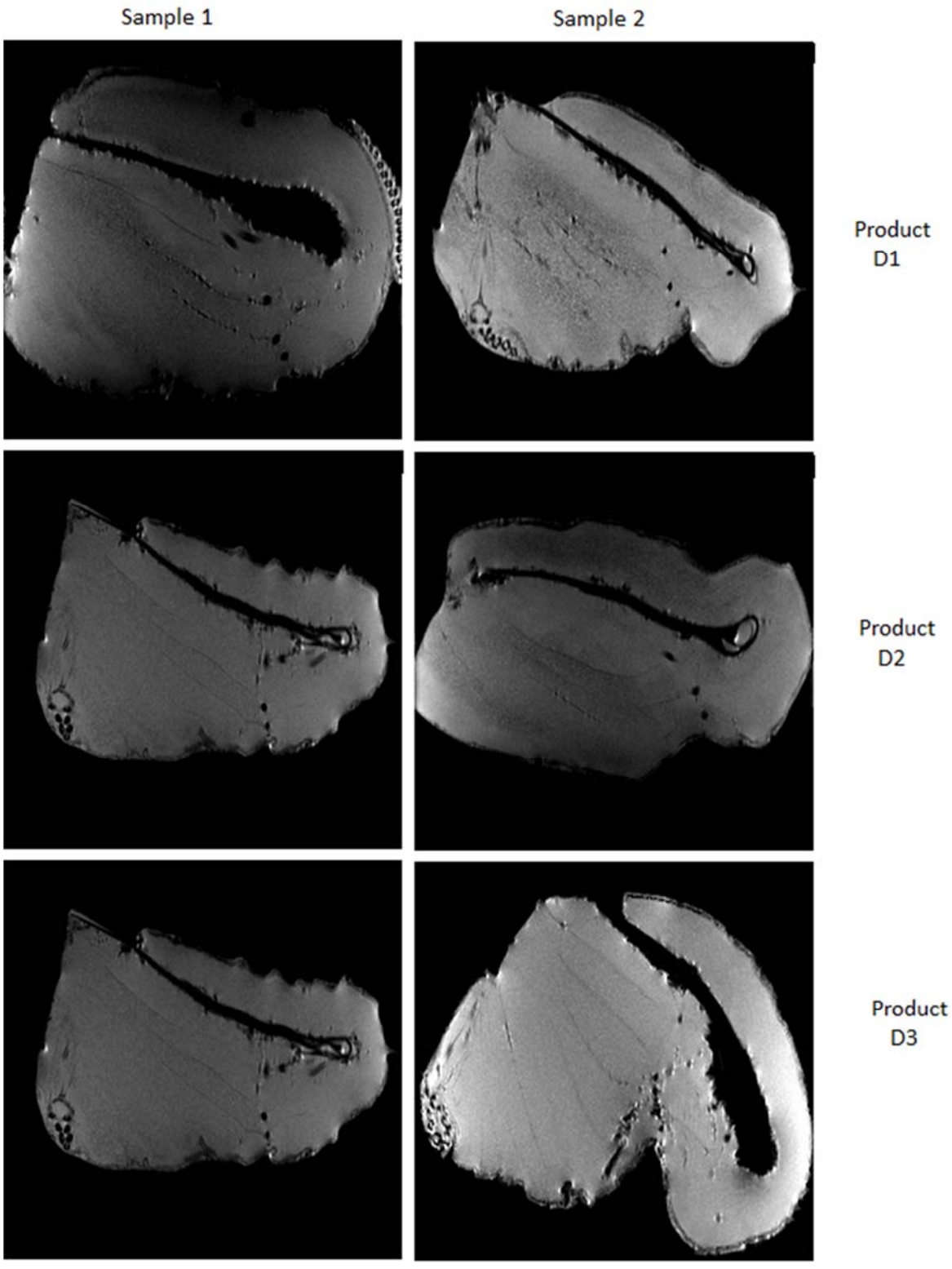

Figure 10: MSME images of products D1 (pre rigor, net caught, RAF injection system), D2 (post rigor, net caught, RAF injection system) and D3 (pre rigor, line caught, RAF injection system). All samples are placed skin-down and with the belly flap folded to the right.

