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Quality changes during seafood processing as studied with NMR and NIR spectroscopy

Thesis for the degree of Philosophiae Doctor

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Abstract

The production of seafood is a complex procedure, including various steps where quality can be lost by during chemical, physical or mechanical treatment during processing or storage. The aim of this study was to obtain a deeper understanding on the changes occurring within the muscle structure during seafood processing and storage and how these changes are affecting the product quality. The processes studied were the processing differences of farmed and wild Atlantic cod (*Gadus morhua*) in the production and storage of lightly salted products, the effect of salting and superchilled storage on the quality of Atlantic cod, the effect of different pre salting methods on the quality of heavily salted cod (bacalao), the effect of protein injection on the quality and stability of saithe (*Pollachius virens*) fillets during frozen storage and the effect of phosphate content during pre brining on cold water shrimp (*Pandalus borealis*) muscle.

Low field nuclear magnetic resonance (LF-NMR) was used to study the water distribution and water holding ability of the muscle. Relaxation time measurements revealed 1-3 water populations in the seafood muscle with relaxation times dependent on the sample treatment. Multivariate analysis was used to compare the spectroscopic results to traditional physicochemical methods. Principal Component Analysis gave indications of which parameters were related and then Partial Linear Square (PLS1) models were used to indicate which physicochemical parameters showed significant correlations to the NMR variables. Strong significant correlations were found to parameters reflecting water behaviour and retention, such as water content, water holding capacity, water activity, storage drip during superchilled and frozen storage and cooking yield, but also to other parameters affecting the muscle structure such as muscle pH, the formation of total volatile base nitrogen (TVB-N) and trimethylamine (TMA) to mention a few. The study then indicated that LF-NMR can be used to indicate different aspects of seafood quality deterioration where the spoilage mechanisms or denaturation affect the water properties of the muscle.

The application of Low field NMR by using a unilateral magnet was studied during shrimp processing. The instrument showed promising characteristics in the control and optimization of shrimp processing and other seafood, although the technique requires further optimization prior to process implementation.

The application of near infrared (NIR) reflectance spectroscopy, using a fibre probe, to online or at-line quality monitoring of shrimp was also studied in this work. Strong correlations were found

to water content and water holding capacity of the shrimp during processing and calibration models were built for these parameters, using traditional measuring methods as a reference.

The study shows that LF-NMR and NIR are very useful methods in online or at-line monitoring of various physicochemical properties during seafood production. However, online or at-line applications of these techniques need to be optimized for each processing line of interest, with regard to the most important quality parameters for each case with respect to type of raw material, concentrations of additives or technical aids and choice of other processing steps.

Key words: Low field Nuclear Magnetic Resonance (LF-NMR), seafood, muscle properties, process optimization, near infrared spectroscopy (NIR), physicochemical properties.

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Table of Contents

2 List of papers

The thesis is based on the following papers referred to in the thesis with their respective Roman numerals. The papers are appended at the end of the thesis.

I. Gudjonsdottir M, Gunnlaugsson VN, Finnbogadottir GA, Sveinsdottir K, Magnusson H, Arason S, Rustad T. 2010. Process control of lightly salted wild and farmed Atlantic cod (*Gadus morhua*) by brine injection, brining and freezing–A low field NMR study. *Journal of Food Science* 75 (8), E527-536.

II. Gudjónsdóttir M, Lauzon HL, Magnússon H, Sveinsdóttir K, Arason S, Martinsdóttir E, Rustad T. 2011. Low field Nuclear Magnetic Resonance study on the effect of salt and modified atmosphere packaging on cod (*Gadus morhua*) during superchilled storage. *Food Research International* 44, 241-249.

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

IV. Gudjónsdóttir M, Karlsdóttir MG, Arason S, Rustad T. 2010. Injection of fish protein solutions to fresh saithe (*Pollachius virens*) fillets studied by low field Nuclear Magnetic Resonance and physicochemical measurements. Submitted to *Journal of Food Science and Technology*.

V. Gudjónsdóttir M, Jónsson Á, Bergsson AB, Arason S, Margeirsson S, Rustad T. 2010. Shrimp processing assessed by low field Nuclear Magnetic Resonance, Near Infrared spectroscopy and physicochemical measurements – The effect of polyphosphate content and length of pre-brining on shrimp muscle. Accepted for publication in *Journal of Food Science*.

3 The author‘s contribution to the papers

I. The author planned the experiments and wrote the paper in collaboration with co-authors. The author carried out the experiment and analysis.

II. The author planned the experiments and wrote the paper in collaboration with co-authors. The author carried out the experiment and analysis.

III. The author planned the NMR measurements and wrote the paper in collaboration with co-authors. The author carried out the NMR experiment and overall analysis.

IV. The author analyzed the data and wrote the paper in collaboration with co-authors.

V. The author planned the experiments and wrote the paper in collaboration with co-authors. The author carried out the experiment and analysis

4 Abbreviations and symbols

A_{2i} = Apparent water population corresponding to the relaxation time T_{2i}, i = 1, 2, 3

a_w = Water activity

B_0 = Applied magnetic field strength

B_1 = Strength of oscillating magnetic field

Br = Cod fillets pre salted by brining (Paper III)

CPMG = Carr Purcell Meiboom Gill pulse sequence

DS = Dummy scans

DS = Dry salted (Paper III)

DSC = Differential Scanning Calorimetry

ΔE = Energy difference between energy levels

Eq. = Equation

F = Farmed cod (Paper I)

FID = Free induction decay

FIDR = Relative free induction decay

FPHyd = Fish protein hydrolyzate (Paper IV)

γ = Gyromagnetic ratio

Gel = Gelatine (Paper V)

h = Planck's constant

^1H = Hydrogen

HFP = Homogenized fish proteins (Paper IV)

I = Spin quantum number

IQF = Individually quick frozen

IR = Inversion recovery pulse sequence

IS = Cod fillets pre salted by salt injection, followed by brining (Paper III)

ISP = Cod fillets pre salted by salt and phosphate injection, followed by brining (Paper III)

k = Boltzmann constant

Ke = Kench salting (Paper III)

LF = Low field

LPh = Shrimp treated with less phosphate (Paper V)

ν = Larmor frequency

MAP = Modified atmosphere packaging

MRI = Magnetic Resonance Imaging

M_{xy} = Transversal magnetization

M_z = Longitudinal magnetization

n = number of measurements/samples

^{23}Na = Sodium

NIR = Near Infrared spectroscopy

N_{lower} = Population of the lower energy level (spin up)

N_{upper} = Population of the higher energy level (spin down)

NMR = Nuclear Magnetic Resonance

NS = Number of scans

PCA = Principal component analysis

Pi = Cod fillets pre salted by pickling (Paper III)

p = Isoelectric point

PLS = Partial least square regression

PS = Pre salted (Paper III)

R = Relaxation rate

R^2 = Correlation coefficient

RD = Recycle delay

RDT = Receiver dead time

Reg = Regularly treated shrimp (Paper V)

RF = Radiofrequency

RG = Receiver gain

RH = Relative humidity

RH = Rehydrated (Paper III)

RM = Raw material (Paper III)

RMSECV = Root mean square error of cross validation

RPD = Residual predictive deviation

SDS-PAGE = Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

SPB = Shrimp treated with shorter pre-brining time (Paper V)

SPP = Sodium Polyphosphate

θ = Pulse angle

t = Time

T = Temperature

τ = Echo time

T_1 = Longitudinal (spin-lattice) relaxation time

T_2 = Transversal (spin-spin) relaxation time

T_{21} = Shorter transversal (spin-spin) relaxation time in a bi-exponential fit

T_{22} = Longer transversal (spin-spin) relaxation time in a bi-exponential fit

TMA = Trimethylamine

TMAO = Trimethylamine oxide

tp = Radiofrequency pulse duration

TVB-N (TVN) = Total volatile base nitrogen

W = Wild cod (Paper I)

WHC = Water holding capacity

5 Introduction

Fish and seafood products are major export product from Iceland. In 2009 about 669 thousand tons of fish and seafood products were exported from Iceland, worth approximately 1.3 billion Euros (Hagstofa Íslands, Statistics Iceland). Consumers worldwide are generally getting more aware of the nutritional benefits of seafood consumption and are therefore making higher expectations regarding product quality, traceability and safety. Therefore optimization and maintenance of quality through the whole value chain is of great interest to Icelandic seafood producers as well as the common consumer.

Processing of seafood includes various steps that affect the protein structure of the muscle, by chemical, physical or mechanical treatment which affect the characteristics of the final product. Cooking, salting and freezing are examples of processing steps that are known to denature and cause aggregation of proteins and lead to loss of water from the muscle (Shenouda 1980; Steen and Lambelet 1997; Jensen and others 2002, 2003). Water is the most abundant component in muscle foods, such as seafood, and changes in content and properties of water during processing influences the quality, texture, sensory attributes and shelf life of the product. It is therefore a major concern of seafood producers to retain the water, both for quality and economical reasons. The effect various processing steps has on a product is however dependent on the characteristics of the raw material, the chosen processing methods and handling, including the effect of various concentrations of additives and technical aids during the processing. The knowledge on the effects of processing and storage on shelf life and quality deterioration of seafood products is therefore the prerequisite for optimizing the quality and value of the final products reaching the consumers.

Standard procedures for the determination of physicochemical properties of fish and meat are generally complex, time-consuming and expensive. Rapid and economical methods for determination of these properties can therefore be an advantage in developing optimized processing of seafood or other muscle foods. In this thesis low field nuclear magnetic resonance (NMR) and near infrared (NIR) spectroscopy were used to get a further insight into the changes occurring in muscle foods during processing by studying the changes in water content and distribution in the muscle at various processing steps. The muscle structure was studied with respect to the characteristics of different raw materials, rigor status during processing, different additives and processing aids and how different handling and processing affected the product during light and heavy salting, freezing, cooking and superchilled storage.

Relaxation time measurement by low field NMR have been found to give strong correlations to various physicochemical properties of fish and meat muscle, such as moisture content (Andersen and Rinnan, 2002), water holding capacities and drip loss (Jepsen and others, 1999; Erikson and others, 2004) and has been used to indicate pH-induced structural changes occurring in the muscle post mortem (Bertram and others, 2000) and the effects of frozen storage on protein denaturation in fish (Steen and Lambelet, 1997). Near infrared spectroscopy has been applied widely in industry due to its simplicity, short sample preparation time and fast measuring time and the method can be used either online or at-line depending on what factors within a process that are to be controlled or monitored (Windhab and Bollinger 1996; Hoyer 1997). These rapid, non-destructive techniques were therefore also used to study correlations to various physicochemical properties of the muscle, as measured with standard determination methods, with the aim of building calibration models to predict important quality parameters in a rapid and non-destructive way and to find the applicability of these methods for online or at-line monitoring of the product quality during the processes studied.

6 Objectives

The objectives of the study was to investigate the structural changes occurring in seafood muscle during processing and storage and gain increased understanding in the water properties and dynamics of the muscle through various processing. This was done by means of the fast non-destructive and non-invasive analytical methods of low field NMR and NIR spectroscopy, in comparison to traditional physicochemical analytical methods. The underlying aims of the study were to investigate:

- the muscle structure as affected by raw material origin (wild or farmed cod, *Gadus morhua*), rigor status and handling during processing (Paper I).
- the effects of superchilled storage and modified atmosphere packaging (MAP) on water binding and spoilage in cod loins (Paper II)
- the effect of light salting on distribution and compartmentalization of water and quality of seafood (Papers I-V).
- the effect of dry salting of cod, as affected by various pre salting methods, on protein denaturation and water distribution through the salting and rehydration process (Paper III).
- the effect of protein injection on muscle structure and storage stability during chilled and frozen storage of saithe (*Pollachius virens*) fillets (Paper IV).
- the effect of salting, cooking and frozen storage on protein denaturation (Papers III-V).
- the online/at-line applicability of the NMR and NIR techniques during seafood processing (Paper V).
- validation and benchmarking of the NMR and NIR techniques by comparing them to known acknowledged methods (Papers I-V).

7 Theoretical background

To understand the complex effects of various processing and storing methods on the quality of seafood muscle, general knowledge of the characteristics of the species and their physicochemical properties of muscle structure must be known. The following chapter deals with the fundamental structure of seafood muscle and how it can be changed by various processing and treatments.

7.1 Raw material

Three main species were studied during this work, Atlantic cod (*Gadus morhua*), saithe (*Pollachius virens*) and cold water shrimp (*Pandalus borealis*), each with different characteristics and processing ability. A more detailed description of the studied species follows in the sections below.

7.1.1 Atlantic cod (*Gadus morhua*)

Atlantic cod (*Gadus morhua*) is a lean fish species that belongs to the gadoid family of fish. Cod can be found all over the boreal region of the North Atlantic, from North-Carolina to Labrador, around Iceland and Greenland and in the North-east Atlantic from the Bay of Biscay up to Svalbard (ICES, 2010). The cod lives in coastal waters down to approximately 600 m depth and mainly feeds on capelin, but also shrimp and other marine animals (Jónsson, 1992). The cod can grow to become up to two meters in length and up to 96 kg in weight. It can become up to 25 years old and generally reach sexual maturity at the age of 4 to 9 years dependent on the temperature of the sea in its habitat (Jónsson, 1992). Spawning in Icelandic waters generally occurs in the warmer sea around the south and south-west coast at a depth of 50-100 m. The spawning begins in March and ends in the beginning of May. The cod prefers sea temperatures of 4-7 °C, but can be found at temperatures ranging from 0°C up to 20°C.

Cod is one of the most important commercial fish species from the North Atlantic Ocean and is exported both as fresh fish, lightly salted fresh fish, frozen and heavily or dry salted fish (bacalao). However, cod stocks have declined rapidly in the last few years and the stocks are fully or even overexploited. To meet an increasing market demand for cod products, cod aquaculture has become a growing industry. Studies have shown that the muscular structure and quality characteristics of the farmed cod are significantly different from the properties of wild cod muscle (Olsson and others, 2006; Herland and others, 2007; Gudjónsdóttir and others, 2009; Paper I), especially when taking seasonal changes of the wild cod into account (Love and others, 1972). Seasonal differences of fish muscle are described in further detail in chapter 8.1.4 on raw material diversity. Due to this difference farmed cod has not been used in all cod products, in which wild cod is used today. Optimization of cooling, freezing, and salting in the processing of lightly salted cod products, of both wild and farmed origin, is therefore important to ensure optimal product quality.

7.1.2 Saithe (*Pollachius virens*)

Saithe (*Pollachius virens*) is also a member of the gadoid family but is a semi-pelagic North Atlantic species. The species is distributed in the Barents Sea and North Atlantic, reaching as far south as to North Carolina in the west and Bay of Biscay in the east. The species is therefore an important commercial species in many European countries. The saithe lives in deep waters over the shelf edge (approximately 200 m depth) and deeper. However, the juvenile saithe, 2-3 years of age, generally occupy inshore habitats. The saithe becomes sexually mature at the age of 4 to 7 and is then approximately 60-80 cm long, but can grow to become up to a maximum of 130 cm long and 25 years of age (ICES, 2010b). The species mainly lives on krill, fry, capelin, herring or juvenile fish of other species. The larger fish occasionally eat squid (Jónsson, 1992). Spawning of saithe in Icelandic waters occurs from January in the south part of the distribution area to March in the northern part of the distribution area. The majority of caught saithe is exported frozen or salted from Iceland (Hagstofa Íslands, Statistics Iceland).

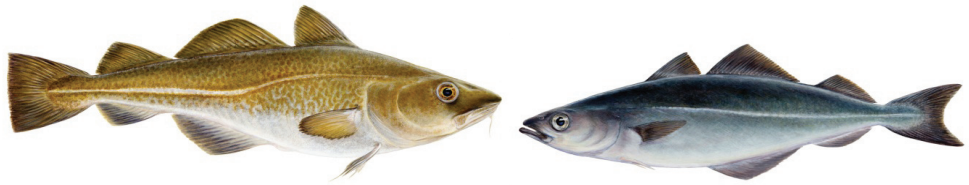

Figure 1: Figure of an Atlantic cod (Gadus morhua) on the left side and a saithe (Pollachius virens) on the right. With courtesy of the artist, Jón Baldur Hliðberg (www.fauna.is).

7.1.3 Cold water shrimp (*Pandalus borealis*)

The cold water shrimp (*Pandalus borealis*) found around Iceland can be divided into inshore shrimp (living at a depth less than 200 m) and deepwater shrimp (living below 200 m of depth) according to their habitat. The shrimp is male from birth and up to 1-6 years of age, when they change sex and become female. The time of this change is dependent on the temperature of the surrounding ocean, but colder habitat leads to a longer male period in a shrimp's life. The shrimp feeds on animal and algae remains, as well as other small animals, i.e. mollusc and worms found on the ocean floor. The shrimp is protected by a calcium-chitin-protein based shell, which surrounds the muscles and the head. The shell does not grow with the shrimp and therefore the shrimp sheds the shell up to 25-30 times during a lifetime (Skúladóttir, 1994). Figure 2 shows the general structure of a cold water shrimp.

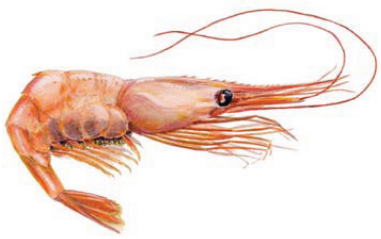

Figure 2: Figure of a cold water shrimp (Pandalus borealis). With courtesy of the artist, Jón Baldur Hliðberg (www.fauna.is).

Iceland is a major producer of cold water shrimp (*Pandalus borealis*) and the main product produced is cooked and peeled shrimp, or so called salad shrimp. Larger shrimp is however exported to Japan. Generally the shrimp is frozen raw onboard the fishing vessels and then processed on land.

7.1.4 Raw material diversity

The composition of the fish muscle varies with multiple factors including species, sex, size, stage of sexual maturity, season, spawning, catching ground and handling on board. Table 1 shows some typical values for the chemical composition in species relevant to this study. Salmon and prawns are here also mentioned for comparative reasons.

Table 1: Chemical composition of a few relevant seafood species.

Species	Scientific name	Water [%]	Fat [%]	Proteins [%]
Atlantic cod	*Gadus morhua*	78-83	0.1-0.9	15.0-19.0
Saithe	*Pollachius virens*	79-81*	0.3-0.6	16.4-20.3
Salmon	*Salmo salar*	67-77	0.3-14.0	21.5
Cold water shrimp	*Pandalus borealis*	77-81[+]	0.7-1.4[#]	17.0-22.1[†]
Prawn, white meat	*Paleamon serratus*	71	1.3	22.8

Values from white muscle (Murray and Burt, 2001). Values from *Smith and Hardy (2001), [+]Zeng and others (2006) and Veigarsson (1999), [#]Iverson and others (2002) and [†]Sikorski (1994a).

The season of catch has a great impact on the distribution of water, fat and proteins in the muscle and thus a great influence on the texture and quality of the fish. Gadoid fish generally regenerate their fat and energy supplies during the summer and early fall. The protein content in cod reaches a maximum in the fall during the months of October and November in Icelandic waters (Jónsson, 1992). The fish is then firm and at an optimal raw material condition. However, during spawning the feed accessibility is low, leading to a soft, watery muscle with a low fat content (Raversu and Krzynowek, 1991). The length of the spawning season is however strongly varying between species (Huss, 1988). Botta and others (1987) also showed that the choice of catching

method (gillnet, handline, longline or trap) significantly affected caloric, moisture and protein content of cod muscle.

7.2 Muscle structure

Fish muscle mainly consists of water, proteins, lipids and connective tissue. The properties and functionality of a muscle vary not only by species, but also the environment in which the animals live and how the muscle is treated and processed post mortem. Basic knowledge of muscle structure is therefore essential to understand the effects of various treatments of muscle food. Muscle proteins are usually divided into the following three categories, based on their solubility characteristics: structural proteins, sarcoplasmic proteins and connective tissue proteins (Huss, 1995; Foegeding and others, 1996). The solubility properties of proteins are also influenced by the pH, ionic strength and processing conditions. The structural proteins (actin, myosin, tropomyosin and actomyosin) are soluble in neutral salt solutions with fairly high ionic strength (≥0.3 M) (Rustad, 2010). These proteins constitute up to 50-60% of the protein content in fish (Shahidi, 1994). Sarcoplasmic proteins (myoalbumin, globulin and enzymes) are soluble in solutions with low ionic strength (<0.15M) (Huss, 1995). The sarcoplasmic proteins make up 25-30% of the total protein content in fish. Denaturation and aggregation of proteins during processing or storage can lead to a loss in protein solubility (Sikorski, 2001). The following chapter illustrates the differences in muscle structure found in the species studied.

7.2.1 Structural proteins

The muscle structure of fish consists of bundles of parallel running multinucleated muscle cells, so called *myotomes*, each connected to adjacent myotomes as well as to the skeleton and skin structure by thin layers of collagenous connective tissue, a so called *myocommata (or myosepta) (*Figure 4*).* These muscle cells contain numerous (up to 1000) myofibrils, which are made up of the contractile myofibrillar proteins. These are arranged in formed structures of thick bands (A-bands), mostly containing *myosin,* and thin bands (I-bands), mostly containing *actin,* between two Z-discs, forming a sarcomere, the basic contractile unit of the muscle (Figure 3).

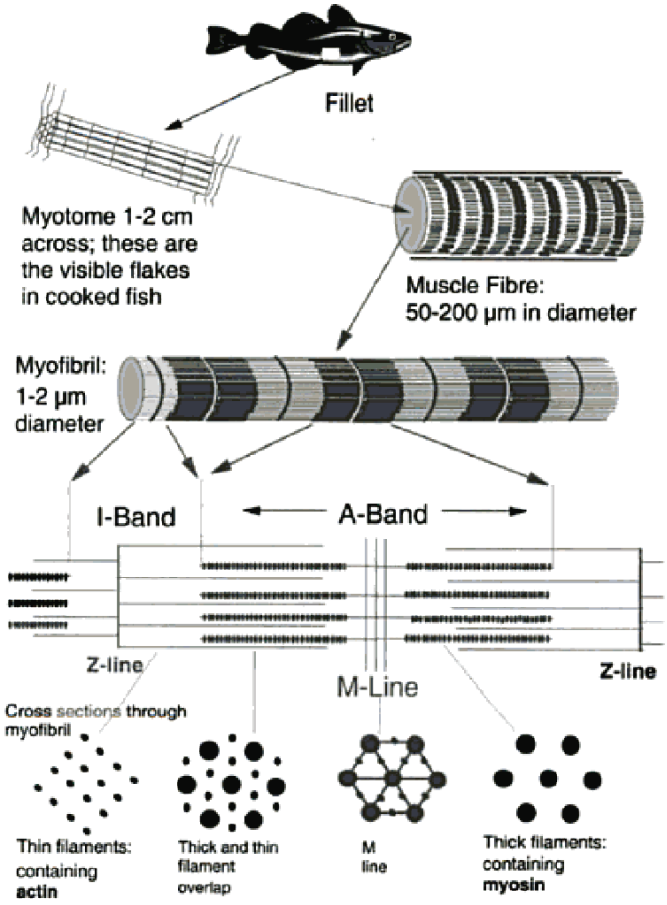

Figure 3: Structure of fish muscle tissue (Goodband, 2002).

The bundles of muscle cells run in the longitudinal direction of the fish, and a muscle cells extends the full length between two myocommata (Huss, 1995) (Figure 4).

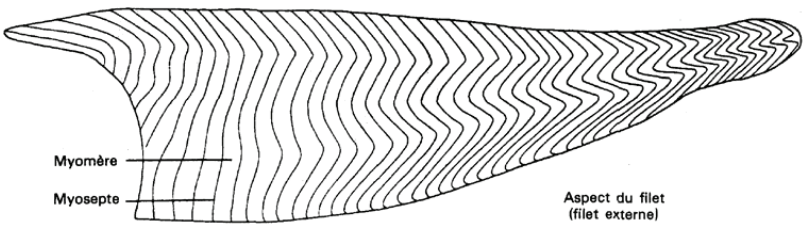

Figure 4: Muscle structure of a gadoid fish fillet, illustrating how parallel running myotomes are connected by myocommatas. Figure adapted from Knorr and others (1974) and later Collignon and others (1984).

The muscle cells in shrimp also run in the longitudinal direction of the shrimp, but the muscle cells are not running between myocommatas, and have in that sense more similarities to the muscle structure of land based animals. The sarcomere length and density of the fibre bundles are however

different with regards to species as well as position in the shrimp (Benjakul and others, 2008) (Figure 5).

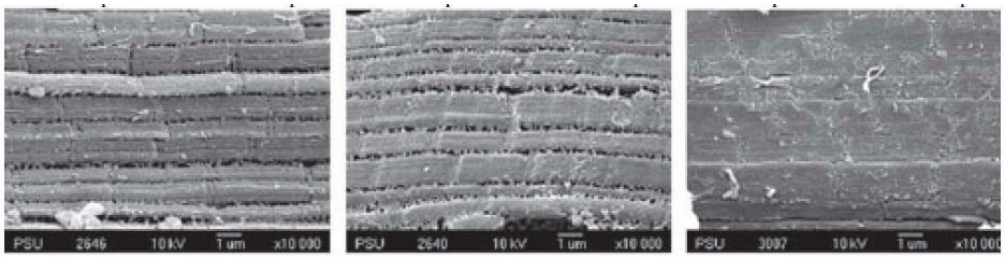

Figure 5: SEM micrographs of a longitudinal section of raw white shrimp meat from the front part (left), middle part (middle) and tail part (right) of the shrimp (Benjakul and others, 2008).

The myofibrillar proteins account for approximately 50-60% of the total protein in the muscle of fish (Shahidi, 1994) and approximately 70% of the total protein in shrimp (Bitsch and others, 1990). The myofibrillar proteins play an important role in binding of water in the muscle, but the amount of water bound to proteins is dependent on the amino acid composition and conformation of the protein, ionic strength, and muscle pH to mention a few. Approximately twenty different types of myofibrillar proteins are known and are divided into three subgroups according to their functionality: i) the major contractile proteins, including myosin and actin, ii) regulatory proteins, such as tropomyosin and troponin and finally iii) cytoskeletal proteins, such as titin or connectin (Xiong, 2000). The major myofibrillar contractile and regulatory proteins are discussed further below.

Myosin, which is the major component in the thick filaments, is the myofibrillar protein that plays the most important role in meat and fish processing. Myosin proteins are large molecules, which consist of six polypeptiede subunits, two large heavy chains and four light chains arranged into an asymmetric molecule. The molecule has two globular heads on a long α-helical rod. Myosins are highly charged molecules, due to negatively charged amino acid residues in the rod part of the molecule. This makes the rod portion relatively hydrophilic, which is the reason why myosin is responsible for most of the water binding within fish muscle. The myosin heads are on the other hand relatively hydrophobic and play a crucial role in binding to actin and is a site for adenosine triphosphatase (ATP-ase) activity generating muscle contraction (Xiong, 2000).

Actin, which is the second most abundant myofibrillar protein, can exist in either monomer form (G-actin) or in fibrillar form (F-actin). The actin in skeletal and cardiac muscle is the fibrillar F-actin, which forms double helical filaments. In a muscle that is in or post rigor the actin is connected to myosin in the actomyosin complex. The properties of the actomyosin complex are influenced by certain food additives, such as salt or phosphates during processing, which in turn affects the physicochemical properties and the quality of the food (Xiong, 2000).

Troponin and tropomyosin are also found in the thin filaments and serve the role of regulating muscle contractions by influencing the binding of myosin to actin (Foegeding and others, 1996). Tropomyosin is made up of long two-stranded helical rods, each consisting of seven G-actin monomers along with a troponin molecule at one end. The troponin lies between the actin filaments in the muscle structure.

7.2.2 Sarcoplasmic proteins

Sarcoplasmic proteins are very diverse (it is estimated that a cell contains between 100 and 200 different sarcoplasmic proteins) and account for 25-30% of the total protein content in fish muscle (Huss, 1995). Most of the enzymes connected to energy metabolism, such as glycolysis, is found in this group. Although sarcoplasmic proteins are so many and diverse, they have some common qualities. Most have relatively low molecular weights, a high isoelectric pH point and globular structures to mention a few (Xiong, 2000). Myoglobin is one of the most important sarcoplasmic proteins in meat and is responsible for the red colour in fresh meat (Molins, 1991). The sarcoplasmic proteins are important for the biochemical processes taking place in the muscle post mortem, including tenderisation processes (Xiong, 2000). The specific activity of different enzymes depends on the fish species, as well as season, life cycle stage etc. (Nakagawa and others, 1988; Søvik and Rustad, 2004, 2005a and b). Determination of sarcoplasmic proteins by electrophoresis can then be used to distinguish between different fish species, but each species has their characteristic band pattern (Lundstrom, 1980; Nakagawa and others, 1988). The sarcoplasmic proteins can affect the quality of fish directly or indirectly through its effects on for example colour, taste, texture or nutritional value to mention a few. Whether the sarcoplasmic proteins affect the water holding capacity in meat is however a matter of discussion (Wilson and van Laack, 1999; den Hertog-Meischke and others, 1997).

7.2.3 Connective tissue proteins

Connective tissue proteins connect muscle cells, fibre bundles and muscles and form structural support to bones, ligaments and tendons. The most abundant connective protein is the extracellular protein collagen. Collagen is a glycoprotein composed of three polypeptide α-chains in a triple helical structure that is stabilized by H-bonds. Sato and others (1986) showed that a high collagen content in fish led to increased flexibility of the body and increased swimming movement ability. Three layers of connective tissue are present in muscle, i.e. endomysium, perimysium and epimysium. Endomysium is the connective tissue layer that sheaths each muscle fiber. Perimysium is the connective tissue surrounding bundles of muscle, while epimysium surrounds the whole muscle.

Each of these layers is made up of different types of collagen. Type I collagen, which consists of two identical α_1(I)-chains and one α_2(I)-chain, is the main component in epimysium. Type I and type III,

which consists of three identical α_1(III)-chains, are the main components in perimysium in meat (Xiong, 2000). The collagen type found in each type of connective tissue in meat and their chain composition can be seen in Table 2 and Table 3 respectively.

Table 2: Description of major collagen components in muscle connective tissue in meat (Light and Champion, 1984).

Connective tissue in muscle	Collagen components
Endomysium	Type I, III, IV and V
Perimysium	Type I and III, traces of type V
Epimysium	Type I and III

Table 3: Chain composition of the different collagen types found in meat and fish.

Collagen type	Chain composition
I*	$[\alpha_1(I)]_2\alpha_2(I)$ or $\alpha_1(I)\ \alpha_2(I)\ \alpha_3(I)$
II	$[\alpha_1(II)]_3$
III	$[\alpha_1(III)]_3$
IV	$[\alpha_1(IV)]_3$
V*	$[\alpha_1(V)]_2\ \alpha_2(V)$ or $\alpha_1(V)\ \alpha_3(V)\ \alpha_4(V)$
VI	α_1(VI)-chain+ α_2(VI)-chain+ α_3(VI)-chain

*Chain compositions identified in fish (Sato and others, 1989; Kimura and Ohno, 1987). Other values from Light and Champion, 1984; Xiong 2000 and Hashimoto and others, 1986.

Although Sato and others (1989) observed type I and type V collagen as major and minor collagens in fish muscle respectively, they did not observe any detectable amount of the type III collagen in the fish species studied, unlike in mammalian and avian intramuscular connective tissue. The localization of molecular species of collagen may therefore differ in fish from the localization of collagen in mammalian and avian muscle. Yata and others (2001) identified type I and type V collagen in fish skin, with a similar distribution of molecular identity of collagens as in ordinary fish muscle. Sato and others (1991, 1997) showed that enzymatic degradation of type V collagen can been related to the postharvest softening of fish muscle such as rainbow trout (*Oncorhychys mykiss*) and sardine (*Sardinops melanosticta*).

7.2.4 Water

Water is the most abundant constituent found in most foods and accounts for approximately 80% in lean fish muscle, such as in cod or saithe. Changes in amount and properties of water therefore play a vital role in the quality changes occurring in fish muscle during processing and storage (Murray and Burt, 2001). The water content affects various quality parameters such as appearance, texture and storage stability of the fish. Water is also the main growth medium for moulds and bacteria, showing that water properties, such as water content and water activity, are highly affecting the shelf life of the product. According to Sikorski (2001) the main factors affecting the muscle structure are properties of the amino acid composition, sequence and the molecular weight. Together with environmental factors such as pH and ionic strength, these factors determine tertiary and

quarternary structures, hydrophobicity net charge and functional properties of the muscle. The behaviour of the major protein constituents in fish muscle, i.e. myofibrillar proteins, the cytosceletal system and connective tissue, is dependent on extrinsic factors, such as solvent characteristics, temperature, pH, ionic strength etc. (Sikorski, 2001). As mentioned in the protein chapter, myofibrillar proteins are the main components responsible for water binding. Changes occurring in muscle proteins during processing therefore influence the water distribution and size of water populations throughout the muscle, as well as influence mass transfer and moisture retention. This in turn influences the quality, texture, sensory attributes and shelf life of the product. It is therefore a major concern of seafood producers to retain the water, both for quality and economical reasons. During processing the protein structure of a food is changed to various extents, affecting the characteristics of the final product. Cooking and freezing are examples of common processing steps that are known to denature and cause aggregation of proteins and lead to loss of water from the muscle (Shenouda 1980; Steen and Lambelet 1997; Jensen and others 2002, 2003). Knowledge on the distribution and behaviour of water in the muscle is therefore important to minimize water loss during processing and storage and improve process control and ensure the stability of the process.

7.2.4.1 Water distribution

Water in muscle tissues can generally be divided into three compartments, depending on its location in the muscle. The amount of water that is restricted to proteins is only about 5% of the water found in muscle (Sikorski, 2001). Water is bound to the protein by interacting with hydrophilic amino acid residues on the protein surface and additional water molecules are bound in the multilayer zone surrounding the hydrophilic amino acid residues (Sikorski, 2001, Almutawah and others, 2009). Generally this water population is referred to as bound water and it does not intervene with the other populations to any extent. The amount of non-freezing water is often considered as a measure of bound water and is generally a strong function of the total water content (Roman-Gutierrez and others, 2002).

The majority of the water in a muscle food can be found within the muscle structure and in the muscle cells, between muscle bundles etc. This population is usually referred to as entrapped water. According to Ofstad and Hermansson (1997) this water is mainly held in the muscle by capillary and tension forces and can partially be removed from the muscle during processing. Finally a small population of free water can be found in the muscle. This free water (or bulk water) is found between the muscle cells and can be easily lost from the muscle through drip. Additional processing affecting the muscle structure, such as mincing, homogenization or possibly protein addition can lead to the formation of more water populations in the sample (Jensen and others, 2002; Andersen and Rinnan, 2002; Paper IV). Continuous osmosis occurs between the entrapped and free water

populations and the amount of water in each population is highly susceptible to the biological properties, post catch handling and different processing and storing treatments. Although shrinkage and expansion of the muscle fibrils affect the water distribution in the muscle, these processes do not necessarily affect the total water content of the myofibrillar lattice (Offer and Knight, 1988).

The number and size of water populations can easily be determined by using low field Nuclear Magnetic Resonance (NMR) relaxation measurements (Bertram and others, 2001; Bertram and others, 2007; Aursand and others, 2008; Erikson and others, 2004). This technique is therefore valuable in quality measurements of fish and meat during processing. The method is described in further detail in chapter 8.5.

7.2.4.2 Water retention

The ability of a muscle based product to retain both endogenous and added water is primarily dependent on its protein content and composition (Sikorski, 2001). In fish and meat the state of water depends on various interactions between water and proteins as well as other solutes. Biochemical and processing factors can lead to changes in spatial arrangements of proteins and tissues, leading to shrinking or swelling of the muscle, and thus exudation or retention of water respectively (Sikorski, 1997). The water holding capacity (WHC) describes the ability of a sample to retain intrinsic or extrinsic fluids under specified conditions (Fennema, 1990) and has an especially large impact on the quality of meat and fish (Sikorski, 1997). The WHC is affected by both internal and external factors. Internal factors include for example species, age, size, muscle type, amount of intramuscular fat and tissue conditions. Ofstad and others (1996) observed that salmon (*Salmo salar*) muscle possessed better liquid holding properties than cod (*Gadus morhua*) muscle, and that wild cod had higher WHC than farmed cod. The salmon had a denser myofibrillar muscle structure and intra- and extracellular spaces were filled by fat or a granulated material. The difference in WHC between the wild and farmed cod was primarily explained by low pH induced denaturation and shrinkage of myofibrils. A similar relationship was also observed in Paper I of this study between wild and farmed cod. External factors include for example feeding patterns, season, location of catching and post slaughter handling. Various processing steps affect the muscular structure by leading to changes in the properties of proteins, resulting in aggregation and changes in protein solubility. This is also affected by changes in the chemical composition of the muscle. Salting concentrations and salting methods are known to influence the WHC. According to Fennema (1990) salt anions bind to the muscle filaments and thus increase the repulsive forces between filaments, leading to muscle swelling in relatively low salt concentrations. The muscle swelling gives rise to increased water diffusion into the muscle structure and thus an increase in the water holding capacity. However, at higher salt concentrations proteins are denatured, will unfold or aggregate, leading to loss of water

from the muscle, due to cross-linking of proteins and myofibrillar shrinkage (Akse and others, 1993). Generally all changes induced by biochemical or chemical processes that tighten the protein structure increase the translational freedom of water molecules and thus decrease the muscle water retention ability, leading to increased muscle drip (Sikorski, 2001). The effects of various processing steps on the muscle structure and water holding capacity are discussed in chapter 8.4.

7.2.5 Lipids

All living species contain lipids, but in various amounts and of various types. Lipids play two critical roles in nature, firstly to form structural barriers to protect the cells from the environment, and secondly to be a source of cellular energy. Most animal lipids contain even-numbered saturated and unsaturated fatty acids combined with glycerol, fatty alcohols, sterols, phosphoric acid and amines. Marine lipid composition differ somewhat from lipids found in land animals in the way that they contain a wider range of fatty acids, longer fatty acid chains and a larger proportion of highly unsaturated fatty acids, especially n-3 and n-6 fatty acids (Ackman, 1989). Fish products are the major source the long chain polyunsaturated n-3 fatty acids in the human diet and the public is constantly getting more aware of the health benefits of fish consumption (Hunter and Roberts, 2000).

Lean fish (such as cod or saithe) store the bulk of the lipids in the liver, while fatty fish such as Atlantic salmon also contains lipids in adipose tissue found within the muscle structure. This adipose tissue is generally found in the subcutaneous tissue, in the belly flap as well as between the light and dark muscle (Kiessling and others, 1991). The amount of lipids is highly dependent on the species, season, sex, sexual maturation etc. The intramuscular fat content is typically below 1% in lean fish muscle, but can vary over a wide range in more fatty species (Murray and Burt, 2001). The presence of fat in the muscle can affect the processing abilities, for example during salting since the fat content represents a barrier for water and salt transport in more fatty species (Gallart-Jornet and others, 2007). Lipid oxidation may also contribute to the spoilage of fish muscle during storage (German and others, 1985; Kaitaranta, 1992).

7.3 Post mortem changes

7.3.1 Rigor mortis

Skeletal muscle contraction and relaxation of muscle in live animals occurs with the sliding filament model, where the thick and thin filaments slide past each other (Figure 6). The process is driven by cross-bridges which expand from the myosin filaments and cyclically interact with the actin filaments as ATP is hydrolyzed by the enzyme ATPase (Brenner and Eisenberg, 1986). Myosin can occur in two major conformations during this process, either with adenosine tri-phosphate (ATP) giving rise to a weak bond to actin or with adenosine di-phosphate (ADP) giving rise to a strong bond to actin, which detaches much less rapidly (Brenner and Eisenberg, 1986). When the myosin-actin detachment occurs, the muscle filaments slide back to their initial position (relaxation).

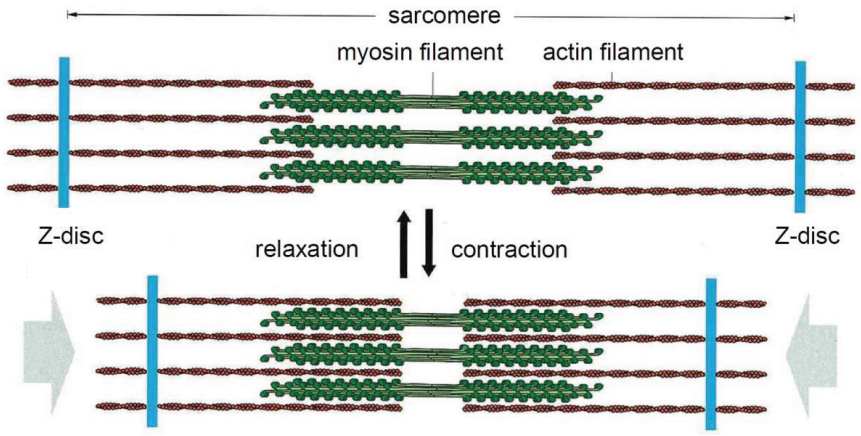

Figure 6: Muscle contraction illustrated with a sliding filament model. Relaxed muscle (above). Myosin and actin filaments slide past each other during contraction occurring during rigor mortis (Alberts and others, 1998).

ATP is regenerated by respiration in live animals and plants. Respiration occurs in three different ways according to where in the cell it occurs: glycolysis, the Kreb's cycle and finally in the cytochrome system. However, postmortem ATP is only regenerated by nonmitochondrial sources, first by dephosphorylation of creatine phosphate, followed by formation of ATP through the degradation of glycogen (Robergs and others 2004, 2006). This means that ATP-induced enzymatic activity, including the activity of the myosin ATPase will keep hydrolyzing ATP according to the sliding filament model while there is still ATP present to use. When no ATP is longer present to catalyze the dissociation of the myosin-actin complex, the myosin and actin becomes irreversibly bound and the muscle enters the rigor state (Sørensen and others, 1995).

Immediately post mortem, the muscle is generally soft and elastic. The muscle is then in the pre rigor state. The muscle will then gradually enter the rigor state, characterized by a stiff muscle with little elasticity. When the stiffness has reached its maximum, the muscle is in full rigor state. The rigor will then loosen gradually due to proteolysis of the Z-lines in the muscle structure (Bendall 1973, Greaser, 1986, Sørensen and others, 1995).The loosened post rigor muscle is then again characterized by a soft and elastic texture (Sørensen and others, 1995).

Robergs and others (2004, 2006) showed that muscle acidosis (drop in muscle pH) post mortem cannot be explained by lactate accumulation, as widely acknowledged, but rather by the hydrolysis of ATP, in which a hydrogen proton is released. The ultimate pH is reached when the glycolysis stops, because the glycogen content has been metabolized or because enzymatic activity in the glycolysis process is inhibited by the lowered pH (Robergs and others 2004; van Meerhaeghe and Velkeniers 2005).

The diameter of the muscle cells decrease during the onset of rigor (Hegarty, 1970; Swatland and Belfry, 1985) due to myofibril shrinkage. Furthermore, as the muscle pH decreases towards the isoelectric point of the major proteins, the net charge of the protein network is zero. This affects the protein solubility, which is lowest close to the corresponding isoelectric point. The isoelectric point (pI) of the structural proteins found in seafood is approximately pH 4.5-5.5 (Spinelli and others, 1972; Gildberg and Raa, 1979; Sikorski, 2001) (Figure 7).

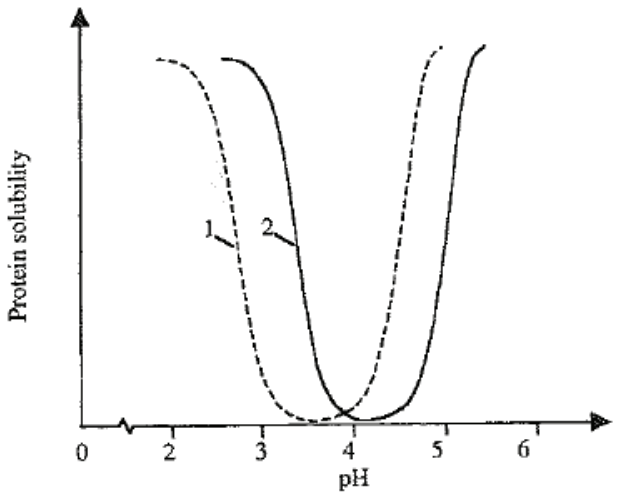

Figure 7: Solubility of proteins which (1) have been acrylated or (2) are untreated for a pH range from 0 to 6. (Sikorski, 2001).

Due to the myofibrillar contraction occurring during rigor mortis, water is expelled from the myofibrils into the extracellular space, due to increased attraction of areas of opposite charges. This

contraction and acidocis-induced protein denaturation leads to a reduction of the myofibrillar diameter and shrinkage of the muscle (Offer 1991, Huff-Lonergan and Lonergan, 2005). Two kinds of intercellular spaces can be found in post mortem muscle as seen in Figure 8. The figure illustrates the state of the muscle at three time points after slaughter. Immediately after slaughter, only slight extracellular water can be observed in the muscle. After some time water starts to appear between fibre bundles and the perimysium and when the muscle has entered rigor mortis water can be seen both between the each fibre within a bundle, as well as between the bundles (Schmidt and others, 2008; Offer and Cousin, 1992).

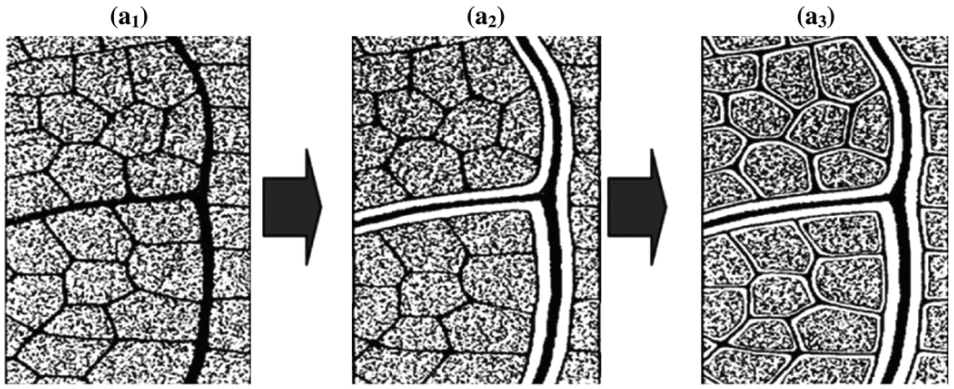

Figure 8: Post mortem changes in muscle. a_1) immediately after slaughter, a_2) some time post mortem a_3) muscle in rigor mortis. Figure from Schmidt and others (2008), adapted from Offer and Cousin (1992).

The time to onset of rigor and the rigor duration is dependent on many factors, including the muscle composition, such as the amount of glycogen in the muscle in vivo. The amount of glycogen in the muscle is dependent on the nutritional status as well as on the level of stress and exercise before death (Huss 1995; Sørensen and others 1995). Generally, well-rested and well-fed fish, such as farmed cod, contains higher levels of stored glycogen than exhausted fish, thus leading to a lower pH in farmed cod muscle post mortem compared to exhausted wild cod (Huss 1995). Other factors affecting the development and duration of rigor are the fish species, size, catching method, handling of the fish, temperature and chemical composition of the muscle (Huss, 1988). Reported values of rigor contraction of Atlantic cod (*Gadus morhua*) are within the range from 7% (Karl and others, 1997) to 25% (Connell, 1990). Stien and others (2005) observed rigor contractions of 15% and 20% in unstressed and stressed Atlantic cod fillets respectively. Veiseth and others (2006) found a similar connection in salmon fillets, where stressed fish contracted 13.8% while unstressed fish contracted 5.4 to 11.2% during the rigor process. Table 4 shows common onset and rigor durations of the species studied in this work. From the table it can be seen that the rigor onset can be postponed by unstressed handling, as well as it leads to less shrinkage of the fillets. Processing during the rigor process should be avoided, since this may have negative effects on the fish quality (Stroud, 2001).

Table 4: Rigor onset and duration of some common stressed and unstressed fish species. All values given for storage temperature 0°C.

Species	Stressed/unstressed	Rigor onset post catch [h]	Rigor duration [h]
Cod	caught by trawl	2-8	20-65
Cod	Unstressed	14-15	75-96
Saithe	caught by trawl	18	110
Cold water shrimp*		0-7	2-5

All values from Sørensen and others, 1995, except *values from Høgh (1989) and Wilaichon and others (1977).