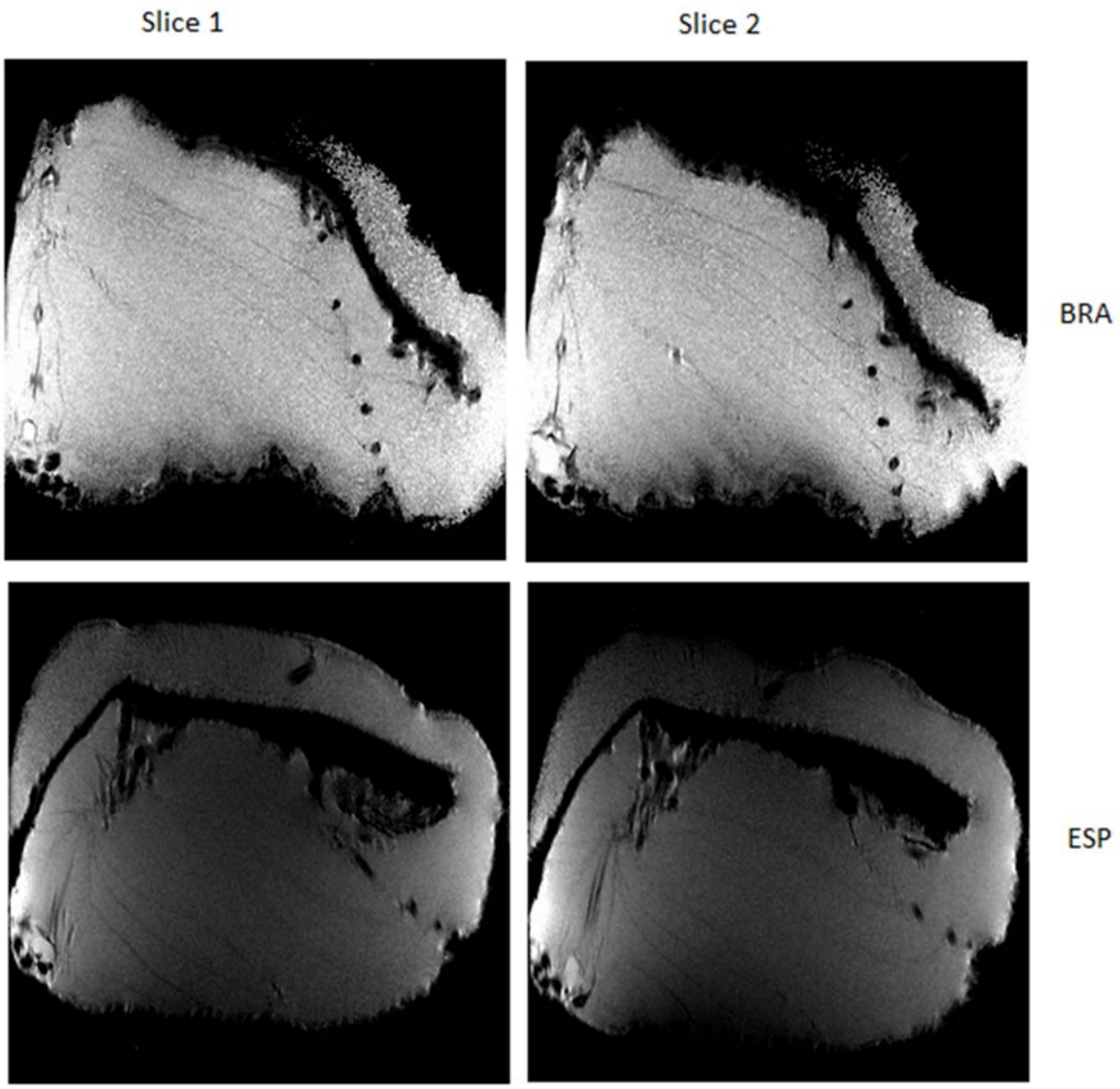

Figure 11: MSME images of the Brazilian (BRA) and Spanish products (ESP). All samples are placed skin-down and with the belly flap folded to the right.