7.4 Processing

7.4.1 Effect of salt addition- Swelling and shrinkage of muscle proteins

During salting the muscle either swells (salting-in effect) or shrinks (salting-out effect), dependent on the salt concentration and its affect on solubility of the myofibrillar proteins. The solubility has a minimum at low salt concentrations (ionic strength 0.025-0.150) similar to the physiological ionic strength of the muscle. Protein solubility increases at higher ionic strengths and reaches a maximum at an ionic strength of approximately 0.75 (Stefansson and Hultin, 1994). However, in the studies of Stefansson and Hultin (1994) higher solubility was always observed at very low ionic strength than at high ionic strength. The changes in solubility are believed to be caused by decreased interaction between proteins, conformational changes, polymerisation and depolymerisation of proteins during salting (Fennema, 1990; Offer and Knight 1988; Stefansson and Hultin, 1994).

Hamm (1960) and later Le Meste and others (2002) studied the role of sodium and chloride ions on muscle swelling by treating meat samples with either sodium acetate or sodium chloride. The studies showed that only the sodium chloride treatment led to increased water holding capacity, indicating that the chloride ions promoted muscle swelling, but the sodium ions did not. Offer and Trinick (1983) indicated that Cl^- ions bind to the actin and myosin filaments, increasing the negative charges of the proteins, and thus amplifies the electro repulsive forces between the filaments. This repulsion of the fibres leads to muscle swelling and more water can flow between the polymers, giving rise to tighter water binding and increased water holding capacity of the muscle (Offer and Trinick 1983; Offer and Knight 1988; Fennema 1990; Bocker and others 2008) (Figure 9). Light salting (salt content below approximately 6%) can therefore be a good method to decrease water drip during storage and is therefore often used prior to freezing to counteract the negative effects of freezing.

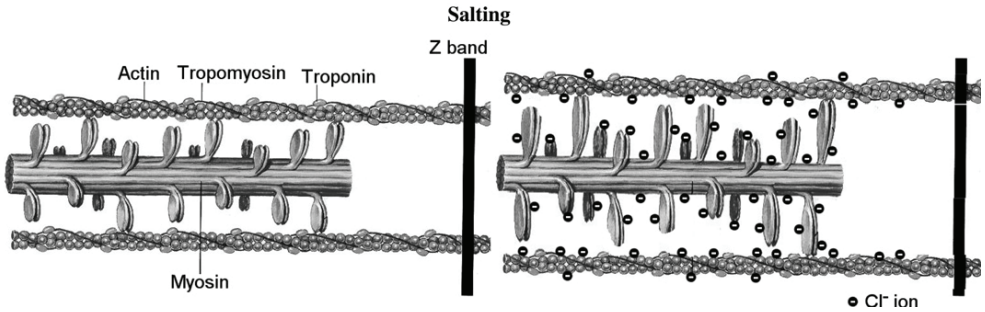

Figure 9: Formation of complex Cl—myofibrillar proteins, leading to electro repulsive forces, causing muscle swelling. Figure from Schmidt and others (2008).

At higher concentrations (>1M) the protein solubility decreases again due to loss of hydrophilic surfaces on the proteins, giving rise to more interaction between exposed hydrophobic areas on the protein surface, thus leading to aggregation and precipitation of the proteins. The repulsive forces of the protein also decrease and protein-protein bonds become stronger than solvent-solute interactions (Stefansson and Hultin, 1994). This protein aggregation leads to decreased water holding capacity of the muscle and increased water loss, believed to correspond to the muscle shrinkage, or the so called salting-out effect (Barat and others, 2003; Offer and Trinick, 1983; Offer and Knight, 1988, Xiong, 2000).

As illustrated above the muscle properties and water distribution in the tissue are altered by the salting-in and salting-out effects. Studies have shown that the salt uptake and muscle properties during salting are dependent on many factors, such as the species, muscle type, fish size, chemical composition, and physiological state of the muscle, rigor state, storage temperature, as well as the choice of salting method and the duration (Ismail and Woottoon 1992; Wang and others 2000; Barat and others 2002; Thorarinsdottir and others, 2004a; Gallart-Jornet and others, 2007). Salt migration by diffusion plays an important role in salting. Wang and others (2000) observed significantly faster salt diffusivities in in-rigor Atlantic salmon muscle than in pre rigor or post rigor muscle. The in-rigor fish also showed a strong linear relatoinship between salt diffusivity and salt concentration, possibly due to gradual protein denaturation during the salting process, resulting in less resistance to diffusion. However, the diffusivity in the pre-rigor fish did not change with increased salt concentrations. Using ^{23}Na Magnetic Resonance Imaging (MRI) Gallart-Jornet and others (2007) observed that the subcutaneus fat layer next to the skin in salmon formed a major barrier for salt diffusion, leading to a salting process basically only from the skin-free side of the fillet. The muscle fat, which is mostly located in the myosepta, was however not considered a major barrier to salt diffusion, possibly due to their parallel orientation with the salt diffusion direction. Slightly lower mass transfer was also observed on the skin side than through the opposite skin-free side in cod

fillets, indicating slight restraining ability of the skin with regard to salt diffusivity. The following chapter gives an overview of the effects of various salting methods on fish muscle.

7.4.2 Salting methods

As already mentioned, salting of food can be performed in numerous ways depending on the wanted characteristics of the final product. During the production of dry salted cod the fish is filleted or butterfly split and then heavily salted. Traditionally the salting has been done by various combinations of pickling, brining and/or kench salting. In *kench salting* the fish is piled into stacks in alternating layers of fish and salt. The fish takes up salt while liquid diffusing from the muscle is allowed to drain away. In *pickle salting* a similar procedure is performed, but in closed vats. The liquid diffusing from the muscle during salting therefore forms a saturated brine solution as the salt dissolves (van Klaveren and Legrende, 1965). It is well known that using high salt concentrations, close to saturation (25% w/w) during brining or dry salting leads to protein denaturation and thus reduction of the water holding capacity of the muscle (Duerr and Dyer, 1952; Sannaveerappa and others, 2004; Thorarinsdottir and others, 2004a; van Nguyen and others, 2010). Such protein denaturation on the fillet surface can “burn” the surface and form a crust, thus preventing the salt and water to diffuse further into the muscle. Wold and others (2001) illustrated how salt and water was prevented from entering through a crust in cod muscle surface formed by protein denaturation during brining in a saturated solution during pickle salting (Figure 10). The thickest parts of the flattened fish therefore had a lower salt content and a water activity high enough in this area to allow the growth of several species of bacteria, yeasts and molds. This problem led to several claims on the product, where consumers complained about the smell of the product.

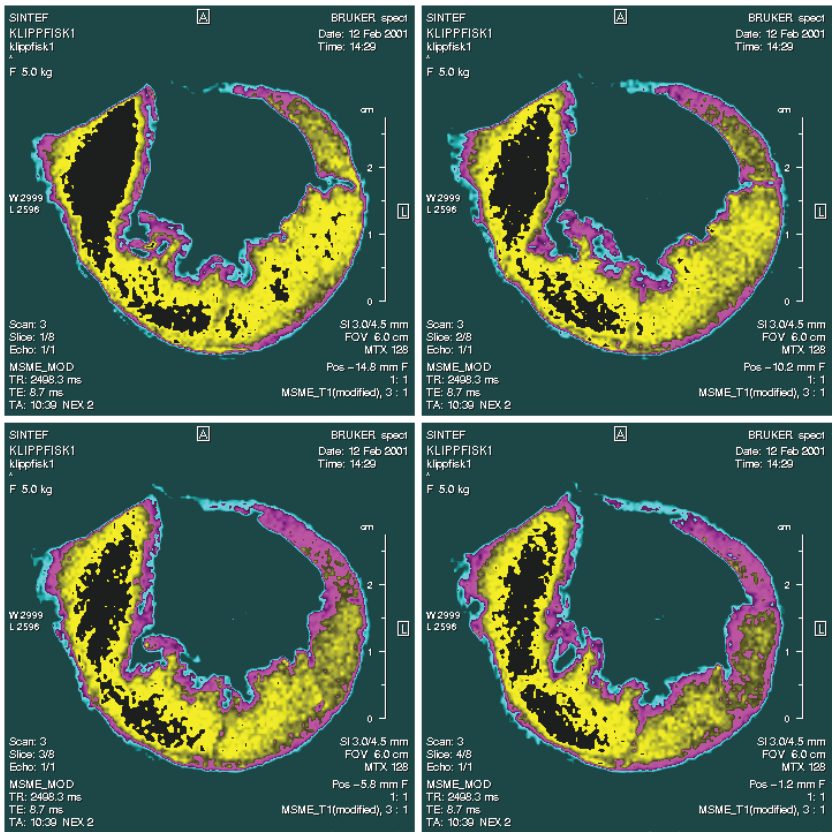

Figure 10: ^{1}H Magnetic Resonance Images (MRI) of four slices from a half flattened bacalao in saturated brine. The figures were obtained with a Bruker Biospec 2.4T instrument at SINTEF, Norway. The sample was adjusted to fit into a round probe. The left part of each slice shows the fillet part, while the right part shows the belly flap. Black areas contain the highest water content, which decreases with the colours yellow, red and finally turquoise, which has the lowest water content (Wold and others, 2001).

The use of lower salt concentrations has however been shown to lead to increased water holding capacity and higher yields (Offer and Trinick, 1983; Wilding and others, 1986; Slabjy and others, 1987; Barat and others, 2002). Thorarinsdottir and others (2004a) observed an increased yield during heavily salted cod (bacalao) production by increasing the salt concentration gradually during the first stages of salting. These observations have lead to a change in the salting methods during the production of heavily salted products in Iceland. Now pre salting by brining with lower unsaturated salt concentrations, followed by dry salting, has become the most popular method in the production of heavily salted cod products (Thorarinsdottir and others, 2004a). Experiments with pre salting by a combination of brine injection and immersion have also recently been made. For brine pre salted fish, the fillets are submerged in brine for 1-4 days before being stacked in alternating layers of salt, where the fish is kept for 10-13 days before packaging and export. It is clear that the salt concentration has a significant effect on the mass transfer and kinetic parameters during brine salting (Poernomo and others, 1992; Bellagha and others, 2007; van Nguyen and others, 2010).

van Nguyen and others (2010) studied the diffusion of water and salt during brining with salt concentrations ranging from 6 to 24% (w/w). Their results showed that the weight gain was highest in the cod loins brined with a low concentration of 6% (w/w), while the diffusion rate was fastest in loins brined with 15% (w/w) brine. Higher salt concentrations lead to earlier mentioned denaturation in the fiber structure.

7.4.3 Effect of phosphate addition

Phosphates, which are salts of phosphoric acid, are naturally occurring in all living things and are an essential component of our diet. Phosphates can connect to each other, forming polyphosphates (Aitken, 2001), which are commonly used as food additives or technical aid during processing. Phosphates have strong anionic properties, which enables interaction with proteins and affects the pH of the proteins. By shifting the pH of the protein away from its isoelectric point, the protein structure unfolds and more negatively charged sites are exposed, leading to muscle swelling and therefore increased water binding ability as well as protein solubility (Sun, 1997; Veigarsson, 1999; Lampila and Godber; 2002). This mechanism is shown in Figure 11. The exact mechanism of the phosphate and actomyosin interactions is however not fully understood.

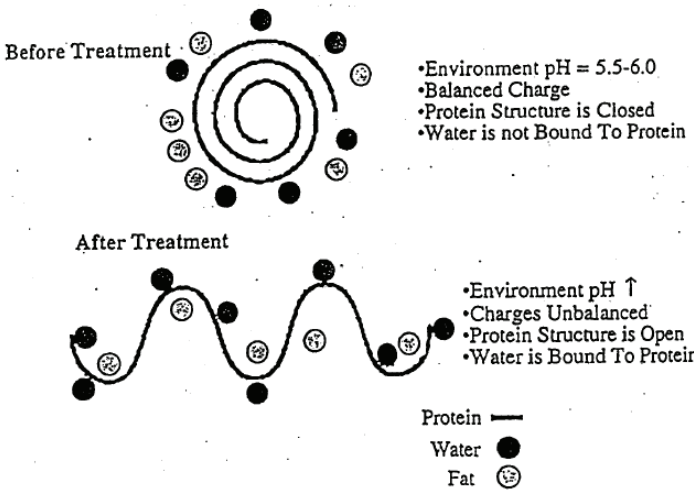

Figure 11: The effect of polyphosphate on muscle Sun (1997).

Phosphates have shown to protect proteins against thermal denaturation in shrimp (Crawford, 1980; Veigarsson, 1999), beef (Trout and Schmidt, 1987) and carp (Yagi and others, 1985). The use of phosphates in food processing has however been a matter of debate, because too much phosphate can have a negative effect on sensory attributes. However when used properly phosphates can retain the natural moisture of the muscle, leading to a higher quality product (Goncalves and Ribeiro 2008). Phosphate has for example been used in bacalao production due to

their positive effects on appearance and weight yield of the products (Lindkvist and others, 2008). Most polyphosphates that are used in food production break down to single phosphate units during digestion of the food. Most added phosphates are therefore nutritionally equivalent to phosphates that are naturally present in food. These substances are therefore not likely to cause any health dangers for consumers. However, polyphosphate use during processing should be not exceed the amount needed to obtain the wanted technological quality of the processed food (Aitken, 2001). Regulations about phosphate addition are under regular review in the European Union and it is possible that stricter restrictions will be imposed in the future.

7.4.4 Effect of functional protein addition

The use of functional proteins as additives in food products has increased over the last years, and these have been found to increase water and fat binding properties of the products and improve texture and stability. The functionality of the proteins is however dependent on their type and origin. Soy proteins have been used in the food industry to improve water- and fat- binding in frozen products (Cunningham and others, 1988). Kristinsson and Rasco (2000) also showed that less drip was observed in frozen salmon patties with added fish protein hydrolyzates than with egg albumin or soy protein concentrate. Fish protein products, which have undergone various isolation methods, are also commercially available as concentrates or dried products, ready to use for the same purpose. Thorarinsdottir and others (2004b) showed that fish proteins injected into cod fillets could improve the water holding capacity of the fillets to a greater extent than fillets injected with soy proteins. Moreover, improving yield and water holding properties of fish fillets by injection of proteins, made from by-products produced during the fillet production, can be an advantage both regarding the quality and value of the final product.

7.4.5 Effect of storage conditions

The temperature during storage and transportation has a large effect on the quality and storage life of food. Optimization of chilling techniques, temperature and physiochemical properties of a food product are therefore crucial factors to ensure good quality of the final products. Furthermore, the food becomes microbiologically more stable as the temperature is lowered (Andersen and others, 1965; Huss, 1995; Lauzon and others, 2009). The superchilling temperature range, which is often defined as the temperatures from 0 °C to the temperature of first ice crystal formation in the product and 1-2 °C below that, is therefore highly dependent on the substances and contents of the sample, especially the muscle salt concentration (Chen, 1985a and b). Using low field Nuclear Magnetic Resonance Relative Free Induction Decay (FIDR) measurements, Gudjónsdóttir (2006) also showed how increased salt content depressed the initial freezing temperature as well as affected the freezing processes and amount of unfrozen water of cod mince (Figure 12).

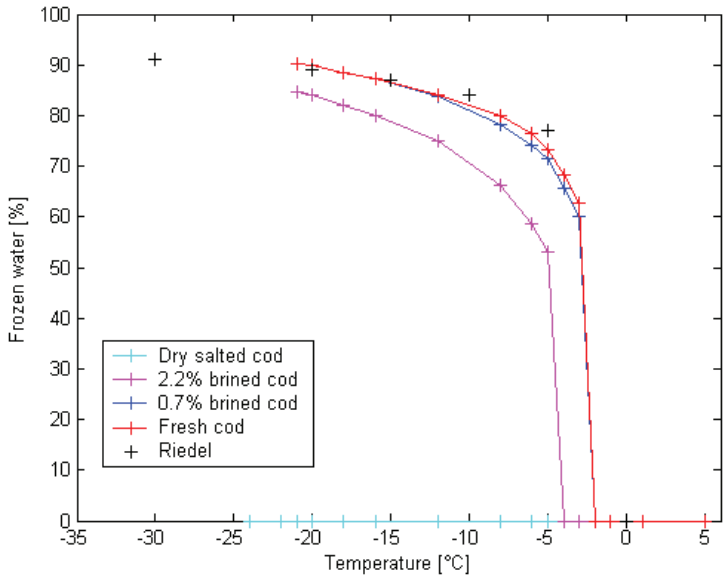

Figure 12: Percentage of frozen water in cod mince as affected by salt concentrations in the muscle measured with relative free induction decay (FIDR) measurements. Water content in the raw material was 82.6 ±0.4 %. Reference measurements from calorimetric analysis are also shown as a comparison (Riedel, 1978). Figure from Gudjónsdóttir (2006).

Storing food at superchilling temperatures, or changing the composition of the surrounding air during storage (i.e. modified atmosphere packaging, MAP) leads to a change in spoilage mechanisms compared to when traditional storage methods (air packaging at 0 to 4°C) are applied. An extended shelf life period, where the fish is neutral in taste and colour, can be gained by using MAP (Martinsdóttir and others, 2005), due to delayed bacterial development (Lauzon and others, 2009), while superchilling can lengthen the overall freshness period (Olafsdottir and others, 2006; Lauzon and Martinsdóttir, 2005; Wang and others, 2008). Dissolution of carbon dioxide CO_2 in MA packed fish can however affect both the texture and taste of the fish (Davis, 1993; Lauzon and others, 2009).

Most studies on superchilling have been focusing on increased shelf life and quality changes of food during storage (Olafsdottir and others, 2006; Sivertsvik and others, 2003; Lauzon and others, 2009). However, recently increased effort has been put into studies of monitoring and process control of physicochemical properties during handling of superchilled and partially frozen products (Duun and Rustad, 2007, 2008; Duun and others, 2008; Stevik and others, 2010; Papers I, II and V). Optimization of physicochemical properties during processing and relating these properties to quality features of the final product is important in the search for optimum superchilling storage temperature of each product. Superchilled storage is especially vulnerable due to the irreversible changes that may occur in the muscle at this temperature range. Ice crystallization can lead to cell

destruction or puncturing of membranes as well as protein denaturation, leading to loss of water holding capacity and increased drip loss (Love, 1968; Fennema, 1990). Also when some of the water freezes out, the concentration of solutes in the unfrozen solution increases. This may lead to increased enzymatic activity, protein denaturation and structural damage of membranes, resulting in increased drip as well as loss in water holding capacity and textural changes (Foegeding and others, 1996). Good thermal control during superchilling is therefore necessary to ensure high product quality.

Freezing is another common preservation method, leading to extended shelf life. However, freezing is often associated with undesirable changes in the physical, chemical or structural characteristics of the muscle due to protein aggregation or denaturation and ice crystallization (Krivchenia and Fennema, 1988; Shenouda, 1980, Love, 1968; Chevalier and others, 2001). The main factors affecting the quality of frozen foods are however the size and location of the formed ice crystals (Love, 1968; Fennema and others, 1973; Shenouda, 1980; Chevalier and others, 2001). According to Love (1968) a slow freezing process lead to the formation of large ice crystals, which highly affects the protein structure, causes rupturing of cell membranes as well as changes the structure of cells and tissues (Shenouda, 1980). During slow freezing the extracellular fluid cools more rapidly than the intracellular water, leading to ice crystallization in the extracellular space, inducing a high salt concentration in the extracellular fluids, leading to diffusion of water from the cells into the extracellular space where further ice crystallization occurs. The extracellular volume thus increases and gaps appear between the muscle fibres (Shenouda, 1980; Foucat and others, 2001). On the other hand fast freezing leads to minimal migration of water between the intracellular and extracellular compartments, leading to the formation of small ice crystals (Love, 1968; Chevalier and others, 2001). Ice crystals are also unstable during frozen storage, where they undergo metamorphic changes or so called recrystallization, including changes in number, size, shape and orientation (Fennema and others, 1973). Thus optimization the freezing technique, by precise temperature control, both during the freezing process and storing, is essential for the quality and value of frozen seafood or meat.

7.4.6 Effect of cooking

Temperature treatments are common in food processing. Heating muscle tissue is known to denature proteins and lead to water loss from the muscle (Wright, 1984; Crawford, 1980; Goncalves and Ribeiro, 2008; Micklander and others, 2002). Crawford (1980) stated that steam pre cooking prior to mechanical peeling of shrimp represented a major site for yield loss during shrimp processing. Schubring (2009) studied the thermal denaturation of shrimp by differential scanning calorimetry (DSC) (Figure 13). The results indicated that myosin (peak I) was significantly denatured at temperatures as low as 35°C. Sarcoplasmic and connective tissue proteins (peak II) were

denatured in samples heated to 50°C, but here an additional peak IV also appeared. In samples heated to 70°C all protein fractions were thermally denatured as displayed by a straight line in the DSC curve. This indicates that myosin, which plays a major role in water holding of the muscle, is most vulnerable to thermal treatment. Several additives, such as sugars, polyols or phosphates, can however be used to stabilize the protein against thermal denaturation (Wright, 1984; Goncalves and Ribeiro, 2008).

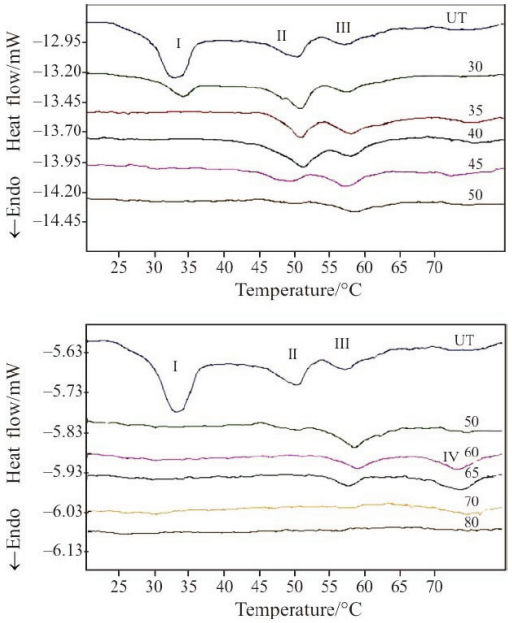

Figure 13: Differential Scanning Calorimetry (DSC) curves of deep-water pink shrimp muscle as affected by elevated temperature (UT-untreated, 30-80°C indicate the temperature to which the samples were heated prior to DSC measurements. Peak I represents myosin or the myosin heavy chain, peak II represents sarcoplasmic and connective tissue proteins and peak III represents actin (Schubring, 2009).

Micklander and others (2002) studied the effects of cooking of meat on protein denaturation and water distribution by using low field ^{1}H Nuclear Magnetic Resonance (NMR). Transversal relaxation time measurements in combination with principal component analysis (PCA) were used to follow the major transitions in the state of water during cooking. The study revealed large changes in the water properties at 46 and 66°C, thought to correspond to transverse shrinkage of the myofibrils and longitudinal contraction respectively, as well as softer changes at 42, 57 and 76°C, thought to correspond to the onset of myosin denaturation, shrinkage parallel to the myofibrils or collagen denaturation and actin denaturation respectively. This shows that the temperatures at which the proteins start denaturing are highly dependent on the type of sample as well as treatment. It should also be kept in mind that seafood proteins are less stable and generally denatured at a lower temperature than proteins from land-living animals, due to a lower temperature in their living

habitat, as well as other properties affecting proteins in vitro, such as muscle pH and differences in ionic strength (Sikorski, 1994b). Exponential fitting of the relaxation data indicated the presence of two water populations in the meat until heated to approximately 60°C, where an additional population with a long relaxation time appeared. This population was assigned to water expelled from the meat matrix due to transverse contraction in the myofibrils and contained high amounts of soluble proteins. The study also revealed that at the initial stage of myosin denaturation around 42°C small amounts of water are squeezed out from the myofibrillar water (T_{21} domain) into the extramyofibrillar space (T_{22} domain). Low field NMR is therefore a valuable tool when assessing protein denaturation and water distribution in muscle foods during processing, such as cooking.

7.5 Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) has become one of the most important analytical tools in a wide range of fields due to its many applications, high efficiency, non-destructive sample characteristics and accuracy. The phenomenon of Nuclear Magnetic Resonance, which was first observed in 1946 (Bloch and others, 1946, Purcell and others, 1946), is based on the ability of some nuclei to have a nuclear spin. Nuclei with odd mass numbers (amount of protons (p) and neutrons (n) combined is even) have half-integral spins (i.e. ^{1}H, ^{13}C, ^{15}N,^{17}O,^{23}Na etc.), while nuclei with both odd amounts of protons and neutrons odd have integral spins (the charge is odd, but mass even) (i.e. ^{2}H, ^{10}B, ^{14}N etc.) and nuclei with both even amounts of protons and neutrons have zero spin (both charges and mass even) (i.e. ^{4}He, ^{12}C, ^{16}O). Only nuclei with non-zero overall spin generate a magnetic moment. When a nucleus with spin quantum number I is placed in a magnetic field, it can take $2I+1$ different orientations according to quantum mechanics. The hydrogen proton, which is the most abundant nucleus in biological tissue and is, therefore, of special importance to food science, has a spin quantum number of ½. It therefore has two possible orientations, spin up or spin down. Outside the magnetic field the two populations are usually degenerate, i.e. have the same energy. However when the nucleus is placed in a magnetic field the degeneracy is uplifted and the protons fill different spin energy levels according to their orientations (Figure 14).

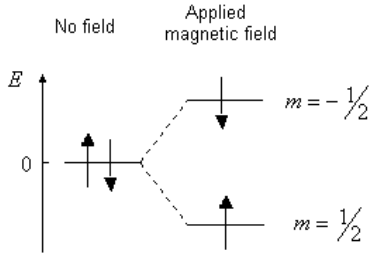

Figure 14: Energy levels of a nucleus with spin quantum number I=1/2 in a magnetic field. When no applied field is present, there is no energy difference between the proton populations with spin up or spin down. When the applied field B_0 is turned on, the energy difference between the populations becomes ΔE as defined by equation 1 (Banwell and McCash, 1994).

These energy levels are separated by an energy difference ΔE given by the equation

$$\Delta E = \frac{h\gamma B_0}{2\pi} \qquad \text{[J]} \qquad \textbf{Eq. 1}$$

where h is the Planck's constant, γ is the gyromagnetic ratio for the nucleus ($2{,}675 \times 10^8$ rad/Ts for protons) and B_0 is the applied magnetic field strength. This means that the stronger the magnetic field the greater is the energy difference between the spin energy levels.

Since the protons have an electric charge and an angular momentum, they behave as small compass needles when placed in a magnetic field. That is, the protons align themselves roughly with the magnetic field. Exact alignment with the applied field is however impossible because of quantum chemistry laws, which state that the magnetic moment must be an integral or a half-integral for the magnetic moment to be perfectly aligned with the field direction. However, the magnetic moment does not take an integral- or half-integral value for an integral or half-integral quantum spin number I and therefore exact alignment is impossible. Instead the nuclei behave in a gyroscope-like process called the Larmor precession where the proton rotates around itself with a resonance frequency ν described with the equation

$$\nu = \frac{\Delta E}{h} = \frac{\gamma B_0}{2\pi} \qquad \text{[Hz]} \qquad \textbf{Eq. 2}$$

At thermal equilibrium the populations of the energy levels are given by the Boltzmann distribution

$$\frac{N_{upper}}{N_{lower}} = \exp\left(\frac{-\Delta E}{kT}\right) \qquad \textbf{Eq. 3}$$

where N_{upper} is the population of the higher energy level (spin down), N_{lower} is the population of the lower energy level (spin up), k is the Boltzmann constant and T is the absolute temperature of the sample. Therefore more nuclei have spin up than down at thermal equilibrium, giving a net magnetization in the same direction as the applied field. If a radio frequency (RF) pulse of the same frequency as the Larmor frequency is applied to the system, transitions between the two energy levels will occur and the system is no longer at energetic equilibrium. If the radiofrequency pulse is

applied for a time t_p an oscillating magnetic field B_1 is generated and the pulse tilts the magnetization with an angle

$$\theta = \gamma B_1 t_p \qquad \text{[rad]} \qquad \text{Eq. 4}$$

The pulse angle is therefore dependent on the duration of the pulse. Figure 15 shows a schematic figure of the processions of protons when a 90° pulse is applied.

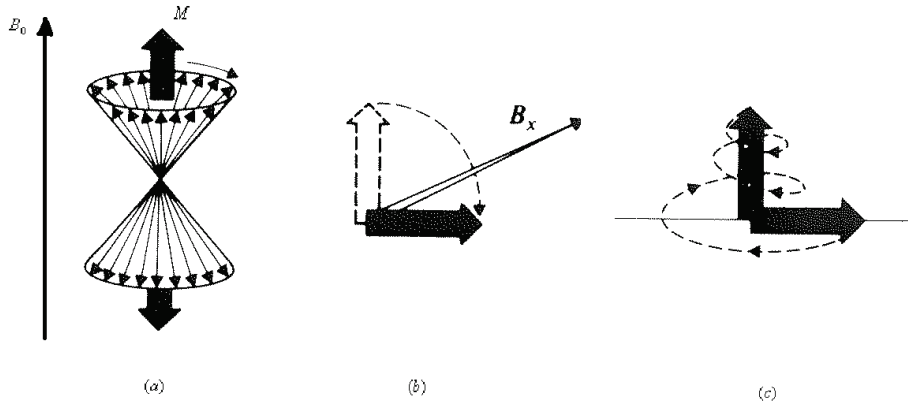

Figure 15: Schematic figure of the precessions of a group of spinning nuclei when a 90° pulse is applied. (a) Before the pulse the net magnetization *M* is aligned with the applied field B_0. (b) At time t = 0 a 90° pulse tilts the magnetization into the transversal plane. (c) The net magnetisation then slowly aligns itself with the applied magnetic field again while processing around the longitudinal axis (Banwell and McCash, 1994).

When the RF pulse is turned off, the system will turn to the lower energetic state again by relaxation. Relaxation of nuclei can take place in two ways, either by spin-lattice relaxation (longitudinal relaxation) or spin-spin relaxation (transverse relaxation), which describe the effect of the environment on the spin of a nuclei (spin-lattice) and the effect of other adjacent rotating nuclei (spin-spin) on a nuclei. Measurements of relaxation times can therefore be used to indicate the phase and state of the atoms and how the environment is influencing the spin. Since the relaxation times are distinctive for the phase and environment of the molecules, the nuclei from different components in a sample can be separated. The oscillations in the magnetic field after excitation lead to an alternation of voltage in the detection coils of the NMR-device. The detected NMR response arises from the excited nuclei in the sample and therefore the amplitude of the signal is a good measurement of the total amount of nuclei in the sample. In order to distinguish between signals from different sources, a pulse sequence fit to the task has to be applied (Banwell and McCash, 1994; Williams and Fleming, 1995).

Several types of pulse sequences exist to distinguish between signals from nuclei from different sources. The Inversion recovery (IR) and Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences

(Carr and Purcell 1954; Meiboom and Gill 1958) were used in this study to measure longitudinal and transversal relaxation respectively. When using a CPMG pulse the nuclei are initially excited with a 90° radio frequency pulse. This pulse tips the magnetic field into the transverse plane. The nuclei begin to precess at various speeds (the signal diffuses) due to heterogeneity in the magnetic field, leading to a smaller net magnetization. At time τ a 180° pulse flips the signal in the transversal plane. The frequencies then refocus again and at time 2τ an echo is obtained. This signal then diffuses again until next pulse is applied. Repeated 180° pulses will generate a train of such echoes. The amplitude of the observed echo signals is used to calculate the transversal relaxation time,T_2, for the sample (Figure 16) (Banwell and McCash, 1994).

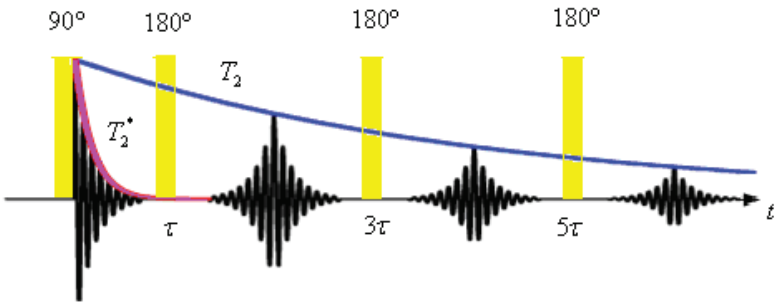

Figure 16: Systematic figure of a CPMG pulse sequence for zero dummy echoes. Yellow rectangles indicate applied radio frequency pulses, while black peaks indicate signal echoes obtained. Modified figure from Lepage and Gore, 2004.

The relaxation signal relaxation decays according to the Bloch equation

$$\frac{\partial M_{xy}}{\partial t} = -\frac{a_{2i}}{T_2} \qquad \text{Eq. 5}$$

The relaxation times are shorter when the nuclei are more tightly bound. It has also been shown that for porous materials, e.g. fish muscle, water in small compartments (e.g. between fibrils) has a faster relaxation decay rate than water in larger pores or compartments. Thus the Bloch equation can be rewritten to express multi-exponential relaxation behaviour as found in fish or meat muscle according to the equation

$$M_{xy} = \sum_{i=1}^{N} a_{2i} \exp(-t/T_{2i}) + \xi(t) \qquad \text{Eq. 6}$$

where N is the number of exponentials fitted, t stands for time, T_{2i} is the relaxation time, a_{2i} its corresponding population and ξ(t) is the model error. The relative amount of nuclei in each population can then be found by the equation:

$$A_{2i} = a_{2i} / \sum_{j=1}^{N} a_{2j} \qquad \text{Eq. 7}$$

Different proton populations exhibit different transversal relaxation properties. This method can therefore be used to distinguish between different proton populations and assess how they are affected by various treatments.

The basic difference between an inversion recovery (IR) pulse sequence and a CPMG pulse sequence lies in the addition of a 180° pulse in front of the CPMG pulse train. This pulse turns the magnetization in the longitudinal plane, reversing the direction of the magnetization. This will leave the spin system in an unstable state, since more spins point against the outer magnetic field than with it. The relaxation of the magnetic field back to its initial state after a pulse can be estimated with the Bloch equation for inversion recovery

$$\frac{\partial M_z}{\partial t} = \frac{M_0 - M_z}{T_1} \qquad \textbf{Eq. 8}$$

and the magnetization at time t can then be expressed as

$$M_z(t) = \sum_i M_i \left(1 - 2\exp\left[-t/T_1 \right]_i \right) \qquad \textbf{Eq. 9}$$

where M_z is the longitudinal magnetization signal, M_i is the size of the nuclei population and T_{1i} is the respective longitudinal relaxation time. Even though fish muscle is a porous material, a mono-exponential relaxation curve is usually used to fit the water relaxation data in a fish samples. As mentioned before the longitudinal relaxation time is a measure of the time interval in which protons exchange energy with their environment (Banwell and McCash, 1994).

Nuclear Magnetic Resonance can be divided into three main categories according to the instrumentation and their applications: high field NMR, low field NMR and Magnetic Resonance Imaging (MRI). The use of low field NMR in food science will be discussed further in the following chapter.

7.5.1 Low field Nuclear Magnetic Resonance in food research

Low field proton NMR relaxation measurements can be used to characterize proton compartmentalization and quantify fat and water in a fast and non-destructible way and is therefore a valuable method in meat and fish research (Ablett, 1992; Gambhir, 1992; Sørland and others, 2004; Aursand and others, 2008; Aursand and others, 2009). The structure of a muscle and water distribution throughout the muscle is highly dependent on the raw material quality as well as pre and post mortem handling and processing. Water distribution measurements with low field NMR therefore gives an opportunity to study the effects of various handling and processing on water distribution and diffusion in the muscle.

Most studies have reported 2 or 3 different water populations in muscle tissue, with transversal relaxation times dependent on the environment of the protons in each water population. The interpretation of the distribution of water throughout the sample is, however, a matter of discussion. Jensen and others (2002) identified 4 water pools with transverse relaxation times of 37, 56, 126, and 361 ms in minced cod samples, where the fastest relaxing group was correlated with the most bound water, that is, water in close contact with proteins. Studies in pork have shown 3 water populations at approximately 1 to 3 ms (T_{2B}) suggested to correlate to water closely associated with macromolecules, 40 to 80 ms (T_{21}) corresponding to myofibrillar water and 200 to 400 ms (T_{22}) corresponding to extramyofibrillar water (Bertram and Andersen 2007; Bertram and others 2009). Erikson and others (2004) studied the production process of heavily salted wild cod from fresh and frozen-thawed wild Atlantic cod by low-field NMR, ^{23}Na Magnetic Resonance Imaging (MRI), and analytical methods. A bi-exponential fit of the transverse relaxation times suggested 2 water populations, with relaxation times of 46 to 69 ms (T_{21}) and 117 to 127 ms (T_{22}) with values dependent on where in the process sampling took place, that is, of the raw material, after salting and after storage.

Low field NMR has also been useful in the prediction of various physicochemical properties associated with the water and fat distribution in the muscle. Studies have shown that good predictions of water holding capacity have been achieved with the method in cod (Jepsen and others, 1999), for water and/or fat content in salmon (Jepsen and others, 1999; Aursand and others, 2008), texture changes in cod muscle during frozen storage (Steen and Lambelet, 1997) and denaturation of whey proteins during heating (Lambelet and others, 1989), as well as indicate water distribution in muscle systems and how it is affected by choice of raw material and processing (Bertram and others, 2007, 2009; Aursand and others, 2008, 2009; Erikson and others, 2004). However, measurements using traditional benchtop instruments with homogenous magnetic fields are restricted to very small sample sizes, making measurements of whole fish or muscle impossible. Recent developments of mobile unilateral magnets have made measurements of larger samples possible. The technique, also often referred to as NMR-MObile Universal Surface Explorer (NMR-MOUSE), has been used for fat content determination in both live (Veliyulin and others, 2005a) and processed salmon (Aursand and others, 2008) as well as for determination of oil and water emulsions in food (Pedersen and others, 2003; Petrov and others, 2008) to mention a few applications.

From this it is clear that low field NMR can give a deeper understanding on the distribution of water and fat throughout the muscle and how their populations are affected by process-induced protein denaturation and diffusion.

7.6 Near Infrared spectroscopy (NIR)

Near infrared (NIR) spectroscopy is based on the principle of quantized absorption of radiation in the near infrared spectrum in the wavelength interval from 700 nm to 2.5 µm. This absorption corresponds to the overtones and combination bands of the fundamental vibrations (bending and stretching vibrations) of covalent bonds in molecules (Figure 17). All molecules have different natural frequencies of vibration, thus leading to the conclusion that no two bonds will give the same absorption pattern. The absorption patterns are also affected by the environment of the bond and thus no two bonds of the same type in different molecules can give the same spectrum. The NIR absorption bands are dependent on the physical and chemical environment of the molecule, such as hydrogen bonding, temperature, moisture content, crystallinity, particle size etc. (Osborne and others, 1993). The position of the fundamental absorption bands in the infrared region (IR) is very well documented (Bellamy, 1975) and these can be used as a starting point in the prediction of the corresponding overtone bands seen in the NIR spectrum.

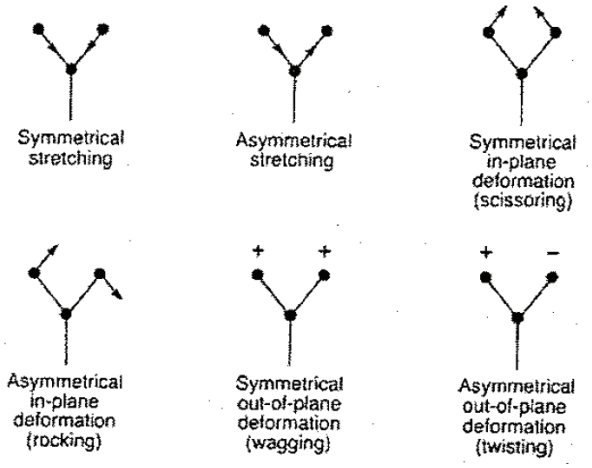

Figure 17: Overview of symmetrical and asymmetrical vibrations in covalent bonds of an AB_2 molecule (Osborne and others, 1993).

Near-infrared spectroscopy is widely used in industry due to its simplicity, short sample preparation time and fast measuring time and can be used either online or at-line depending on what factors within a process that are to be controlled (Windhab and Bollinger, 1996; Hoyer, 1997). Numerous studies have been made on the application of NIR in meat (Mishiro and others, 1995; Egelandsdal and others, 2007; Ortiz-Somovilla and others, 2005; Rason and others, 2007) and fish studies (Bøknæs and others, 2002; Nilsen and others, 2002; Sollid and Solberg, 1992; Wold and others, 1996, 1997, 2006; Jørgensen and Jensen, 1997). Mishiro and others (1995) showed that NIR transmittance measurements with fibre optics gave good predictions of water content and water activity and therefore concluded that the method was well suited for process control during drying of

sausage. Song and Otto (1995) showed that transmittance NIR gave good predictions of water, fat, crude protein and connective tissue protein in sausages. Ellekjær and others (2006) also showed the advantages of predicting sensory attributes, such as colour, juiciness and greasiness of sausages with the NIR technigque. Studies have also shown the usefulness of NIR spectroscopy within the studies of fisheries products i.e. in evaluating fish freshness (Bøknæs and others, 2002; Nilsen and others, 2002, Nilsen and Esaiassen, 2005), predicting moisture content in dry salted coalfish (Wold and others, 2006) and moisture and fat content of cod (Sollid and Solberg, 1992; Wold and others, 1996; Jørgensen and Jensen, 1997) and salmon (Wold and others, 1997). Recent studies also include determination of ice fraction in superchilled salmon fillets by studying the shift in the water peak found at a wavelength of approximately 1000 nm (Stevik and others, 2010).

From the above chapter it is clear that due to its fast measuring time, non-destructive sample abilities and robustness NIR spectroscopy is highly suitable for industrial implementation and highly applicable for online monitoring of various physical and chemical properties during processing and can thus be used to aid process control.

8 Materials and methods

8.1 Experimental design

The experimental design of the project is described in the following chapter as well as in papers I-V, presented in the appendices. Low field Nuclear Magnetic Resonance was used to study the differences in muscle structure in seafood due to varying origin of the raw material (Paper I), as well as changes in the muscle structure caused by various processing (Papers I-V). Physicochemical properties were analyzed for comparison to and interpretation of NMR results. Table 5 summarizes the main parameters studied in the project.

Table 5: Overview of the main parameters studied in the project.

Paper	Species	Wild vs. farmed	Salting	Phosphate addition	Protein addition	Superchilling	Freezing	MAP
I	Cod	x	x			x	x	
II	Cod		x	x		x		x
III	Cod		x	x				
IV	Saithe		x		x		x	
V	Cold water shrimp		x	x		x	x	

8.1.1 Paper I

Paper I was divided into two experiments. The study was designed to evaluate the structural differences in the musculature of farmed and wild cod (*Gadus morhua*) and how these differences are affected by pre or post rigor processing, with regards to salt uptake and moisture retention of cod fillets during processing, frozen storage (experiment 1) and superchilled storage (experiment 2).

In experiment 1 wild and farmed Atlantic cod (*Gadus morhua*), slaughtered in November 2006, were filleted either pre rigor (d0 post mortem) or post rigor (d3 post mortem). The fillets were brine injected with 1.5% (w/w) NaCl salt solution and successively immersed in 1.5% (w/w) brine for 10-30 min and individually quick frozen (IQF) before packaging and frozen storage for 6 months (Figure 18).

In experiment 2 wild and farmed Atlantic cod, slaughtered in March 2007, were processed and filleted either pre rigor mortis og post rigor mortis, injected with a 5.5% (w/w) NaCl solution and successively immersed in a 4% (w/w) brine for 2d. The fillets were packed in Styrofoam boxes and stored at a superchilling temperature of -1.9°C for 17d (Figure 19).

The effect of varying brine concentrations, salting method (brine immersion and/or injection) on yield, chemical composition, water holding capacity, pH, water distribution and relaxation behaviour of the water pools found in the muscle was evaluated.

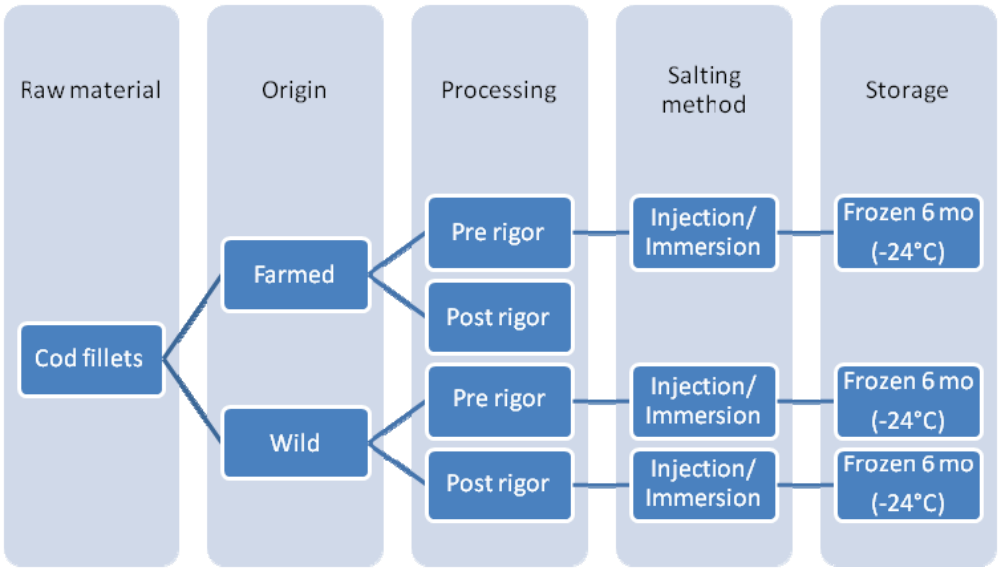

Figure 18: Experimental setup of experiment 1 in Paper I. Comparison of the physicochemical properties of pre and post rigor farmed cod to pre and post rigor wild cod fillets for production of lightly salted cod fillets undergoing frozen storage. The post rigor farmed cod showed excessive gaping during filleting and was therefore not considered for further processing or storage.

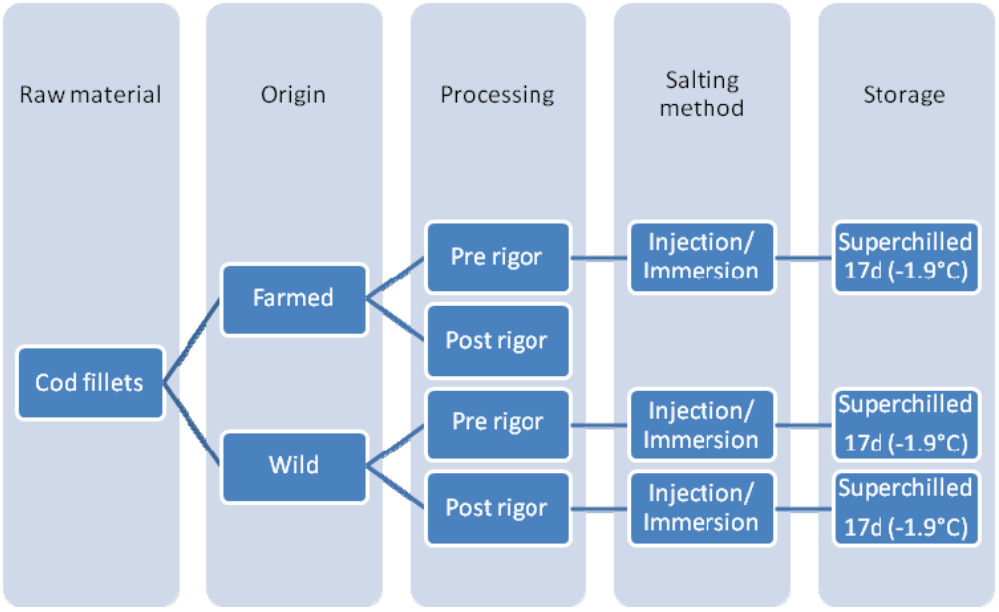

Figure 19: Experimental setup for experiment 2 in Paper I. Comparison of the physicochemical properties and shelf life of pre and post rigor farmed cod to pre and post rigor wild cod fillets for production of lightly salted cod fillets undergoing superchilled storage. As in sub trial 1 the post rigor farmed cod showed excessive gaping during filleting and was therefore not considered for further processing or storage.

8.1.2 Paper II

Wild Atlantic Cod (*Gadus morhua*), caught by trawl in September 2006, was filleted and trimmed into loins after undergoing brine injection or immersion of high or low salt concentrations. The brine immersed loins were packed in either 5kg expanded polystyrene (EPS) boxes or under modified atmosphere packaging (MAP) with a gas mixture of 50% CO_2, 5% O_2 and 45% N_2. The loins were then stored at chilled and superchilled conditions for up to 28 days (Figure 20).

The effect of these experimental parameters on the quality and shelf life of the cod loins was studied by measuring yield, chemical composition, microbial counts, water holding capacity, pH and water distribution and relaxation times throughout the storage time.