3.3.3 MSME T_2 relaxation time analysis

T_2 relaxation times were calculated from the T_2 distribution maps at four regions of interest (ROIs); the first region at the fillet surface (ROI 1), the second in the middle of the fillet (ROI 2), the third close to the skin (ROI 3) and the fourth in the belly flap area of each slice. The results from this analysis can be seen in figures 12 to 14. The mono-exponential T_2 data obtained with the MSME method, ranging between 24.9 to 43.2 ms on average, correlated well with the weighted average relaxation time from the low field relaxation time analysis. The T_2 MSME relaxation time was also positively correlated to the water content of the samples (R^2=0,834). Large variations, both in T_2 relaxation times standard deviations and max-min range, within a fish/treatment indicated that the water distribution was quite heterogeneous, but the level of heterogeneity was dependent on the treatments. Whether the products were filleted (B1 and C) or flattened did not seem to have a significant effect on the homogeneity of the water distribution.

Pickle salting (product A, BRA and ESP) seemed to lead to a fairly homogeneous water distribution. However, as mentioned before lower T_2 values, as seen in these products, can be correlated to more denatured proteins in the muscle. This was especially evident in the dried Spanish product (ESP), where the lower T_2 value relates well with both the lower water content and higher degree of muscle protein denaturation. A trend of higher water content close to the skin than at the surface was observed in the pickle salted products, indicating this method may dry or “burn” the surface through salt-induced myosin aggregation (Thorarinsdottir et al., 2011). However, these differences were not significant for all samples.

The fine needles used for the RAF injection system (products B1, D1-D3) seemed to have a positive effect leading to higher homogeneity of the water distribution as assessed by the T_2 MSME distribution, compared to the brine injected products using other injection devices (B2, C and E1). However, here net catching (D1 and D2) seemed to increase the heterogeneity in the water distribution (figure 14) compared to the line caught products (B1 and D3).