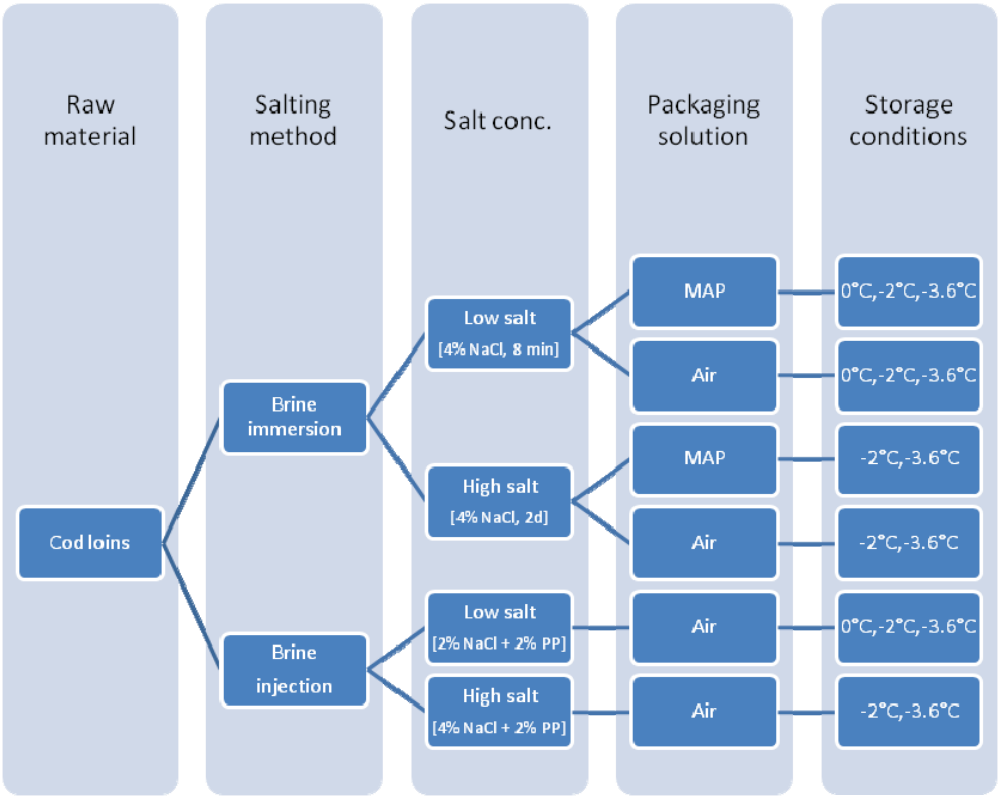

Figure 20: Experimental design of Paper II. The effect of salt concentration, brining method, choice of packaging (air or modified atmosphere packaging, MAP) as well as choice of chilled or superchilled storage temperature on the quality of cod loins was studied.

8.1.3 Paper III

The effect of various pre salting methods during the production of dry salted cod (*Gadus morhua*) was studied with LF-NMR, yield and physicochemical analyzing methods, as well as differential scanning calorimetry (DSC) and electrophoresis (SDS-PAGE). Yield and physicochemical results of the study were published by Thorarinsdottir and others (2010) and DSC and SDS-PAGE results were published by Thorarinsdottir and others (2011).

Wild Atlantic cod, caught by long-line in February 2006, was processed 3-4 d post catch. Fillets were equally divided into five groups of 45-50 fillets each, and each fillet identified with a plastic tag. The fillets were i) injected with 25% NaCl and successively immersed in a 12% brine for 2 days, ii) injected with 22.5% NaCl and 2.5% polyphosphate and successively immersed in a 12% salt brine for 2 days, iii) brined in 12% NaCl, iv) pickle salted for 3 d by stacking the fillets in a closed tubs and v) no pre salting. During brine immersion a fish-to-brine ratio of 1:1 was used. After the pre salting step all fillets were dry salted by stacking them with salt in alternating layers in open tubs for 23-36 days. After two weeks of storage the fillets were rehydrated again in a two step process (Figure 21).

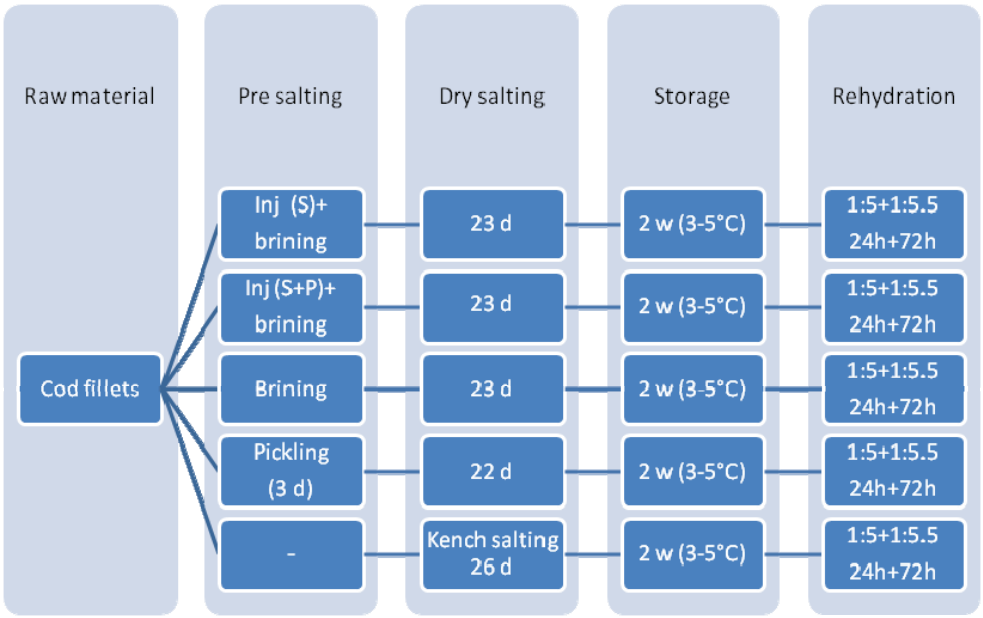

Figure 21: Experimental setup of Paper III. The effect of various pre salting methods during dry salting of cod was investigated.

8.1.4 Paper IV

Saithe (*Pollachius virens*), caught in May 2009 by trawl, were filleted and injected with various combinations of salt and protein solutions with the aim of studying if addition of functional salt- or protein solutions could make the fillets more stable through chilled and/or frozen storage (Figure 22).

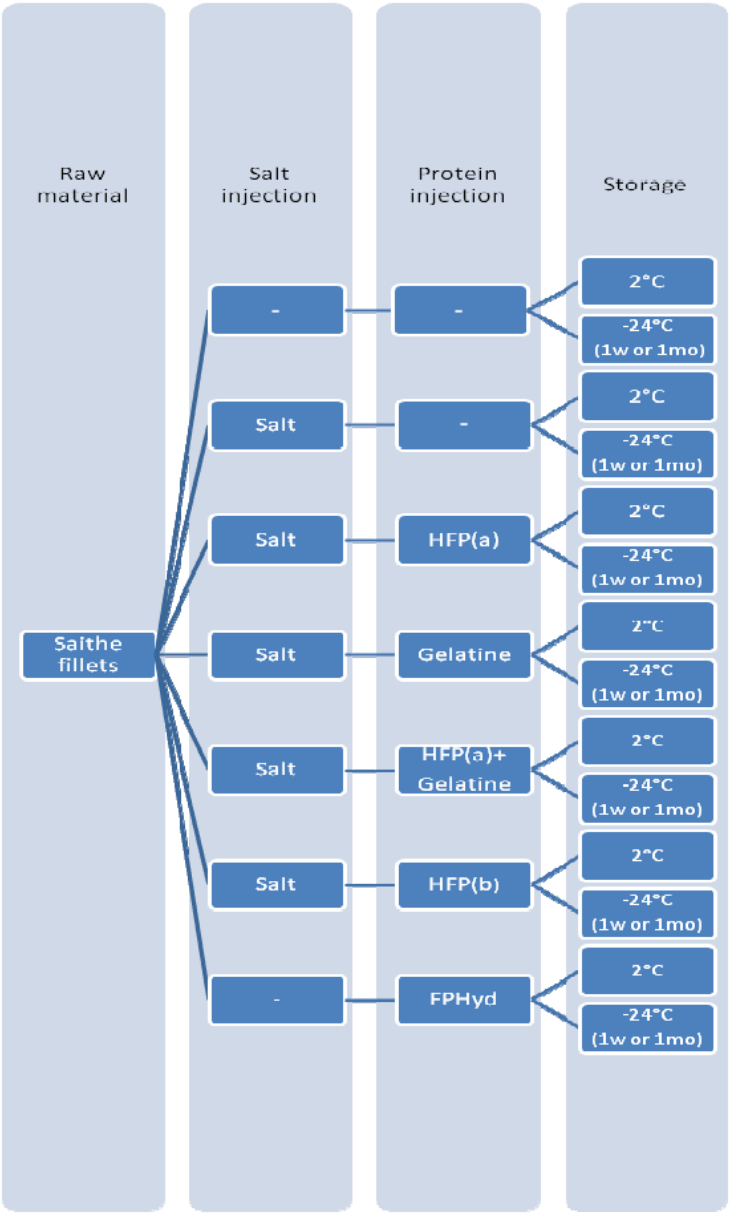

Figure 22: Experimental setup of Paper IV. The effect of protein injection on physicochemical properties and stability of saithe fillets through chilled and frozen storage was studied.

The protein solutions studied were homogenized fish protein solutions, gelatine solutions and a fish protein hydrolyzate solution. The homogenized fish proteins HFP(a) and HFP(b) were made from fresh and frozen mince from cut-offs respectively. Dried fish collagen peptides (gelatine) were produced to form a 2% (w/w) gelatine concentration. Injection of the commercial fish protein hydrolyzate (FPHyd) concentrate was used for comparison.

8.1.5 Paper V

Cold water shrimp (*Pandalus borealis*) caught by shrimp warp in February and March 2010 was frozen on board the fishing vessel and processed in June 2010. The shrimp was brined in three separate tanks with i) 1.4% (w/w) NaCl and 3.6mg/g sodium polyphosphate (SPP) for 24h (Regular treatment, Reg), ii) 1.4% (w/w) NaCl and 3.6mg/g sodium polyphosphate (SPP) for 19h (Shorter pre brine, SPB) iii) 1.4% (w/w) NaCl and 2.8 mg/g SPP for 24h (Less phosphate, LPh). All shrimps then underwent the same treatment, including cooking, peeling, brining, freezing and glazing (Figure 23).

The state of the shrimp muscle was evaluated through the entire process with LF-NMR, both benchtop and unilateral instruments, near infrared (NIR) spectroscopy and physicochemical analyzing methods.

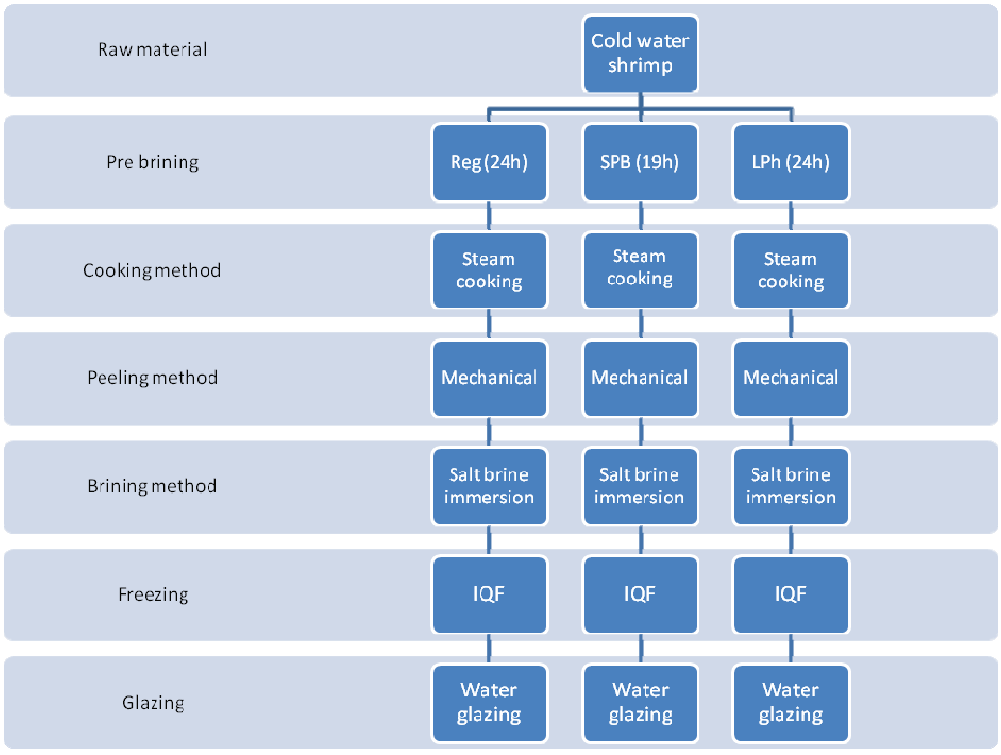

Figure 23: Experimental design of Paper V. The effect of pre brining treatment on the physicochemical characteristics of cold water shrimp was studied.

8.2 Analytical methods

Various analytical methods were used in the papers to evaluate the physical and chemical changes occurring in the muscle during processing. The weight yield and physicochemical methods chosen are commonly used methods for evaluation of quality and physicochemical properties. These measurements were compared to the NMR and NIR results to simplify and improve interpretation of the data from these novel technologies and to relate to the behaviour of the muscle during processing. Table 6 summarizes the analytical methods used in each paper. A detailed description of the methods can be seen in the following chapters as well as in Papers I-V.

Table 6: Overview of analytical methods used in the papers.

Paper	LF-NMR (Minispec)	LF-NMR (Unilateral Profiler)	NIR	Moisture, salt and WHC	Yield	Muscle pH	Protein content	Water activity A_w	Microbial analysis	DSC, SDS-PAGE*	Colour
I	x			x	x	x			x		
II	x			x	x	x			x		
III	x			x	x	x	x	x		x	
IV	x			x	x		x				
V	x	x	x	x		x					x

*DSC and SDS-PAGE results were presented by Thorarinsdottir and others (2011)

8.2.1 Low field Nuclear Magnetic Resonance analysis

8.2.1.1 Instruments

Two low-field NMR instruments were used in the papers, firstly a Bruker mq 20 benchtop instrument (Papers I-V) (Figure 24) and secondly a unilateral Bruker Profiler (Paper V) (Figure 25) (Bruker Optics GmbH, Rheinstetten, Germany). Both instruments have a working proton frequency of 20 MHz, but differ greatly in homogeneity, and thus also in precision and signal-to-noise (SNR) ratio as well as the measurable sample size. The main reasons for this difference lies in the highly inhomogeneous magnetic field found in the Profiler, with a constant gradient of approximately 10 T/m at the surface, while the benchtop instrument has a very homogeneous magnetic field, but can only allow measurements of very small samples (10 or 18 mm in diameter, depending on the probe chosen and 5 mm in height) (Table 7).

Figure 24: Low field benchtop Bruker mq 20 NMR Analyzer used in Papers I-V.

Figure 25: Low field unilateral Bruker Profiler NMR surface scanner used in Paper V.

Table 7: Main characteristics of the NMR instruments used in the study.

	Benchtop mq 20 instrument	**Unilateral Profiler surface scanner**
Magnetic field	**20 MHz**	**20 MHz**
Magnetic field	Homogeneous	Inhomogeneous (10T/m at surface)
Temperature	Variable from -70 to +200°C	ambient
Sample size	10 or 18 mm in diameter, 5 mm in height	5x5 mm in plane and approx. 2.5 mm in depth
Sample weight	0.5 g or 2 g in 10 and 18 mm diameter probe respectively	Unlimited

8.2.1.2 Relaxation time measurements

Measurements of longitudinal T_1 (Papers I-III) and transversal relaxation times T_2 (Papers I-V) were made using the inversion recovery (IR) and Carr-Purcell-Meiboom-Gill (CPMG) (Carr and Purcell 1954; Meiboom and Gill 1958) pulse sequences respectively. Measurement settings were varying between papers, and can be seen in further detail in the materials and methods of each paper.

8.2.1.3 Low field NMR data processing – CPMG/IR curve fitting

By using exponential fitting of the data set the relaxation times can easily be calculated as well as the water distribution. The method is a robust and classic method and makes comparison of results to literature values easy. However there is a risk of overfitting the data, making residual analysis essential (Aursand, 2009).

NMR data was collected with the Bruker Minispec software and successively maximum-normalized to allow comparison of samples with different size and water content. The data was normalized by setting the maximum echo to a value of 100 and scaling the other echoes accordingly. Transversal relaxation data was fitted to a multi-exponential curve by using the Low-field NMR toolbox for Matlab (The Mathworks Inc. Natric, MA), as described by Pedersen and others (2002) according to the equations 7-9 in chapter 8.5. Residual analysis of the exponential fittings was used to decide the number of exponential used for the fittings. The weighted amount of water in each population compared to the total amount of observed water was calculated according to equation 8. Longitudinal relaxation data was fitted to a mono-exponential curve according to equation 9.

8.2.2 Near Infrared (NIR) analysis

Near Infrared reflectance measurements were performed over the wavelength range from 800 to 2500 nm using a Bruker Multi Purpose Analyzer (MPA) system with a fibre probe (Bruker Optics, Rheinstetten, Germany) (Figure 26). Measurements were done on intact, peeled shrimp muscle directly after sampling at the factory. Five scans were used to build each absorption spectrum and all samples were measured in triplicate.

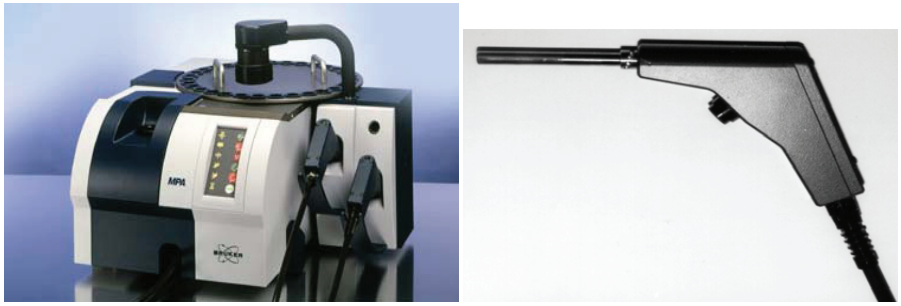

Figure 26: Bruker Multi Purpose Analyzer (MPA) with a fibre probe.

Near Infrared calibrations for prediction of moisture content and water holding capacity (WHC) were made in the Bruker Opus software. The whole NIR spectral range (800-2500 nm) was used for the calibrations, without any further data processing and compared to measurements of moisture content and WHC as measured by the drying and centrifugal methods respectively as described in the physicochemical measurements description. Calibrations for other physicochemical properties were on the other hand not adequate for further use in the study. Several data processing methods were also tried, such as min-max normalization, calculations of derivatives etc., but these did not improve the results to any extent.

8.2.3 Reference measurements

8.2.3.1 Weight yield analysis

The fillets or loins in Papers I-IV were weighed prior to and after each processing step. The weight yield was determined by comparing the weight of the fillets/loins after each processing step to the weight of the raw material according to the equation:

$$Yield_i\ (\%) = \frac{m_i}{m_{raw\ material}} \times 100 \quad \text{(eq. 10)}$$

where $m_{raw\ material}$ and m_i represents the weight of the fillet/loin before processing (raw material) and after each processing step respectively. Drip loss during storage was calculated according to the equation:

$$Drip\ loss(\%) = \frac{m_{before\ storage} - m_{after\ storage}}{m_{before\ storage}} \times 100 \quad \text{(eq. 11)}$$

where $m_{before\ storage}$ and $m_{after\ storage}$ represented the weight of the fillets before and after storage respectively.

The cooking yield was evaluated by steam cooking three pieces from each sample group at 95-100°C for 8 min in a Convostar oven (Convotherm, Elektrogeräte GmbH, Eglfing, Germany). The pieces were taken from the middle part of the fillet. The samples were cooled down to room temperature prior to weighing. The cooking yield was calculated as the ratio between the weight of the pieces after and before cooking (Papers II-IV).

The total yield of the fillets was calculated by multiplying the storage yield and the cooking yield (Paper IV):

$$Total\ yield(\%) = \frac{Storage\ yield \times Cooking\ yield}{100} \quad \text{(eq. 12)}$$

8.2.3.2 Physicochemical analysis

The moisture content was measured by drying 5 g of the sample, mixed with sand, in a ceramic bowl at 103 ± 2 °C for four h (ISO 6496, 1999). The moisture content was calculated as the weight loss after drying, compared to the sample weight prior to drying. The salt content of the samples was measured with the Volhard titration method (AOAC 976.18, 2000) and the protein content was obtained from the total nitrogen content (TN*6.25) and analyzed with the Kjeldahl method (ISO-5983, 2005). Phosphorus content was determined as phosphate vanadomolybdate by spectrometric colorimetric absorbance at 420 nm (AOAC 969.31, 1990; Hanson, 1950; Sutton and Ogilvie, 1967). The method is based on the reaction of orthophosphate (PO_4^{-3}) in an acidic solution with ammonium molybdate and ammonium vanadate in nitric acid. The absorbance of the solution was compared to a calibration curve of standard solutions (vanado-molybdate reagents). The phosphate content, presented as phosphorous oxide (P_2O_5/g sample), was obtained by multiplying the phosphate content by 2.2914. The water holding capacity (WHC) of the samples was measured with the centrifugal method described by Eide and others (1982). Approximately 2 g of the samples were weighed precisely into a vial and centrifuged (Sorvall RC-5B, Dupont Company, Wilmington, Delaware, USA) at 210 g (1300 rpm) at 2-5 °C for 5 min. WHC (%) was calculated as the ratio of water in the sample after centrifugation to water in the sample before centrifugation. TVB-N and TMA was measured as described by Malle and Tao (1987) by steam distillation (Struer TVN distillatory, STRUERS, Copenhagen, Denmark) and titration after extracting the fish muscle with 7.5% aqueous trichloracetic acid (TCA) solution. TMA was measured in TCA extract by adding 20 mL of 35% formaldehyde. pH measurements were performed with a pH electrode (SE 104 Mettler Toledo GmbH, Greifensee, die Schweiz) connected to a Knick pH meter (Portames 913 pH, Knick, Berlin, Germany). The electrode was immersed in the minced samples at 20 ± 2 °C during measurements. Water activity of the muscle was measured in triplicate in a Novasina Aw-center (Novasina AG, Axair Ltd., Pfäffiko, Switzerland) at 25°C. All chemical analyses were performed on minced samples. TVB-N and TMA measurements were done in duplicate, but all other chemical measurements in triplicate. Results are presented as an average of these measurements with standard deviations.

8.2.3.3 Colour measurements

The intensity of the flesh colour was measured by using Minolta type CR-300 (Konika Minolta, Tokyo, Japan), using the D65 light source. The instrument records the L- (lightness – intensity of white colour), a- (redness – intensity of red colour) and b- (yellowness – intensity of yellow colour) values. Each sample was measured in triplicate.

8.2.3.4 Microbiological analysis

Total viable psychrotrophic counts (TVC) and H_2S-producing bacteria were obtained using iron agar (IA), as described by Gram and others (1987), but with 1% NaCl and no overlay. Surface plating was performed, followed by incubation at 15 °C for 4-5 d at 15°C. Cooled Maximum Recovery Dilutent (MRD, Oxoid) was used for tenfold serial dilution. All results are presented as an average of duplicate samples.

8.3 Statistical analysis

Various multivariate analysis methods were used in the project using Unscrambler ® (Version 9.8, CAMO ASA, Trondheim, Norway). Principal component analysis (PCA) was performed on either weighted principal components (PCA) of the fitted NMR parameters and physicochemical quality parameters of the muscle to identify similarities and differences between samples (Papers I-V) or directly on maximum normalized raw T_2 relaxation data (Paper III). All variables in the PCA were weighed with the inverse of the standard deviation to correct for different scales of the variables and the data was centred prior to analysis.

Individual Partial Least Square Regression (PLS1) models, with Martens Uncertainty Test (Martens and Martens, 2001) were then made to identify the significant effect of the NMR parameters on each physicochemical quality property. The obtained NMR parameters were set as the X-matrix while each individual physicochemical quality parameter was set as the Y-matrix. All models were fully cross validated. All significant levels were set to $p < 0.05$.

9 Results and discussion

The aims of the study were to investigate the possibilities that lie in the use of the fast measuring techniques of low field Nuclear Magnetic Resonance and near infrared spectroscopy during seafood processing. The methods were used to give a further insight and understanding to the changes in muscle structure and water properties during processing as well as used to build calibration prediction models for various physicochemical quality properties of the muscle. The main results of the study are presented in the following chapter and their importance to the seafood production industry is discussed. More detailed results can be seen in Papers I-V.

9.1 Water distribution and tissue structure in seafood muscle

The connection between relaxation behaviour and tissue structure has mainly been studied on meat (Bertam and others, 2007, 2009). Although some studies have been made on fish (Steen and Lambelet, 1997; Jensen and others, 2002; Aursand and others, 2008, 2009) the present study gives added knowledge about the connection between these parameters in fish and seafood. According to transverse relaxation measurements in the study the technique can be used to study and follow changes in tissue microstructure during processing and storage as affected by rigor status, choice of raw material, salt-induced swelling dependent on salting method and concentration, as well as protein and phosphate addition.

9.1.1 Raw material differences

Several water populations have been identified in muscle foods by using transversal relaxation time measurements. However, the number of populations is a matter of discussion. Most studies on fish or meat indicated the presence of two relaxing components, T_{21} and T_{22} (Lambelet and others, 1995; Erikson and others, 2004; Aursand and others, 2008, 2009). More populations have also been identified in other studies. Jensen and others (2002) identified four water pools with transverse relaxation times of 37, 56, 126, and 361 ms in minced cod samples, where the fastest relaxing group was correlated with the most bound water, that is, water in close contact with proteins. Studies in pork have then shown three water populations at approximately 1 to 3 ms (T_{2B}) suggested to correlate to water closely associated with macromolecules, 40 to 80 ms (T_{21}) corresponding to myofibrillar water and 200 to 400 ms (T_{22}) corresponding to extra-myofibrillar water (Bertram and Andersen 2007; Bertram and others 2009).

The aim of this work was not only to identify the number of populations present in the muscle but rather to study the diffusion of water between compartments as affected by processing. In this study 1-3 populations were observed in the muscle, as affected by choice of raw material and processing. Two populations were sufficient to describe the water distribution of most of the Atlantic cod samples in papers I-III, as well as in the cold water shrimp in paper V. Mono-exponential

behaviour was observed in brined high salted cod fillets after 13 d of storage, due to a high degree of gelation of these samples. Protein injected saithe fillets in Paper IV showed tri-exponential relaxation behaviour, possibly due to the injection of the protein solutions or due to mincing of the samples. Table 8 gives a summary of the mono- and bi-exponential results from papers I-III and V.

Table 8: NMR relaxation time results for longitudinal relaxation, mono- and bi-exponential transversal relaxation from papers I-III and V.

Paper	Sample	T_1 [ms]	T_{21} [ms]	A_{21} [%]	T_{22} [ms]	T_{2mono} [ms]
I	Wild cod	528.4-718.6	60.1-96.5	34.8-73.2	110.9-222.9	
	Farmed cod	437.8-504.4	34.0-59.1	5.7-72.9	79.5-170.6	
II	Brined low salt	682-779	38.2-44.4	68.9-82.6	100-182	
	Brined high salt	684-863	48.7-85.9	18.5-89.3	86-158	77.3-92.9
	Brine injected low salt	718-824	44.4-49.3	66.1-76.0	87-195	
	Brine injected high salt	780-812	47.6-54.4	50.3-78.6	94-196	
III	Wild cod raw material	597±5	51.3±1.1	94.0±1.9	197±14	
	Pre salted	296-703	30.9-94.2	85.9-95.9	138-233	
	Dry salted	270-325	23.1-29.8	75.4-81.2	160-239	
	Rehydrated	553-669	70.2-93.4	66.0-78.0	126-187	
V	Cold water shrimp	-	42.0-80.4	91.2-98.0	460-1230	

The table shows that the relaxation behaviour is highly dependent on the choice of raw material. As shown in Paper I farmed cod generally show shorter relaxation times than the wild cod. In addition a smaller proportion of water in the less restrained population (A_{22}) was observed in the pre rigor farmed cod compared to the post rigor wild cod. This is in agreement with the observations of Gudjónsdóttir and others (2009), who using light microscopy found a smaller extracellular spacing in farmed prerigor cod muscle compared to wild post rigor muscle (Figure 27). The study also showed longer relaxation times in both pre and post rigor processed wild cod fillets compared to farmed cod fillets.

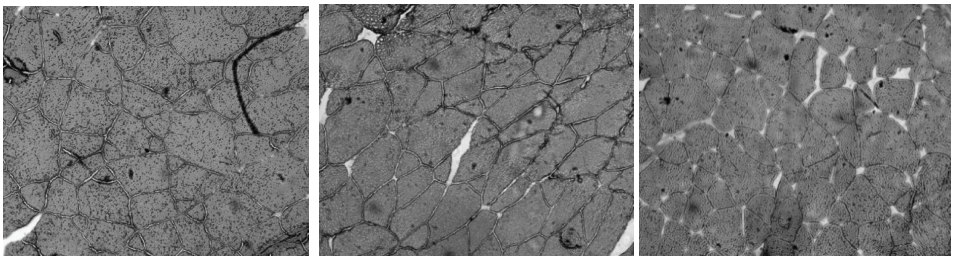

Figure 27: Microscopic images of chilled cod muscle after slaughter/catch of farmed pre rigor mortis muscle (left), wild pre rigor mortis muscle (middle) and wild post rigor mortis muscle (right). More extracellular water was found in chilled post rigor wild cod (16.6 ± 3.2%) than in the pre rigor wild cod (10.6 ± 2.2%) and the farmed cod (10.3 ± 2.3%). No significant difference was found in extracellular fluids between the wild and farmed cod in pre rigor samples. Figure from Gudjónsdóttir and others (2009).

Table 8 also indicates the structural differences between the muscle of cod fillets and cold water shrimp. The cold water shrimp has a significantly higher amount of restricted water (91.2-98.0% water in A_{21} population) than the cod fillets. The relaxation time of this major relaxing T_{21} component is however comparable between the species. However, the T_{22} relaxation component observed in the shrimp has a significantly slower relaxation than the cod, indicating the presence of a small population of very mobile water in the shrimp muscle.

9.1.2 Rigor onset and relaxation behaviour

The rigor onset in farmed cod was followed in Paper I. The study showed that the transverse relaxation times (T_{21} and T_{22}) decreased between day 0 and day 6 of superchilled storage, as well as the apparent population A_{21}, correlating to the shorter relaxation time T_{21}. This observation is consistent with the study of Renou and others (1989) who showed a significant decrease in the T_{21} exponential transverse relaxation time and its corresponding population A_{21} during rigor onset. However, Renou and others (1989) also observed an increase in the longer T_{22} relaxation time during rigor onset in disagreement with the present study. This can possibly be explained by the added salt, which complicates the interpretation of the results. However, the decrease in the T_{21} relaxation time and the amount of the more restricted population, A_{21}, during superchilled storage can be explained by the fact that during rigor mortis, the myofibrils contract, increasing the tension, causing diffusion of water from the myofibrils out to the extracellular space, resulting in a lower water content within the myofibrils post rigor. As the rigor tension is reduced, water can start to diffuse back into the myofibrils again, resulting in a slow increase in the transverse relaxation times (T_{21} and T_{22}) as well as in the amount of water in the more restricted water population (A_{21}), as observed after day 6 of superchilled storage in Paper I.

9.1.3 Salting and relaxation behaviour

The effects of salting on muscle structure were studied in all papers of the study. The results showed that the salting method and the salt concentration both have large influence on the properties of the final product. This was most clearly seen in Paper III, where different pre salting methods were compared during the production of heavily salted cod fillets (bacalao). Figure 28 shows a principal component analysis (PCA) score plot from the normalized transverse relaxation curves, which illustrates the differences in relaxation behaviour of the samples with different treatments. Salt-induced swelling of the muscle was correlated to an increase in the faster relaxing component T_{21} for salt concentrations below 6% in agreement with earlier studies on the effects of salt-induced muscle swelling in fish muscle (Aursand and others, 2008, 2009; Erikson and others, 2004). The largest muscle swelling was observed in fillets pre salted by brine injecting followed by brining, while fillets pre salted by brining followed closely in muscle swelling. Injection of phosphates in combination with salt did not give an added effect on muscle swelling or water holding capacity compared to fillets injected by salt alone. Increased muscle swelling during pre salting was shown to be beneficial for the quality of the fillets through further processing.

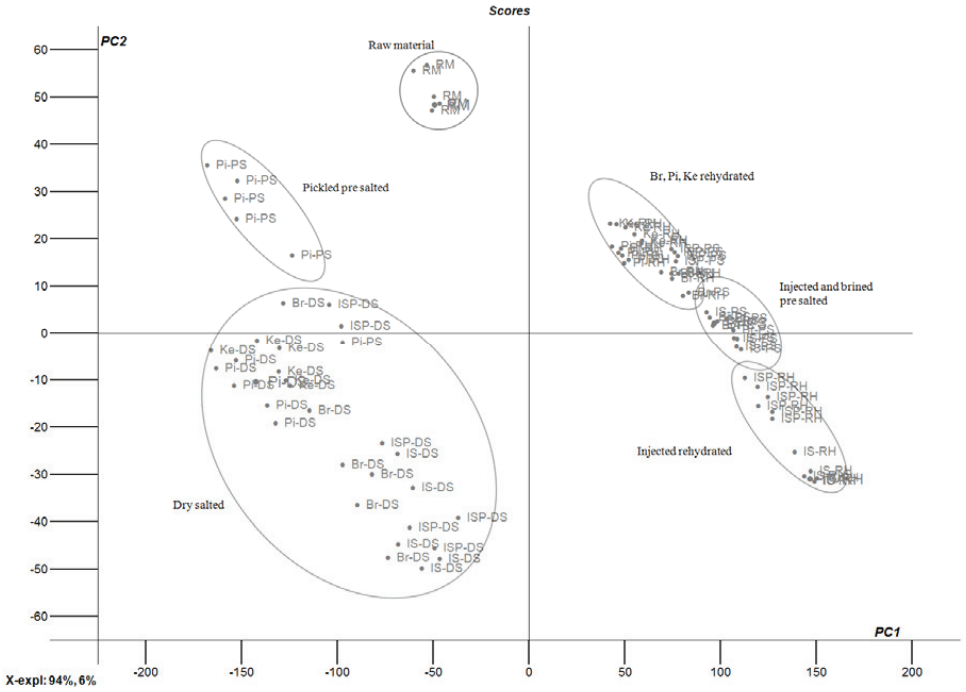

Figure 28: Principal component analysis (PCA) figure of scores of of maximum normalized raw T_2 data of all samples at all salting stages. Explained variances by PC1 and PC2 were 94% and 6% respectively. RM indicates the raw material, while PS indicates samples after the pre salting step, DS samples after the dry salting step and RH after the rehydration step. IS refers to salt injected samples, ISP to salt and phosphate injected samples, Br to brined samples, Pi to pickled salted samples and Ke to kench salted samples.

Higher salt concentrations led to salting-out effects and loss of moisture. This is in agreement with earlier observations, which state that the maximum water holding capacity is reached at approximately 6% salt concentration in the muscle, while concentrations above 9-10% lead to loss of water holding capacity (Duerr and Dyer, 1952; Offer and Knight, 1988; Offer and Trinick, 1983). After dry salting, all groups had a salt content in the range from 20.4 to 21.4% (Thorarinsdottir and others, 2010), leading to a corresponding decrease in the T_{21} relaxation times from 86.4-94.2 ms in the brine injected and brined fillets after the pre salting step to a range of 23.1-29.8 ms in the dry salted fillets. Significantly higher T_{21} relaxation times were observed in the brine injected samples compared to the other pre salting treatments after the dry salting and rehydration steps, indicating less protein denaturation in the brine injected fillets. This is in agreement with Differential Scanning Calorimetry (DSC) and electrophoresis (SDS-PAGE) results, which indicated less protein aggregation, mainly in the heavy myosin chain, in the brine injected fillets compared to fillets of the other treatments, especially the pickled and kench salted groups (Thorarinsdottir and others, 2011). The study also showed that although a lower average salt content was observed in the brined samples than in the brine injected samples, the initial salt concentration of the fillet surface was higher during in the brined fillets. This gave rise to more myosin aggregation in the brined fillets, explaining the differences observed in the T_{21} relaxation times between the fillets pre salted by brining alone or brine injection followed by brining. To get an even salt distribution and minimum protein denaturation during the production of heavily salted cod, pre salting by brine injection followed by brining can therefore be recommended.

A general decrease observed in the A_{21} population of the fillets between the processing steps indicated that the water distribution could not be explained by a simple myofibrillar and extra-myofibrillar model, but rather with a model associating A_{21} and the corresponding relaxation time T_{21} to water in close association with muscle proteins as suggested by Bertram and others (2001) and Erikson and others (2004). Furthermore this decrease in the A_{21} population between the processing steps suggests that the salting process had an irreversible effect on the water distribution of the fillet.

Brining and brine injection during the production of lightly salted products were also compared in paper II. Salting by brining with the higher salt solution led to substantial gelling of the muscle, resulting in the observation of only one water population in these samples. This is in agreement with earlier studies, which state that brining can cause protein coagulation and lead to formation of a gel network, in which water can be entrapped (Nagashima and Suzuki, 1981). According to Nagashima and Suzuki (1981) most of this water in this gelled network of macromolecules is loosely bound. However, longer T_1 relaxation times observed in the brine injected

loins indicated that the muscle structure was affected more than if salting the loins by brining, probably due to cell puncturing and destruction during injection. This is in agreement with the increased drip observed in the brine injected loins compared to their brined counterparts

Differences in the raw materials also influence the salt-induced swelling of the muscle. This was seen in Paper I, where differences in the muscle structure of wild and farmed cod led to insufficient salt uptake in the farmed cod fillets compared to the wild cod fillets, both when brined and brine injected. This indicates that if farmed cod is to be used in the production of lightly salted products in the future, as wild cod is used today, the processing methods has to be studied and optimized further with these differences in muscle structure kept in mind.

9.1.4 Protein addition and relaxation behaviour

Injection of functional protein solutions in saithe fillets were studied in Paper IV. Saithe fillets injected with various combinations of salt, homogenized fish proteins, gelatine and fish protein hydrolyzate, were compared to the properties of untreated fillets during chilled and frozen storage. Tri-exponential relaxation behaviour was observed in these fillets with relaxation times of 27-45 ms, 60-99 ms and 187-341 ms, taking values dependent on the treatment of the fillets. This observation of three water pools indicated that a simple model of myofibrillar and extra-myofibrillar water was not applicable for the present study either. This can possibly be explained by the injection of proteins, which might give rise to the formation of additional water pools in the fillets.

The amount of water in the middle relaxing water pool (A_{2b}) increased as a result of muscle swelling and water uptake caused by the electro-repulsive forces within the muscle structure caused by added salt (Erikson and others, 2004), while the amount of water in the shortest relaxing water pool (A_{2a}) decreased. Injection of salt in combination with homogenized fish proteins (HFP(a)) and/or gelatine resulted in the longest relaxation times, indicating that the injection of these proteins had the highest influence on the characteristics of these water pools compared to the control fillets. Aursand and others (2009) showed an increasing trend in the faster relaxing component T_{21} and its water population coupled to the salt-induced swelling of myofibers in frozen-thawed salmon, but this can only partly explain the observed relaxation behaviour of the varying treatments in Paper IV, since no significant difference was found in the salt content of the fillets after injection. However, as earlier mentioned most of the water in a gelled network of macromolecules is loosely bound (Nagashima and Suzuki, 1981), which could explain the increase in the more restricted relaxation times and associated amount of water in each water pool in the gelatine injected fillets. No significant difference was seen between the relaxation times or water populations of the fish protein hydrolyzate (FPHyd) injected fillets and the control group, in agreement with the similar moisture content observed in these groups.

A general increasing trend was observed in the shortest relaxing water population (A_{2a}) during frozen storage, with a clear negative linear correlation to the water population of the second fastest relaxing water population (A_{2b}) (R^2=0.906, n=84, P<0.0001). This is agreement with Jensen and Jørgensen (2003) who found four water populations in frozen cod mince (37, 56, 126 and 361 ms respectively), where the amount of water associated with the two fastest relaxing components were directly correlated to the changes obtained in the denaturation profiles during storage, as assessed by Differential scanning calorimetry (DSC). The study suggested that the increased amount of water in the shortest relaxing water pool at the expense of the second fastest relaxing water pool was due to denaturation of myosin and sarcoplasmatic proteins. This indicates that the highest degree of denaturation of myosin and sarcoplasmic proteins after one week of frozen storage was observed in the fillets injected with frozen-thawed homogenized fish protein mince (HFP(b)), while gelatine injected fillets, solely or in combination with fresh homogenized fish protein mince (HFP(a)), were the most denatured after one month of frozen storage.

Injection of the commercial FPHyd resulted in the most stable yield during chilled and frozen storage. Addition of gelatine, alone or in combination with homogenized fish proteins (HFP(a)) had no additional effects on the weight gain compared to fillets only injected with salt or HFP(a) respectively. The NMR results also indicated that the FPHyd fillets fillets most resembled the control fillets, while the water distribution and muscle structure was most affected in the HFP(b) injected fillets frozen for 1 week and the HFP(a)+Gel injected fillets frozen stored for 1 month. The study therefore showed that protein addition can be used to stabilize and improve quality of saithe fillets, but the isolation processes, choice of concentrations etc. need to be optimized further to reach the desired functional properties of the muscle.

9.1.5 Phosphate addition and relaxation behaviour

The effects of phosphate addition on seafood muscle were investigated in Atlantic cod and cold water shrimp in Papers III and V respectively.

In Paper III the effect of salt and phosphate injection, followed by salt brining as a pre salting method was compared to six other pre salting methods during the production of heavily salted cod (bacalao). Comparison of pre brining by injection of salt and phosphates to injection of salt solely showed no significant difference on the water distribution of the cod fillets with the phosphate concentrations used. However, added phosphate increased the yield during salting but not after rehydration in agreement with earlier studies (Thorarinsdottir and others, 2010, Thorarinsdottir and others, 2001). The phosphates also lead to positive effects on colour and quality scores, mainly due to a lighter appearance (Thorarinsdottir and others, 2010).

The results from Paper V indicated that phosphates added to pre brining solutions improved shell peeling after cooking by aiding the separation of the muscle from the shell. The phosphate content was however washed away from the muscle during processing and was lower in the final product than in the raw material. It is therefore clear that phosphate use prior to cooking during shrimp processing is in this case rather a technical aid than an additive. The effects of phosphate on muscle swelling during pre brining are somewhat difficult to interpret from the obtained results. No significant difference was seen in the relaxation times between pre brining treatments during the pre brining step although a difference were seen in the water distribution. A lower amount of the more restricted population (A_{21}) was observed in shrimp with higher phosphate content during pre brining than in the other groups. It is possible that this higher proportion of less restricted water (A_{22}), due to the higher phosphate content, leads to added muscle swelling and thus contributes to easier separation of the muscle from the shell during cooking and peeling.

9.1.6 Storage conditions and relaxation behaviour

9.1.6.1 Freezing

Several studies have been made on the effects of freezing on fish muscle by using LF-NMR in Atlantic cod (Lambelet and others, 1995; Jensen and others, 2002; Burgaard and Jørgensen, 2010) and Atlantic salmon (Veliyulin and others, 2005b; Aursand and others, 2009). Steen and Lambelet (1997) also studied the effects on frozen storage on Atlantic cod mince. The effects of freezing on muscle structure were evaluated in papers I, IV and V in Atlantic cod, saithe and cold water shrimp respectively in this study.

When the results from the frozen samples from Paper I were compared to the fresh samples studied by Gudjónsdóttir and others (2009) a decreasing trend in T_1, due to freezing, was seen in both the untreated and brine injected wild post rigor processed cod. Using MRI relaxation measurements, Evans and others (1998) showed that protein denaturation during freezing of meat was consistent with a decrease in the longitudinal relaxation time T_1. It was suggested that an increase in molecular weight of the proteins, due to protein aggregation, would reduce the mobility of the proteins and enhance cross-relaxation, thus leading to reduced T_1 values. This trend was however not observed in other groups analyzed, possibly due to a firmer muscular structure of the pre rigor processed farmed and wild cod and due to lower water content and WHC, which might lead to smaller changes in these parameters during frozen storage in the pre rigor processed fillets compare to the wild post rigor processed cod.

Slightly lower T_2 values were reported by Erikson and others (2004) for frozen-thawed wild cod, where unbrined samples gave a T_{21} of 50 ± 2 ms and a T_{22} of 177 ± 8 ms than those observed in Paper I. The fish in their study was frozen for one week at −30 °C, while in this study the fish was

frozen for six months, thus leading to further protein denaturation and thus also longer T_{22} values. This is in agreement with the observations of Steen and Lambelet (1997), who studied the texture changes in cod mince during frozen storage at −10, −20, and −70 °C. They observed longer T_{22} values with longer storage time and higher (less negative) storage temperature. On the other hand, frozen storage had no significant effect on the two shorter relaxation times, T_2' and T_2'' observed in their study.

The results of Paper IV indicated that injection of functional proteins can stabilize saithe fillets during frozen storage. Injection of a commercial fish protein hydrolyzate (FPHyd) turned out to be the most effective functional protein solution for this task of the solutions investigated.

9.1.6.2 Superchilling

There are few studies where LF-NMR has been used to study the effect of superchilling on water distribution. As mentioned in the introduction chapter, superchilling storage is especially vulnerable due to irreversible changes to the muscle structure that may occur at this temperature range. Since LF-NMR is an excellent method for the studies of water distribution this technique was applied to study the effect of superchilling on water distribution and quality attributes of fish muscle in Papers I and II. The shrimp of Paper V were then pre brined in a superchilled brine solution.

The effect of storage temperature was observed in the weighted water population distribution (A_{21} and A_{22}) and the longer relaxation time T_{22} in the superchilled modified atmosphere packed (MAP) samples in paper II. In these samples the water population, relating to the slower relaxing component A_{22}, was larger in MAP samples at −3.6 °C than at the other temperatures, indicating diffusion of water from the myofibers into the extracellular space, probably due to cellular damage and protein denaturation caused by the partial freezing observed in the samples at this temperature. Lambelet and others (1995) showed that frozen storage of cod at −10 °C had a significant effect on the transitions related to myosin, as measured by Differencial Scanning Calorimetry (DSC). These changes in myosin, which increased with storage time, was coupled to longer T_{22} values and decreased WHC, similar to the behavior observed in the samples stored at −3.6 °C in this study. Transitions associated with sarcoplasmic proteins and actin were on the other hand almost unaltered during frozen storage at −10 °C. This indicates that partial freezing in the MAP samples stored at −3.6 °C had a significant effect on the myosin structure.

The study therefore showed that LF-NMR can be used to indicate freeze denaturation of the muscle and that the temperature recommended for fresh and lightly salted cod products should not be stored below -2°C.

9.1.7 Muscle cooking and relaxation behaviour

Lambelet and others (1989) showed that the transverse relaxation parameter (T_2) was sensitive to protein denaturation during heating of whey proteins by following the changes in the relaxation rate ($R=T_2^{-1}$) during heating. A similar increasing trend, as observed by Lambelet and others (1989), was found in the relaxation rate during cooking of shrimp in Paper V, indicating protein denaturation in the shrimp muscle during the cooking process (Figure 29).

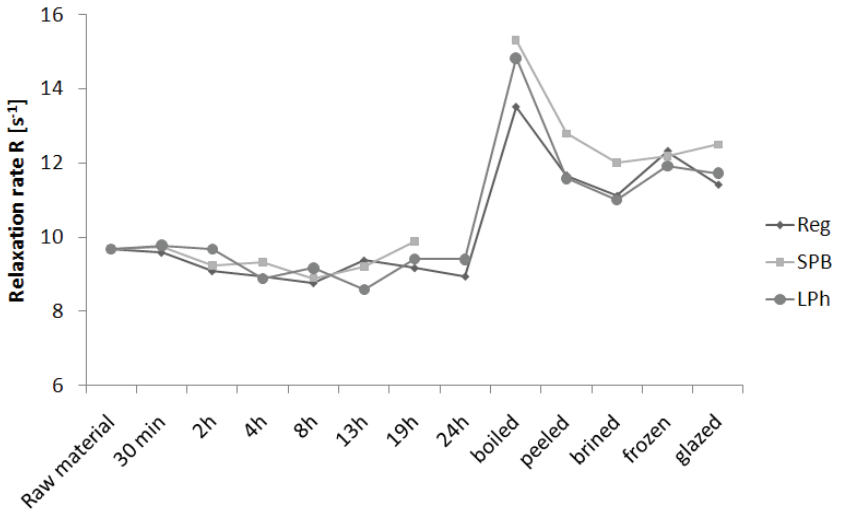

Figure 29: Relaxation rate R of cold water shrimp through processing as assessed by equation 13.