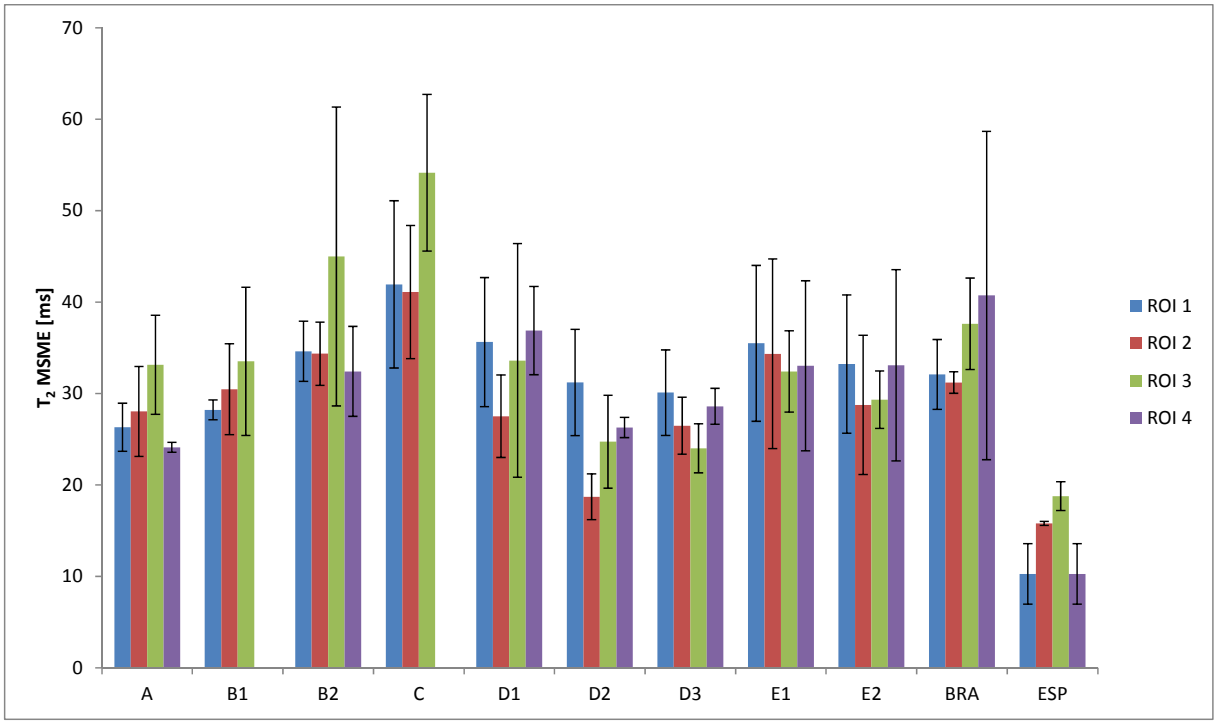

Figure 12: T_2 relaxation times obtained with MSME pulse sequence for four regions of interest in heavily salted cod muscle: ROI 1 at muscle surface, ROI 2 at middle of muscle, ROI 3 close to skin and ROI 4 at the belly flap.

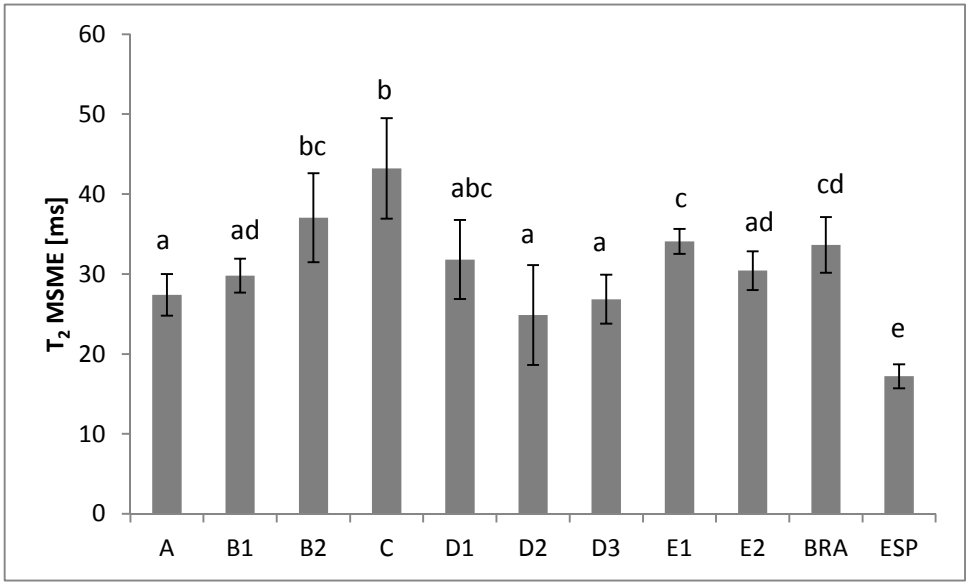

Figure 13: Average T_2 MSME values for each product.