Slightly longer relaxation times were observed for the regularly treated shrimp (Reg) than the other treatments after cooking (and thus slower relaxation rate), indicating that the proteins of the regularly treated shrimp were slightly less denatured than in the other groups. This could contribute to the higher peeling yield observed in the regularly treated shrimp. Muscle swelling was observed again during the peeling step and brining, particularly in the regularly treated shrimp indicating that the highest water uptake was observed in that group both prior to and after cooking. The relaxation rates increased again during freezing, indicating further protein denaturation and aggregation of the shrimp muscle as shown in earlier studies (Sikorski and others 1976; Shenouda 1980; Steen and Lambelet 2002). This observation is also in agreement with the moisture loss observed during the cooking stage.

9.2 Comparison to physicochemical properties

Several studies have used LF-NMR and NIR to predict physicochemical properties of seafood. The LF-NMR studies have however mostly been focusing on water and fat content (Ablett, 1992; Gambhir, 1992; Sørland and others, 2004; Aursand and others, 2008, 2009) as well as water holding capacity (Jepsen and others, 1999). It was our goal to investigate if significant correlations to other important physicochemical properties could be found, using LF-NMR and/or NIR where appropriate.

Principal Component Analysis (PCA) was used to gain an overview of the associated parameters, while Partial Least Square (PLS) linear regression models were used to find significant correlation between the spectroscopic measurements and the physicochemical quality properties.

In this study exponential fitting of the NMR relaxation curves were used prior to linear modelling to give further information on the underlying reasons why correlations can be found between the NMR relaxation parameters to certain physicochemical properties and therefore gain further information about different muscle behaviour during processing. Table 9 gives a summary of the correlations found between LF-NMR measurements, using the Minispec instrument, and various physicochemical and microbiological reference measurements observed when all samples in each paper were analyzed together.

Parameters reflecting water behaviour and retention, such as water content, water holding capacity and water activity were highly correlated with the NMR parameters in the different papers of the study, and this is in agreement with earlier findings (Aursand and others, 2008, 2009; Jepsen and others, 1999). However, strong correlations were also found to several other important quality parameters, such as muscle pH, the formation of total volatile base nitrogen (TVB-N) and trimethylamine (TMA) to mention a few. Strong significant correlations were found to the muscle pH during the production of heavily salted cod (bacalao) in Paper III and during shrimp processing in Paper V. Strong correlations were found between the water distribution and TVB-N and TMA respectively in Paper I and a weak, but still significant, correlation was found to H_2S-producing bacteria in brine injected samples in Paper II. These properties are however often affected by the water properties of the muscle. From this summary it can be seen that LF-NMR can be used to indicate different aspects of seafood quality deterioration where the spoilage mechanisms or denaturation affect the water properties of the muscle.

Table 9: Summary of NMR relaxation parameters, obtained with the Minispec benchtop system, with significant correlation to various physicochemical quality parameters in Papers I-V. All regressions are obtained using all samples in the study. Correlation factors (R^2) and root mean square errors of cross validation (RMSECV) of the models are stated in the table.

Physicochemical property	Paper I Farmed/wild cod	Paper II Superchilled cod	Paper III Bacalao	Paper IV Protein injected cod*	Paper V Shrimp processing
Water	T_1, T_{21}, A_{21}/A_{22}, T_{22} (R^2=0.953, RMSECV=0.43)	-	T_1, T_{21} (R^2=0.916, RMSECV=3.52)	A_{2a}, T_{2a}, A_{2b}, T_{2b}, T_{2c} (R^2=0.826, RMSECV=0.61)	A_{21}, A_{22}, T_{22} (R^2=0.558, RMSECV=1.45)
Salt	T_1, T_{21}, A_{21}/A_{22}, T_{22} (R^2=0.858, RMSECV=0.30)	T_{21}, A_{21}/A_{22} (R^2=0.719, RMSE=0.48)	T_1, T_{21} (R^2=0.852, RMSECV=3.44)	-	T_{21} (R^2=0.219, RMSECV=0.27)
WHC	T_1, A_{21}/A_{22}, T_{22} (R^2=0.906, RMSECV=3.98)	-	T_1, T_{21},T_{22} (R^2=0.894, RMSECV=2.88)	A_{2a}, A_{2b}, T_{2b}, T_{2c} (R^2=0.862, RMSECV=3.45)	T_{21}, T_{22} (R^2=0.492, RMSECV=3.59)
pH	-	-	T_1, A_{21}/A_{22} (R^2=0.691, RMSECV=0.10)	NA	T_{21}, A_{21}/A_{22}, T_{22} (R^2=0.828, RMSECV=0.19)
Protein	NA	NA	T_1, T_{21}, A_{21}/A_{22}, T_{22} (R^2=0.981, RMSECV=0.51)	A_{2a},T_{2a},A_{2b},T_{2b} (R^2=0.816, RMSECV=0.54)	T_{21}, A_{21}/A_{22}, T_{22} (R^2=0.848, RMSECV=0.80)
TCA	NA	NA	T_1, T_{21}, A_{21}/A_{22} (R^2=0.908, RMSECV=0.06)	NA	NA
Water activity	NA	NA	T_1, T_{21}, T_{22} (R^2=0.960, RMSECV=0.03)	NA	NA
Drip	-	-	NA	A_{2a},A_{2b},T_{2b} (R^2=0.341, RMSECV=3.30)	NA
Cooking yield	NA	-	-	-	NA
Total yield	NA	NA	T_1, T_{21}, T_{22} (R^2=0.785, RMSECV=11.31)	A_{2b}, A_{2c} (R^2=0.542, RMSECV=2.80)	NA
TVB-N	A_{21}/A_{22} (R^2=0.951, RMSECV=2.48)	-	NA	NA	NA
TMA	A_{21}/A_{22} (R^2=0.984, RMSECV=1.44)	-	NA	NA	NA
Phosphate	NA	NA	NA	NA	T_{21}, T_{22} (R^2=0.842, RMSECV=0.46)

*Tri-exponential fitting was the most appropriate in paper IV, while bi-exponential fitting gave the best results in the other studies.

9.3 Online or at-line applications

This study has focused on finding significant correlations between LF-NMR measurements and various physicochemical reference methods, which are commonly used today to assess the quality of seafood during processing and storage. However, in Paper V the technology was tried at-line, with the use of a unilateral magnet (Bruker Profiler) in combination with near infrared (NIR) spectroscopy. These measurements were then compared to LF-NMR measurements done with the benchtop Minispec magnet and several physicochemical reference methods.

Measurements of Near Infrared (NIR) reflectance of the samples showed strong correlation to moisture content and WHC of the shrimp muscle, as measured with traditional drying and centrifugal methods respectively. Successively calibration models were built for these two parameters using the whole spectra, without any further data treatment. Further data treatment and choosing more restricted spectral ranges were also tried, but these did not improve the results in this study. The resulting moisture content calibration had a correlation coefficient of 0.8475, a root-mean-square error of cross validation RMSECV of 0.875, a number of samples n=52 and residual predictive deviation RPD of 1.88, while the corresponding coefficients for the WHC calibration were 0.8610, 2.71, 52 and 1.96 respectively. The study showed that NIR is a rapid, reliable and convenient analyzing method for moisture and WHC during shrimp processing and can be used to adjust the processing parameters. Brodersen and Bremner (2001) successfully used NIR reflectance spectroscopy to distinguish between frozen and thawed shrimp, salt concentration of brines used, pH in the flesh and cooking period of whole and minced shrimp. However, comparison to various physicochemical properties indicated that their NIR data could not predict dry matter content, salt content, peeling ability or WHC. No significant correlations were observed in dry matter or salt content in the present study in agreement with Brodersen and Bremner (2001), while good correlations were found for the WHC as discussed earlier.

Strong significant correlation was observed between all NMR parameters obtained with the benchtop Minispec system to the protein content (R^2=0.848, RMSECV=0.804) and muscle pH (0.828, RMSECV= 0.189) respectively. The latter is in agreement with the observations of Larsson and Tornberg (1988) which showed that T_2 values correlating to perimysial water in porcine meat correlated well with the ultimate pH, indicating that pH can affect the pore size distribution within the muscle. Strong correlations were also observed for the relaxation times to the phosphate content (R^2=0.842, RMSECV=0.459). Moisture content showed significant correlation to the T_{22} relaxation time as well as the water distribution between the populations, when the moisture content was measured using NIR (R^2=0.517, RMSECV=1.519). Correlations were only found to the T_{22} when the

traditional drying method was used to measure the moisture content. Significant correlation was observed between WHC and the relaxation times T_{21} and T_{22} measured with the Minispec device. When the WHC was assessed with the NIR technique the water distribution also gave significant correlation to the WHC. This is in agreement with Offer and Knight (1988) who stated that the water holding capacity of meat depended primarily on the extent of the post mortem myofibrillar shrinkage and the successive changes occurring in the extracellular compartments. Only weak correlations were observed between the NMR parameters and salt content and the colour parameters respectively. This is in agreement with the fact that salt content or colour values alone do not reflect the state of the muscle. Other factors, such as the cooking or freezing steps, have a greater effect on the water properties of the muscle.

Significantly lower signal-to-noise (SNR) ratio was observed in the unilateral Profiler magnet, compared to the benchtop Minispec magnet, due to the heterogeneous magnetic field and the low number of scans chosen. The sensitivity and SNR can however probably be improved by adding repetitive scans and sample replicates. Large irregular fluctuations observed in the relaxation parameters between samples through processing made interpretation of the data difficult. However a similar decrease in the slower relaxation time was observed in both LF-NMR methods during pre brining, indicating that this parameter was sensitive to the changes occurring on the muscle structure during pre brining using both methods. Significant correlations were also found between the relaxation parameters, using the unilateral NMR Profiler magnet, and several reference measurements, such as muscle pH, water content, water holding capacity and colour L-value. However, the penetration length of the signal was very short for the probe used and therefore it was necessary to peel the shrimp prior to analysis. By choosing a probe with a longer penetration length, shell peeling prior to analysis would no longer be necessary, making the technique even more efficient as an online or at-line process control method.

The study shows that LF-NMR and NIR are very useful methods in online or at-line monitoring of various physicochemical properties during seafood production. The main disadvantage of LF-NMR is that the equipment is complex and results are often difficult to interpret. NIR might therefore be preferred for relatively homogeneous samples, due to its fast, non-invasive characteristics. However, NIR only penetrates a short distance into the sample while all protons in the sample volume are measured with LF-NMR. LF-NMR is therefore a better choice for heterogeneous samples, since a larger quantity of each sample is measured with the LF-NMR method than by NIR. However, both methods require multivariate analysis for interpretation of the data in relation to physicochemical quality properties of the muscle.

Unilateral magnets are promising instruments in the control and optimization of shrimp processing or other seafood, but the technique requires further optimization before being implemented into the processing lines. Online or at-line applications of these techniques need to be optimized for each processing line and the most important quality parameters need to be chosen for each processing line with respect to type of raw material, concentrations of additives or technical aids and choice of other processing steps.

10 Conclusions

The study showed that low-field NMR measurements give valuable information about the state and behavior of the water in muscle systems during processing with regard to different raw material, processing methods and handling. The method gives a deeper understanding of the changes occurring in muscle during processing by indicating their effect on water distribution and relaxation behaviour of water protons in the muscle. The method also gave strong correlations to various physicochemical properties of the muscle, such as water content, water holding capacity, muscle pH, salt content and many more dependent on the process studied. The method was also efficient with regard to indicating various aspects of seafood quality deterioration, where the spoilage mechanisms or denaturation affected the water properties of the muscle.

Although the unilateral magnets had a significantly lower signal-to-noise (SNR) ratio than the benchtop Minispec system, the method showed promising correlations to water content, water holding capacity and muscle pH. However the method needs further optimization with regards to measurement settings, number of sample replicates to improve standard variation and size of analyzing surface. The penetration length of the signal is also very short for the probe used and therefore it was necessary to peel the shrimp prior to analysis. By choosing a probe with a longer penetration length shell peeling prior to analysis would no longer be necessary, making the technique even more affective as an online or at-line process control method.

NIR spectroscopy was shown to be an excellent predictor of water content and water holding capacity in shrimp muscle. Several trials done on fish and meat muscle samples not reported in this work indicate that numerous other parameters show strong correlations to the NIR reflectance curves. Although the untreated spectra gave the best prediction models in this study it must be kept in mind that this is not always the case and that more data handling may be necessary when the technique is implemented into a processing line.

Online or at-line applications of these techniques need to be optimized for each processing line and the most important quality parameters need to be chosen for each process with respect to type of raw material, concentrations of additives or technical aids and choice of other processing steps. However the study illustrated the wide applicability of these techniques and indicated what parameters can be expected to be predicted with high certainty.

11 Future prospects

As shown in this study LF-NMR and NIR spectroscopy can be used to gain further insight and understanding of how processing affects the muscle structure by studying the water distribution and water holding properties of the muscle. The future prospects are to collect all NMR and NIR data from this study as well as other studies performed at Matís in an overall database with connections to all reference measurements that have been made on these samples. This data will be used to build calibrations for fast determination of various quality parameters, which can then easily be applied as a quality control tool in a wide range of processes, either online or at-line. This will be linked to further optimization of the measuring parameters of the Bruker Profiler or other unilateral devices. This optimization is necessary for this technique to be implemented into online monitoring in a sufficient way. Further work is also needed to simplify the procedure and to make it accessible for the quality managers in the industry, who supervise the processing lines on a daily basis. By implementing these techniques into Icelandic fish and seafood processing plants improved quality management can be achieved, resulting in improved and more valuable products.

Continued research on the muscle structure and how it is affected by processing with regard to different raw material, processing methods and handling is highly important and necessary to develop new and improve existing techniques. This is an endless field of research and I look forward to face more challenges within the field in the future.

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13 Original Papers I-V

Paper I

Process control of lightly salted wild and farmed Atlantic cod (*Gadus morhua*) by brine injection, brining and freezing

A low field NMR study

Gudjonsdottir M, Gunnlaugsson VN, Finnbogadottir GA, Sveinsdottir K, Magnusson H, Arason S, Rustad T.

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Paper II

Low field Nuclear Magnetic Resonance study on the effect of salt and modified atmosphere packaging on cod (*Gadus morhua*) during superchilled storage

Gudjónsdóttir M, Lauzon HL, Magnússon H, Sveinsdóttir K, Arason S, Martinsdóttir E, Rustad T.

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Low field Nuclear Magnetic Resonance on the effect of salt and modified atmosphere packaging on cod (*Gadus morhua*) during superchilled storage

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ABSTRACT

Low field Nuclear Magnetic Resonance was used to evaluate the effect of salt and modified atmosphere packaging (MAP) on cod loins during superchilled storage. Transversal and longitudinal proton relaxation times of the cod loins were measured with Carr–Purcell–Meiboom–Gill (CPMG) and Inversion Recovery (IR) pulse sequences respectively. The relaxation parameters reflected the observed differences in the muscle caused by variation in salt concentration, the choice of salting method (brining or brine injection) and packaging (air or MAP), as well as superchilled storage temperature and storage time. Significant correlations were found between the NMR parameters and parameters describing the water dynamics of the muscle (moisture and salt content, water holding capacity, drip and cooking yield), as well as muscle pH and counts of H_2S-producing bacteria in chosen sample groups. The study showed the possibility of using low field NMR to indicate fish quality deterioration, when the spoilage mechanisms affect the water properties and muscle structure.

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1. Introduction

In recent years the amount and value of exported fresh fish products have increased rapidly in Iceland. The transport of both raw materials and fish products, both domestically and abroad has increased. This lengthens the value chain and at the same time increases the risk of quality loss or contamination of the product. The knowledge on the effects of processing and transport on shelf life and quality deterioration of fresh cod products is the prerequisite for optimizing the quality and value of fresh fish products.

Salting of foods has been a traditional method for preserving food for centuries. By increasing the salt content, the initial freezing point of the food can be lowered (Chen, 1985a,b). Moreover salting of food also leads to diffusion of water and swelling of the muscle fibers (Offer & Knight, 1988; Offer & Trinick, 1983). The uptake of salt in the fish muscle thus affects the properties of the muscle, such as the water holding capacity (WHC), which increases with an increasing salt concentration up to 6% (Bocker, Kohler, Aursand, & Ofstad, 2008; Fennema, 1990; Offer & Knight, 1988; Offer & Trinick, 1983). Light salting of fish is therefore a method that has been widely used to increase the moisture content in foods and to reduce drip (Thorarinsdottir, Arason, & Thorkelsson, 2002). This method is also often used prior to freezing to counteract the negative effects of freezing. Lightly salted products are generally salted by brine immersion or injection, resulting in a muscle salt concentration up to 2% (Gudjonsdottir et al., 2010). The effect of these two salting methods, as well as the effect of different salt concentrations was examined in this study.

The temperature during storage and transportation of fish has a large effect on the quality of fish. The shelf life of lightly salted cod or desalted products is considered to be very short, i.e. 6–10 days for the latter (Magnússon, Sveinsdóttir, Lauzon, Thorkelsdóttir, & Martinsdóttir, 2006). Cooling and freezing of fish slow down the metabolic degradation of the fish and hence lengthens its storage time. Optimization of chilling techniques and physiochemical properties of the fish is therefore crucial to ensure good quality of fish products to the consumers. Furthermore, the fish becomes microbiologically more stable as the temperature is lowered (Andersen, Jul, & Riemann, 1965; Huss, 1995; Lauzon, Magnússon, Sveinsdóttir, Gudjónsdóttir, & Martinsdóttir, 2009). To ensure optimal quality of fresh fish it is important that no water in the muscle freezes during storage, since freezing is known to cause aggregation and destruction of muscle proteins (Love, 1968; Sikorski, Olley, & Kostuch, 1976). The optimal storage temperature for fresh fish is therefore within the superchilling temperature range, given that good temperature control can be guaranteed, so that partial freezing and temperature fluctuations in the muscle can be prevented. The superchilling temperature range, which

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is often defined as the temperatures from 0 °C to the temperature of first ice crystal formation in the product and 1–2 °C below that, is therefore very dependent on the substances and contents of the sample, especially the muscle salt concentration (Chen, 1985a,b).

At superchilled storage, as well as when using modified atmosphere packaging (MAP), different spoilage mechanisms are observed, compared to when traditional storage methods (air packaging at 0 to 4 °C) are used. An extended shelf life period, where the fish is neutral in taste and color, can be gained by using MAP (Martinsdóttir, Lauzon, & Tryggvadóttir, 2005), due to delayed bacterial development (Lauzon et al., 2009), while superchilling can lengthen the overall freshness period (Lauzon & Martinsdóttir, 2005; Olafsdottir, Lauzon, Martinsdottir, & Kristbergsson, 2006; Wang, Tang, & Correia, 2000). Dissolution of carbon dioxide CO_2 in MA-packed fish can however affect both the texture and taste of the fish (Davis, 1993; Lauzon et al., 2009).

The objectives of the study was to investigate the effects of MAP, superchilling and salting methods on the quality of lightly salted cod loins during superchilled storage examined with regard to physico-chemical and microbial quality and spoilage indicators, as well as NMR relaxation time studies on the mobility and distribution of water into populations.

2. Materials and methods

2.1. Experimental design

Wild Atlantic cod caught by trawl north of Iceland in September 2006 was used for the experiment. The fish was bled and gutted in one operation and placed in running seawater in a bleeding tub on board. The cod was washed thoroughly and stored in tubs on flake ice until processing 2–3 days post catch. A part of the lot was filleted in a Baader 252 machine and skinned in a Baader 51 machine. These fillets were immersed in a 4% (w/w) brine for 2 day at 1 °C (hereafter referred to as brined high salt cod, with a 2.5% salt concentration). The other part was kept whole on ice for the same period at 1 °C in an air blast cooling chamber (Celcius ehf, Iceland). This batch was filleted and skinned in the same way as the other samples and then divided into 3 groups, i) brined in a 4% (w/w) brine for 8 min (referred to as brined low salt, with a measured 0.4% salt concentration), ii) injected with brine containing 2% (w/w) salt and 2% (w/w) phosphates (referred to as brine injected low salt, with a measured salt concentration of 0.4%) and iii) injected with a brine containing 4% (w/w) salt and 2% (w/w) phosphates (referred to as brine injected high salt, with a measured 0.7% salt concentration). The salt used in the experiment was Esco food grade pure dried NaCl salt and the phosphate used was food grade sodium tripolyphosphate. Brine injection was performed in a Traust 0518 brine injection machine (Traust, Lækjarkot, 311 Borgarnes, Iceland) with a band speed of 14 mm/s and a brine injection pressure of 2 bar. Fillets of all groups were trimmed into 350–550 g loins and packed in plastic bags placed in 3 kg polystyrene (EPS) boxes, with 2 cooling mats placed on top and an absorbent pad underneath the fish bulk. Samples from the brined groups were also packed under modified atmosphere into retail packaging with a gas mixture of 50% CO_2, 5% O_2 and 45% N_2 as described by Lauzon et al. (2009). All samples were transported to the laboratory in a refrigerated truck and finally stored in air blast cooling chambers (Celcius ehf, Iceland) set to 0 °C, −2 °C and −3.6 °C (measured temperature). The low salt groups were stored at all previously mentioned storage temperatures, while high salt groups were only kept at −2 °C and −3.6 °C. Analysis of physicochemical, microbiological and NMR analysis were performed on the low salt brined samples and high salt MAP samples on days 1, 6, 12, 15, 21 and 28 or to the end of shelf life as analyzed by sensory analysis (data not shown). Analysis of the high salt brined air samples and brine injected samples were made on days 1, 7, 13 and 16. Sensory and microbiological results from the brined groups were reported by Lauzon et al. (2009).

2.2. Temperature and gas measurements

The sample temperature was monitored using iButton DS 1922L data loggers, with an accuracy of ±0.5 °C, a resolution of 0.0625 °C and an operating range from −40 to +85 °C. The temperature was measured in 90 s intervals. Average environmental temperature in the cooling chambers were calculated from temperature measurements recorded by the built-in chamber system every 2 min.

The gas composition in the head space of two MA-packs was measured in each group during sampling. Septums were put on the MA-packs and the gas composition measured with a PBI Dansensor (CheckMate 9900, Denmark). The gas content was measured twice, and the latter measurement recorded. The results of the temperature and gas measurements were discussed in detail by Lauzon et al. (2009).

2.3. Yield measurements

The storage yield of the fish products, presented as water drip, during chilled (0 °C) and superchilled (−2 and −3.6 °C) storage in the experiment was assessed by comparing the sample weight of the fish before packaging and the weight of the fish in selected packages on each sampling day. The drip on each sampling day was calculated as the percent weight change compared to the initial weight before packaging.

The cooking yield of the loins was estimated by steam cooking three pieces from each group for 12 min at 95–100 °C in a Convostar steam oven (Convotherm, Elektrogeräte GmbH, Eglfing, Germany) on each sampling day. The samples were weighed and cooked on a grid whereby the water extruded from the muscle during cooking could leak away. Samples were allowed to cool down to 25 °C before being weighed again (Mettler Toledo SB 16001 DR, ±0.01 g, Mettler Instruments AB, Greifensee, Switzerland).

2.4. Physicochemical and microbiological analysis

The moisture content was measured by drying 5 g of the sample, mixed with sand, in a ceramic bowl at 103±2 °C for 4 h (ISO, 1999). The salt content of the samples was measured with the Volhard titration method (AOAC 976.18, 2000). The water holding capacity (WHC) of the samples was measured with the centrifugal method described by Eide, Børresen, & Ström (1982). Approximately 2 g of the samples were weighed precisely into a vial and centrifuged (Sorvall RC-5B, Dupoint Company, USA) at 210 g (1300 rpm) at 2–5 °C for 5 min. WHC (%) was calculated as the ratio of water in the sample after centrifugation to water in the sample before centrifugation. TVB-N and TMA was measured as described by Malle & Tao (1987) by steam distillation (Struer TVN distillatory, STRUERS, Copenhagen, Denmark) and titration after extracting the fish muscle with 7.5% aqueous trichloracetic acid (TCA) solution. TMA was measured in TCA extract by adding 20 mL of 35% formaldehyde. TVB-N and TMA measurements were done in duplicate, but all other chemical measurements in triplicate. Results are presented as an average of these measurements with standard deviations. pH measurements were performed with a pH electrode (SE 104 Mettler Toledo GmbH, Greifensee, die Schweiz) connected to a Knick pH meter (Portames 913 pH, Knick, Berlin, Germany). The electrode was immersed in the minced samples at 20 ±2 °C during measurements. Microbiological counts of total viable psychrotropic (TVC) and H_2S-producing bacteria were described by Lauzon et al. (2009).