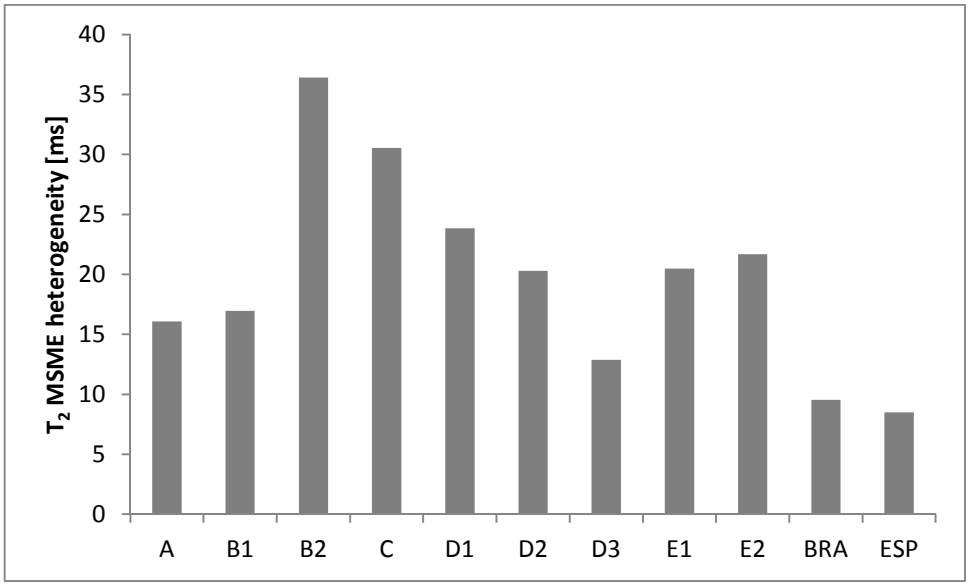

Figure 14: T_2 MSME heterogeneity as assessed by difference between the maximum and minimum T_2 relaxation times within a sample.

3.3.4 Diffusion

Proton diffusion was analysed in products A, B1 and B2 by generating Apparent Diffusion Coefficient (ADC) maps using the MRI instrument (Figures 15-16). ADC parameters were assessed at three regions of interest for these samples, ROI 1-3, positioned in the same was as for the T_2 MSME analysis. Generally higher diffusion coefficients were observed in the brine injected samples (B1 and B2) compared to the pickle salted product A, in agreement with the higher water content and higher water mobility in brine injected products. Veliyulin & Aursand (2007), have earlier 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. Brine injection, with the fine needles in the RAF injection device (B1), lead to a larger variation in the diffusion coefficient, dependent on the region of interest during analysis than injection with the TRAUST device (B2).

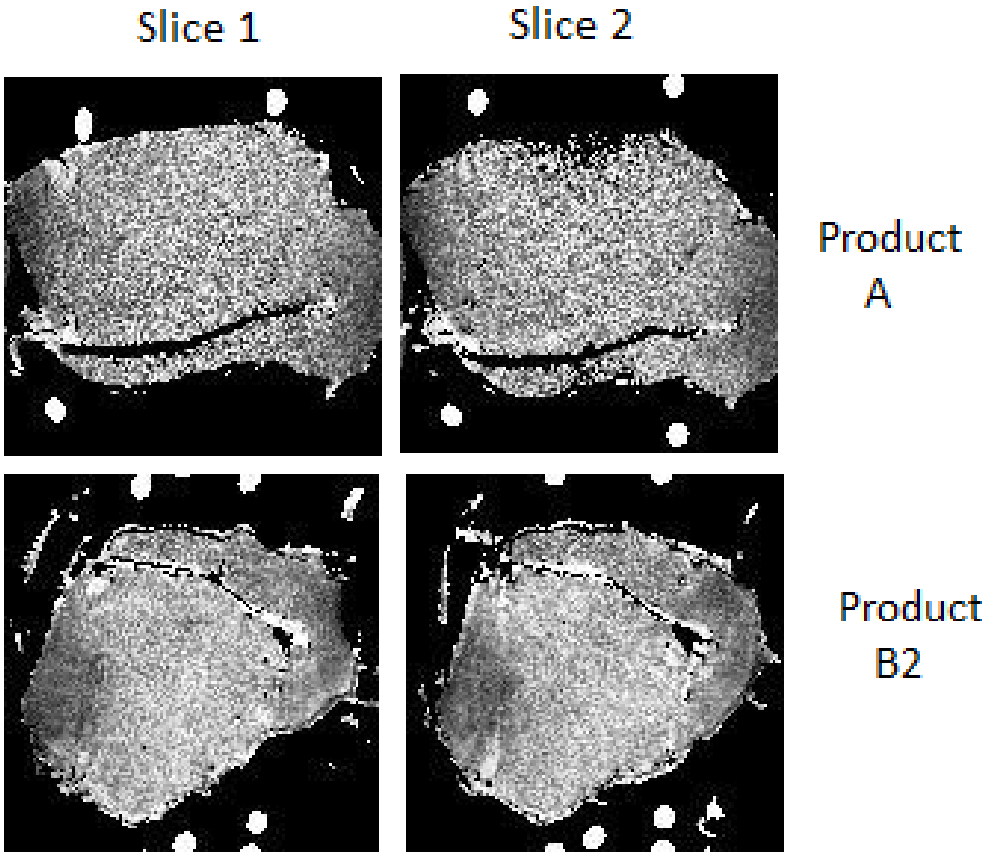

Figure 15: Apparent Diffusion Coefficient (ADC) maps of three dry salted cod products A, B1 and B2.

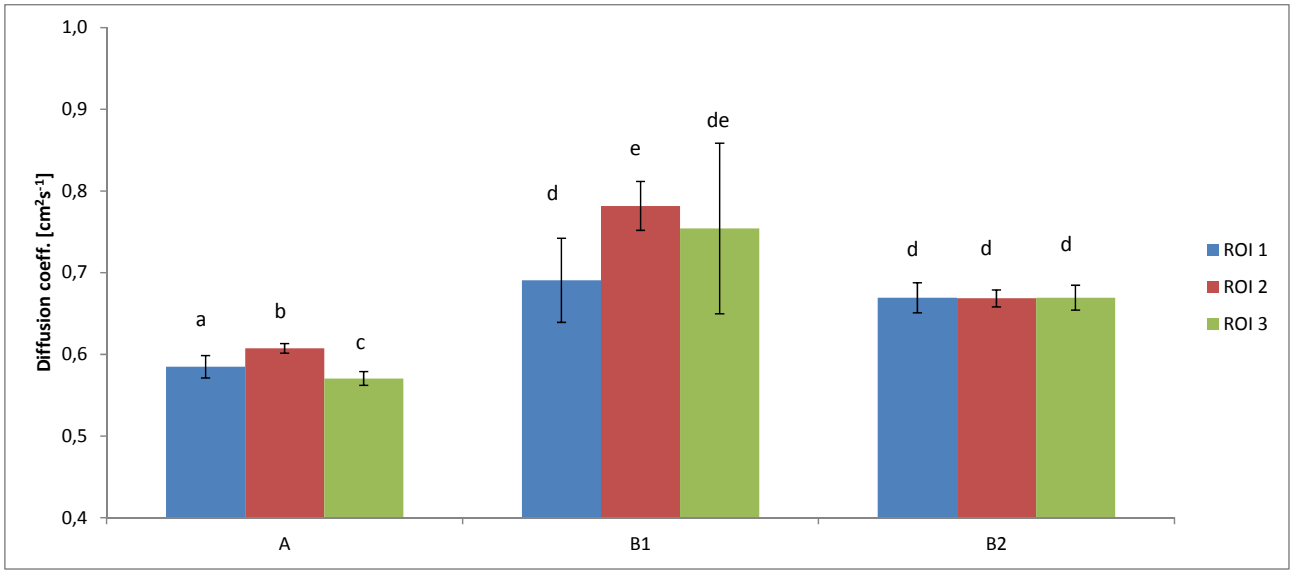

Figure 16: Apparent Diffusion Coefficients from products A, B1 and B2 at three regions of interest: ROI 1 at the muscle surface, ROI 2 at the middle of the muscle and ROI 3 close to the skin.

3.4 Multivariate analysis

A multivariable analysis of the Icelandic products resulted in a principal component analysis where the first two components described 85.9% of the variation between the products. Diffusion results were left out of the analysis, since it was only applied to three of the studied products. The first principal component (PC1 describing 60.8% of the variation) mainly described the differences in the products based on their water content and WHC. Fillets (B1 and C) which contained higher amount of water and salt were seen on the extreme negative axis, while the pickle salted product A, with a low water and salt concentration but a high WHC was located high on the positive axis. The analysis also indicated a positive correlation between increased water and salt content with a longer T_{21} relaxation time. The PC1 also shows an effect of stress prior to catching, since the products which underwent high stress (products A (caught by trawler), D1 and D2 (caught by net)) were grouped together on the positive end of PC1, characterized by a low water and salt content, high WHC and short low field T_{21} and MSME T_2 relaxation times. The products, where the fish was caught by line, were more distributed according to their pre-salting methods. The second principal component (PC2, describing 25.1 % of the variation) described the effect of the water mobility and water distribution within the products. No significant differences were observed in any parameters between the brined product E2 to the brine injected product from the same raw material (E1), except in the phosphate content. However, this indicated that the phosphate content did not have significant effect on the water distribution or retention abilities of the muscle.

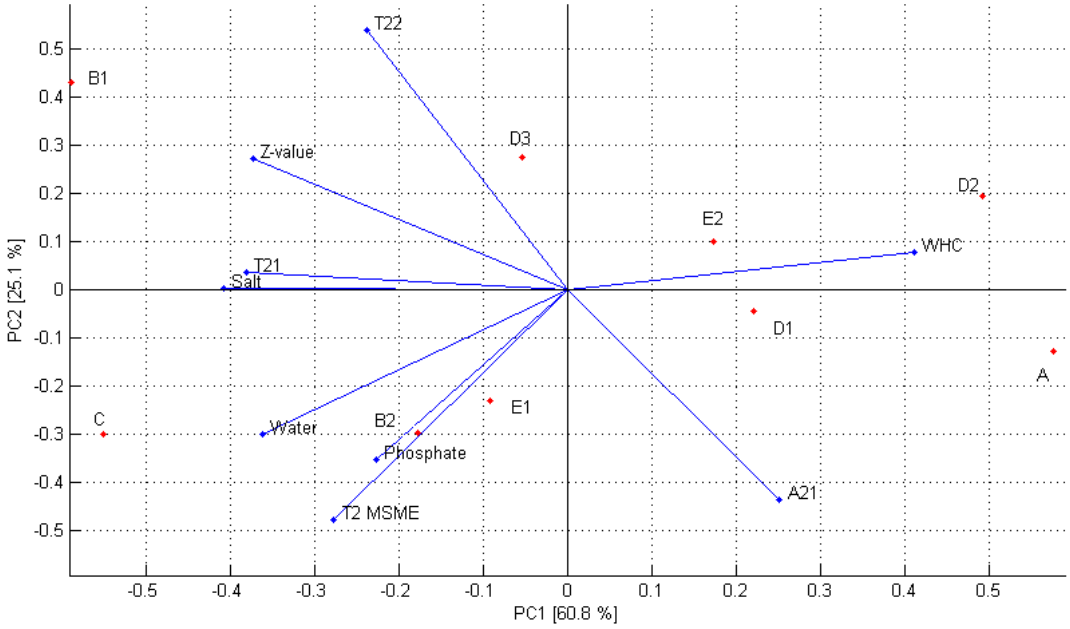

Figure 17: Principal Component Analysis (PCA) of analysed parameters for the Icelandic products. The first two components describe 85.9% of the variation between the products.

To clarify how strongly the analysed parameters were correlated a correlation table for all average results was generated (Table 2). The table indicated that strong positive correlations were obtained between the water content and salt content, as well as the relaxation times from the MSME analysis and the dominating T_{21} relaxation time. However a negative correlation was observed between the water content and the WHC in the products. Several other interesting correlations can be viewed in the summary table.

Table 2. Correlation between variables.

	Water	*Salt*	*WHC*	*Phosphate*	Z^{NaCl}	T_2 *MSME*	T_{21}	T_{22}	A_{21}
Water	1								
Salt	0,888	1							
WHC	-0,843	-0,887	1						
Phosphate	0,602	0,424	-0,516	1					
Z^{NaCl}	0,624	0,914	-0,764	0,184	1				
T_2 *MSME*	0,834	0,578	-0,717	0,680	0,243	1			
T_{21}	0,673	0,770	-0,910	0,320	0,719	0,609	1		
T_{22}	0,086	0,508	-0,439	-0,121	0,789	-0,201	0,594	1	
A_{21}	-0,128	-0,497	0,461	-0,240	-0,728	0,083	-0,477	-0,839	1

4 Conclusions

The study showed that all the analysed experimental parameters had a significant effect on the water and salt uptake and water distribution. Stress induced catching methods, such as trawling or catching by net had a negative affect on the homogeneity of the water in the muscle. Pre rigor processing made the muscle more delicate and sensitive to the formation of brine injection marks. Analysis of the pre-salting methods indicated that a higher water content was gained by using brine injection during pre-salting, while pickle salting lead to a higher degree of protein denaturation during kench salting. Double injections should be avoided since clear injection marks were still visible in these products after kench salting and a large heterogeneity was observed in the water distribution of these samples.

5 Acknowledgements

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7 Appendix

Product	Water [%]	Salt [%]	WHC [%]	Phosphate [mg P_2S_5/g]	Z-value [%]	T_2 MSME [ms]	T_2 homogeneity range [ms]	T_{21} [ms]	T_{22} [ms]	A_{21} [%]	A_{22} [%]
A	56,4 ± 1,3^{ac}	19,5 ± 0,6^{a}	91,8 ± 2,4^{a}	1,3 ± 0,1^{a}	25,7 ± 0,3a	27,4 ± 2,6^{a}	16,1	24,6 ± 0,9^{a}	168 ± 14^{a}	87,7 ± 0,6^{a}	12,3 ± 0,6^{a}
B1	57,8 ± 0,6^{ab}	21,3 ± 0,6^{b}	78,0 ± 3,6^{b}	1,5 ± 0,3^{ac}	27,0 ± 0,6b	29,8 ± 2,1^{a}	16,9	29,2 ± 0,7^{be}	342 ± 30^{b}	81,4 ± 0,4^{b}	18,6 ± 0,4^{b}
B2	58,2 ± 0,4^{b}	20,6 ± 0,4^{bc}	78,3 ± 2,0^{b}	1,5 ± 0,3^{ac}	26,1 ± 0,3ab	37,0 ± 5,6^{b}	36,4	28,4 ± 0,7^{e}	183 ± 30^{ae}	86,8 ± 0,4^{a}	13,2 ± 0,4^{a}
C	58,5 ± 0,3^{b}	20,8 ± 0,2^{b}	76,5 ± 3,0^{b}	2,3 ± 0,2^{b}	26,2 ± 0,2b	43,2 ± 6,3^{b}	30,5	30,5 ± 0,7^{b}	237 ± 2^{c}	84,6 ± 0,7^{ce}	15,4 ± 0,7^{ce}
D1	56,4 ± 0,5^{ac}	19,6 ± 1,0^{ac}	84,7 ± 2,5^{cd}	1,3 ± 0,1a	25,8 ± 1,0ab	31,8 ± 4,9^{ab}	23,9	27,5 ± 1,1^{cde}	222 ± 11^{d}	86,4 ± 1,2^{ac}	13,6 ± 1,2^{ac}
D2	55,1 ± 1,5^{c}	19,0 ± 0,1^{a}	89,1 ± 2,4^{ac}	1,4 ± 0,4^{ac}	25,6 ± 0,6a	24,9 ± 6,3^{a}	20,3	26,0 ± 0,5^{d}	226 ± 2^{d}	84,0 ± 0,5^{c}	16,0 ± 0,5^{c}
D3	56,7 ± 0,5^{ac}	20,1 ± 0,2^{c}	82,3 ± 1,5^{d}	1,2 ± 0,2^{a}	26,2 ± 0,3ab	26,9 ± 3,1^{a}	12,9	29,2 ± 1,3^{be}	299 ± 24^{be}	84,9 ± 0,4^{e}	15,1 ± 0,4^{e}
E1	57,9 ± 1,3^{ab}	20,3 ± 0,9^{bc}	82,9 ± 4,5^{bcd}	2,2 ± 0,7^{bc}	26,0 ± 0,4a	34,1 ± 1,6^{b}	20,5	27,4 ± 0,1^{c}	204 ± 6^{e}	85,4 ± 0,4^{de}	14,6 ± 0,4^{de}
E2	56,7 ± 1,6^{abc}	19,9 ± 0,8^{bc}	86,2 ± 4,1^{acd}	0,8 ±0,2^{d}	26,0 ± 0,5a	30,4 ±2,4^{b}	21,7	28,1 ± 0,7^{ce}	241 ± 50^{cde}	86,2 ± 0,6^{d}	13,8 ± 0,6^{d}