Physicochemical data are stated as mean values±standard deviation from triplicate measurements, while microbiological results were gained from duplicate measurements. Microbiological data (number of colony forming units, CFU, per gram) is presented in its logarithmic form (log CFU/g) in order to compare values of several orders of magnitude.

2.5. NMR measurements

For the NMR measurements a low field Bruker Minispec mq 20, bench top NMR analyzer, with a working frequency of 20 MHz, was used (Bruker Optics GmbH, Am Silberstreifen D-76287 Rheinstetten, Germany). Test tubes of 18 mm width were used in the study. For all NMR measurements 4 scans, a receiver gain of 70 dB, a delay between repetitive scans of 4 s and 0 dummy scans were used.

Relaxation time measurements were performed on the samples in the experiment. The transverse relaxation time, T_2, was measured with a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (Carr & Purcell, 1954; Meiboom & Gill, 1958), with an interpulse spacing of 500 μs. The number of measuring points was 200 for the CPMG measurements. The longitudinal relaxation time, T_1, was measured with an Inversion Recovery (IR) pulse sequence. The number of IR measuring points was 30 and the duration factor used was 1.322. Samples were minced before being placed in the NMR sample tubes. Four replicates were made from each sample group. The NMR measurements were performed at ambient temperature.

2.6. Data analysis

Data handling and plotting of histograms was performed in Microsoft Excel 2007 (Microsoft Corporation, US).

All NMR data were collected with the Bruker Minispec software. The NMR data were normalized by setting the strongest sampled echo to a value of 100 and the following echo train was successively scaled accordingly. This normalization was performed to allow comparison of samples with different size and water content. The transversal (spin–spin) relaxation data were fitted to a multi-exponential curve by using the low field NMR toolbox for Matlab (The Mathworks Inc. Natric, MA), as described by Pedersen, Bro, and Engelsen (2002). Residual analysis of the exponential fittings indicated that two exponentials were sufficient to describe the system for all samples except the brined samples with high salt concentration from day 13 of storage, but this group showed mono-exponential behavior. A bi-exponential fitting, characteristic of most of the samples, thus resulted in two water populations A_{21} and A_{22}, with corresponding relaxation times T_{21} and T_{22}. The relaxation populations have shown to be proportional to the total amount of sample or water or fat in the muscle (Aursand et al., 2009) Therefore the relative amount of water in each population compared to the total amount of observed water as $A_{21}/(A_{21}+A_{22})$ was calculated. Longitudinal relaxation times (spin-lattice), measured with an Inversion Recovery (IR) pulse sequence, were fitted with a mono-exponential function.

Multivariate analysis on weighted principal components (PCA) was performed on all data obtained in the experiment using Unscrambler® (Version 9.8, CAMO ASA, Trondheim, Norway) to identify similarities and differences between samples. All variables were weighted with the inverse of the standard deviation to correct for different scales of the variables and the data were centered prior to analysis. Partial Least Square Regression (PLS1) models, with Martens Uncertainty Test (Martens & Martens, 2001) were made to identify the significant effect of the experimental variables of salt addition (brine immersion or injection), storage time, storage temperature and air or MA packaging on the quality parameters measured in the study. Separate PLS1 models were made for each quality parameter by setting each quality parameter as the Y-matrix, and the experimental variables as the X-matrix. Similar PLS1 models were made to estimate significant correlations between the observed NMR parameters and measured physicochemical and microbiological parameters. Here the NMR variables were set as the X-matrix, while each individual chemical or microbial parameter was set as the Y-matrix. All multivariate models were fully cross-validated and an uncertainty test was used. All significant levels were set to $P<0.05$.

3. Results and discussion

3.1. Physicochemical and microbiological analysis

The range of the quality parameters of yield, physicochemical, microbial and NMR data obtained during the superchilled storage is given in Tables 1 and 2 respectively. In order to get an overview of the main variation between the samples and the effect of the experimental variables (storage temperature, storage time, salting method (brine immersion/injection) and packaging solution (air/MAP) on the samples, a weighted principal component analysis (PCA) was performed on the quality parameters (drip, cooking yield, moisture content, salt content, WHC, pH, TVB-N, TMA, counts of TVC and H_2S-producing bacteria) as well as the NMR parameters (T_1, A_{21}, T_{21}, A_{22} and T_{22}). Seven principal components described 91% of the variation between the samples. The correlation loadings from the first three PCs can be seen in Fig. 1. The first principal component, PC1, representing 27% of the total variation, mainly described the effect of salting on the samples. The second principal component, PC2, representing 20% of the total variation, described the variation in the samples due to storage conditions (storage temperature and storage time). The third principal component, PC3, representing 14% of the total variation between the samples described the effect of the chosen packaging solution (air or MAP). According to PC1 increased salt content in the loins lead to an increase in WHC, an increase in the faster relaxing component T_{21} and the slower relaxing water population A_{22}, as well as a simultaneously lead to a decrease in muscle drip. This is in accordance with earlier studies (Aursand et al., 2009; Erikson, Veliyulin, Singstad, & Aursand, 2004; Esaiassen et al., 2005, Jepsen, Pedersen, & Engelsen, 1999; Offer & Knight, 1988). In the same manner PC2 showed close correlation between storage time and temperature to increased bacterial growth and production of the chemical spoilage indicators TVB-N and TMA.

To analyze which experimental variables (storage temperature, storage time, salting method (brine immersion/injection) and packaging solution (air/MAP)) had a significant effect on the quality parameters several PLS1 models were made. The experimental variables were set as the X-matrix while each individual measured quality parameter was set as the Y-matrix. The Martens uncertainty test (Martens & Martens, 2001) was applied to assess the significance of each experimental parameter on the quality parameters (Table 3a and b).

Overall data analysis indicated that salt addition and longer storage time had a significant effect on the drip loss of the samples. Drip loss in the low salt brined cod loins stored aerobically was the lowest at −3.6 °C, but may be explained by the fact that the fish stored at −3.6 °C was partly frozen, underestimating the drip at that temperature. Comparison of brined loins with different salt content showed that significantly less drip was observed in high salt than low salt loins, independently of the packaging method. The effects of brining on reduced weight loss of fish products during storage has been investigated in earlier studies for both air-packed (Esaiassen, Dahl, Eilertsen, Gundersen, & Sivertsvik, 2008; Goulas & Kontominas, 2007) and MA-packed (Pastoriza, Sampedra, Herrera, & Cabo, 1998) fish products. MAP loins generally lost more water than their air-stored counterparts, in agreement with Fey & Regenstein, 1982; Layrisse & Matches, 1984; Dalgaard, Gram, & Huss, 1993). According to Table 3b the choice of packaging only significantly affected the drip in the high salted brined samples. Brine injected loins showed a significantly higher drip loss compared to those brined by immersion. It is therefore evident that storage temperatures had no significant effect on the drip loss when all samples were analyzed together, in contrast to storage time and salt content. Brine injection had a significant effect on the drip. This is in agreement with the fact that during brine injection there is an increased risk of puncturing cells with the needles, as well as other destruction of the muscle due to too

Table 1
Range of the quality parameters measured in the study during storage of cod loins processed by different salting methods (brine immersion/injection), salt concentrations (high or low salt conc.) and different packaging solutions (air/MAP) at different storage temperatures.

Sample description	Low/high salt	Storage conditions	Analyzed on storage day [d]	Drip [%]	Cooking yield [%]	Moisture content [%]	Salt content (NaCl) [%]	WHC [%]	pH	TVB-N [mg/100 g sample]	TMA [mg/100 g sample]	TVC [log (CFU/g)]	H_2S-prod. bact [log(CFU/g)]
Raw material		–	0	–	76.8 ± 1.3	81.8 ± 0.4	0.2 ± 0.1	91.1	6.97	14.7 ± 0.7	0.0	3.74	1.80
Brined (NaCl)	Low salt	Air 0 °C	1.6.12.15	0.2–4.9$^+$	70.4–76.6$^+$	82.0–82.4	0.3–0.5	85.4–90.8	6.71–7.03$^+$	10.6–30.3$^+$	0.0–20.0$^+$	3.56–6.78$^+$	0.65–5.24$^+$
		Air − 2 °C	1.6.12.15	0.2–4.5$^+$	71.7–73.0$^+$	81.3–82.2	0.2–0.5	86.2–90.5	6.71–6.91$^+$	11.8–27.9$^+$	0.5–12.6$^+$	3.74–7.08$^+$	1.80–6.18$^+$
		Air − 3.6 °C	1.6.12.15	0.2–2.0$^+$	69.8–77.1$^+$	81.4–82.2	0.2–0.5	81.4–90.4$^-$	6.67–6.87$^+$	12.3–15.9$^+$	0.5–2.6$^+$	3.74–4.76$^+$	1.80–3.30$^+$
		MAP 0 °C	1.6.12.15.21	0.2–9.0$^+$	68.0–74.3$^+$	81.3–82.2	0.2–0.5	80.7–90.4$^-$	6.71–6.85$^+$	13.6–77.7$^+$	0.0–51.7$^+$	4.27–7.54$^+$	1.95–6.65$^+$
		MAP − 2 °C	1.6.12.15.21.28	0.2–6.3$^+$	63.1–77.6$^+$	81.7–82.2	0.3–0.5	81.1–94.5	6.62–7.02$^+$	12.3–60.9$^+$	0.0–30.4$^+$	3.94–6.02$^+$	0.80–5.18$^+$
		MAP − 3.6 °C	1.6.12.15.21.28	0.2–6.6$^+$	66.3–76.6$^+$	81.0–82.4	0.2–0.5	81.0–90.4$^-$	6.61–7.05$^+$	11.6–26.0$^+$	0.0–1.4	3.74–7.45$^+$	1.80–5.62$^+$
	High salt	Air − 2 °C	1.7.13.16	1.3–2.8$^+$	74.5–86.0$^+$	81.5–83.0	1.8–3.2	82.0–99.6	6.57–6.74$^+$	9.6–38.9$^+$	0.0–20.30$^+$	3.48–6.01$^+$	1.80–4.72$^+$
		Air − 3.6 °C	1.7.13.16	2.1–2.8$^+$	74.5–87.15$^+$	75.4–83.2	1.8–7.9	99.4–100.0	6.32–6.62	8.8–12.0$^+$	0.0–1.1	3.30–5.27$^+$	1.80–3.30$^+$
		MAP − 2 °C	1.6.12.15.21.28	0.9–2.0$^+$	69.2–81.8$^+$	80.3–83.5	1.7–4.0	99.1–100.0	6.36–6.57$^-$	9.4–44.9$^+$	0.0–3.7$^+$	4.10–5.01$^+$	3.12–3.69$^+$
		MAP − 3.6 °C	1.6.12.15.21.28	0.8–1.9$^+$	67.4–74.8$^-$	81.0–83.0	1.8–3.6	95.9–99.9	6.28–6.57$^-$	10.0–11.6	0.0–2.0	4.05–4.77$^+$	1.30–3.13$^+$
Brine injected	Low salt	Air 0 °C	1.7.13.16	3.0–7.3$^+$	69.7–83.4$^+$	82.6–83.5	0.4–0.5	87.6–98.1	6.73–7.19$^+$	12.5–68.0$^+$	0.4–53.1$^+$	3.74–7.89$^+$	1.80–7.78$^+$
(NaCl +		Air − 2 °C	1.7.13.16	4.6–7.4$^+$	70.3–74.1$^+$	82.6–83.4	0.4–0.5	85.8–95.3	6.73–7.02$^+$	12.0–38.8$^+$	0.0–19.9$^+$	3.74–7.19$^+$	1.80–7.19$^+$
phosphates)		Air − 3.6 °C	1.7.13.16	2.5–3.0$^+$	71.9–75.9$^+$	81.5–82.6	0.4–0.5	77.8–92.0$^-$	6.72–6.89$^+$	12.8–13.3$^+$	0.0	3.74–4.68$^+$	1.80–3.87$^+$
	High salt	Air − 2 °C	1.7.13.16	1.2–6.6$^+$	74.3–86.1$^+$	82.7–83.9	0.5–0.8	88.8–99.4	6.69–7.20$^+$	12.1–82.1$^+$	0.0–66.5$^+$	3.74–8.00$^+$	1.80–6.80$^+$
		Air − 3.6 °C	1.7.13.16	2.1–5.8$^+$	73.8–77.5$^+$	82.4–83.4	0.7–0.8	77.5–97.8$^-$	6.64–6.81$^+$	12.2–14.3$^+$	0.0–0.6$^+$	3.74–4.95$^+$	1.80–4.21$^+$

$^+$ indicates an increasing trend in a variable with storage time.
$^-$ indicates a decreasing trend in a variable with storage time.

high injection pressure. This possible destruction of the muscle can lead to reduced water holding properties and thus increased drip. This corresponds to the observation that salt content had a significant effect on both drip and WHC in the experiment. It is also possible that water added by brine injection is more susceptible to drip than water already present in the muscle, leading to increased drip in the injected groups.

The cooking yield of the samples was significantly affected by the muscle salt concentration and the choice of packaging, but higher salt concentrations led to higher cooking yield while MAP reduced the cooking yield compared to air storage. The effect of salting was especially evident in the air packaging. No significant difference was found between the brined and brine injected samples, neither at lower nor at higher salt concentrations. No significant effect of storage time on the cooking yield was found, except in the brine injected samples, where a general increase in cooking yield was observed with longer storage time.

The moisture content was significantly influenced by the storage temperature when all samples were analyzed together, but lower moisture content was observed in the samples stored at −3.6 °C than in those stored at higher temperatures. This effect was most evident in the high salt samples (both brined and brine injected). When the brine injected samples and samples grouped after air or MAP packaging were studied separately, no effect of the experimental design variables was found on the moisture content. An effect of added salt on the moisture content was however observed when the low salt and the high salt samples were analyzed separately, independently of the brining method.

WHC was significantly affected by the muscle salt concentration when all samples were analyzed together. As stated earlier, this is in agreement with the fact that by adding salt, the muscle swells. The water retention in the muscle is increased because the water molecules bind more easily to the myosin in the swelling muscle, thus increasing the WHC (Erikson et al., 2004). When the WHC was studied separately in the brined, brine injected, air and MA-packed samples respectively, no effects of the experimental design variables were found. In the low salt group an effect of the storage temperature was however found, where the WHC decreased with lower storage temperature, especially samples stored at −3.6 °C. As mentioned before these samples were partially frozen during the storage. Cell destruction, due to the formation of ice crystals, as well as protein aggregation and destruction result in lowering of the WHC (Love, 1968). No signs of partial freezing or protein damage thereof were found in the superchilled samples at −2 °C, based on the WHC or NMR parameters obtained.

The muscle pH in all samples was influenced by the storage temperature, salt concentrations and choice of packaging. MA packaging generally led to a decrease in the muscle pH, corresponding to 0.2–0.3 units. This is in agreement with Huss (1995) stating that CO_2 is dissolved in the water phase of the MAP fish leading to a pH decrease of 0.2–0.3 units, depending on the CO_2 concentration. Addition of salt had a lowering effect on the pH in agreement with earlier studies on meat (Hamm, 1986; Offer & Knight, 1988) and fish (Aursand et al. (2009) Also, a higher pH was observed with increasing storage time, especially in the air-packed loins, corresponding to the increased growth of H_2S-producing bacteria (mainly *Shewanella putrefaciens*), which is believed to use lactic acid and amino acids as main substrates (Ringø, Stenberg, & Strøm, 1984) leading to a decrease in lactic acid content in the muscle during storage. The chemical spoilage indicator TVB-N was affected by storage time and temperature when all samples were analyzed together, while TMA was only affected by the storage temperature. No significant effect of salting was found on the two parameters except on the TVB-N formation in the MA-packed samples, but the formation of TVB-N increased faster in MAP than in air-stored loins at 0 °C, while TVB-N increased faster in air-packed loins than in MAP loins at −2 °C. The latter is in agreement with the observations of Goulas & Kontominas

Table 2
Range of the NMR parameters obtained in the study during storage of cod loins processed by different salting methods (brine immersion/injection), salt concentrations (high or low salt conc.) and different packaging solutions (air/MAP) at different storage temperatures.

Sample description	Low/high salt	Storage conditions	Analyzed on storage day [d]	T_1 [ms]	T_2 mono [ms]	A_{21} [%]	T_{21} [ms]	T_{22} [ms]
Raw material	–	–	0	682 ± 16		75.9 ± 1.2	40.8 ± 1.0	101 ± 8
Brined (NaCl)	Low salt	Air 0 °C	1,6,12,15	718–779^{+}		72.2–75.4	43.3–44.4^{-}	113–122^{+}
		Air−2 °C	1,6,12,15	688–764		72.2–75.0^{+}	41.3–44.4^{-}	100–120^{+}
		Air −3.6 °C	1,6,12,15	721–751^{+}		72.2–75.1	41.1–44.4^{-}	116–164^{+}
		MAP 0 °C	1,6,12,15,21	699–741		72.2–82.6^{+}	40.4–44.4^{-}	105–118^{-}
		MAP −2 °C	1,6,12,15,21,28	688–731^{-}		72.2–78.9^{+}	38.2–44.4^{-}	118–136^{+}
		MAP −3.6 °C	1,6,12,15,21,28	682–731^{-}		68.9–78.1^{-}	38.5–44.4^{-}	118–182^{+}
	High salt	Air −2 °C[a]	1,7,13,16	787–831	77.3–91.9^{+}	18.5–89.3^{+}	48.7–85.9^{+}	86–105^{+}
		Air −3.6 °C[a]	1,7,13,16	684–801^{-}	74.9–91.7^{+}	22.9–67.1^{+}	48.7–71.9^{+}	86–128^{+}
		MAP −2 °C[a]	1,6,12,15,21,28	774–863^{+}	77.3–91.4^{+}	22.9–67.7^{+}	48.7–82.1^{+}	86–126^{+}
		MAP −3.6 °C[a]	1,6,12,15,21,28	793–801^{-}	77.3–92.9^{+}	22.9–53.5^{+}	48.7–71.99^{+}	86–158^{+}
Brine injected (NaCl + phosphates)	Low salt	Air 0 °C	1,7,13,16	751–777^{+}		66.1–76.0^{-}	45.0–47.3^{+}	87–130^{+}
		Air −2 °C	1,7,13,16	751–824^{+}		66.3–73.0^{-}	44.9–49.3^{+}	95–131^{-}
		Air −3.6 °C	1,7,13,16	718–770^{+}		68.6–74.9^{+}	44.4–45.8	117–195^{+}
	High salt	Air −2 °C	1,7,13,16	780–812^{-}		50.3–78.6^{+}	47.6–54.4^{-}	94–138^{+}
		Air −3.6 °C	1,7,13,16	786–812^{-}		50.3–69.9^{+}	47.9–52.3^{-}	94–196^{+}

$^{+}$ indicates an increasing trend in a variable with storage time.
$^{-}$ indicates a decreasing trend in a variable with storage time.
[a] Bi-exponential behavior was observed in the brined high salt loins until day 13, while mono-exponential behavior was observed loins after day 13.

(2007), who observed faster TVB-N formation in salted, air-stored sea bream products than in MAP products at 4 °C. No increase in TVB-N or TMA was observed in the MAP loins stored at −3.6 °C during the studied storage time, indicating a low bacterial activity at these storage conditions. However, no significant effect of type of packaging was seen on the formation of TVB-N or TMA when all samples were analyzed together. When the ratio between the measured value of TMA and TVB-N (p-ratio) was calculated a very low value was found in the loins stored at −3.6 °C (p-ratio = 0.1–0.2), indicating a very low activity of TMA-producing agents, both bacterial and chemical. At the end of storage the p-ratio in loins stored at 0 °C and −2 °C was lower (0.5–0.7) than in MAP products (0.7–0.8). This could be expected since TMA formation generally occurs faster under low oxygen tension (Dalgaard, Gram & Huss, 1993; Gram & Huss, 1996; Lauzon et al., 2009). The results

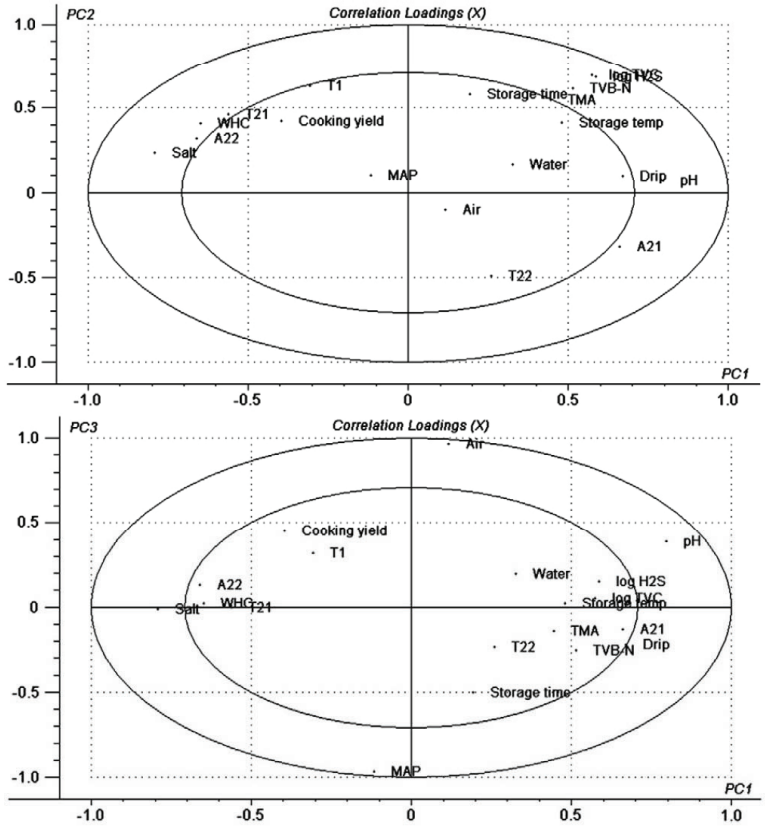

Fig. 1. Correlation loadings from PC1, PC2 and PC3 from principal component analysis (PCA) of all samples and variables in the study. Seven PCs described 91% of the sample variation. PC1, PC2 and PC3 described 27% (effect of salting), 20% (effect of storage conditions) and 14% (effect of air/MAP packaging) respectively.

Table 3
Experimental variables with significant effect on quality parameters in the study.

(a)

Quality parameter	All samples	Brined (HS + LS)	Brine injected (BrInj LS + HS)
Drip loss	NaCl, storage time	Storage temp, NaCl	None
Cooking yield	NaCl, air/MAP	Air/MAP, NaCl	Storage time, NaCl
Moisture content	Storage temp	Storage temp	None
WHC	NaCl	None	None
pH	Air/MAP, NaCl, storage temp	Storage temp, Air/MAP, NaCl	Storage temp
TVB-N	Storage time, Storage temp	None	Storage time
TMA	Storage temp	None	None
TVC	None	Storage time, Storage temp, Air/MAP	storage temp
H_2S-prod. bact.	Storage time, storage temp, air/MAP, NaCl	Storage time, storage temp, air/MAP	Storage temp
T_1	None	None	NaCl
T_2 mono	None	None	NA[a]
A_{21}	None	None	None
T_{21}	None	None	NaCl
A_{22}	None	None	None
T_{22}	None	None	None

(b)

Quality parameter	Low salt (Br LS + BrInj LS)	High salt (Br HS + BrInj HS)	Air	MAP
Drip loss	NaCl	NaCl, air/MAP	NaCl	NaCl
Cooking yield	None	None	NaCl	None
Moisture content	NaCl, air/MAP	NaCl, storage temp	None	None
WHC	Storage temp	NaCl	None	None
pH	Storage time, storage temp, air/MAP	Storage temp, air/MAP, NaCl	Storage time, storage temp, NaCl	NaCl, storage temp
TVB-N	None	None	None	NaCl, storage temp
TMA	None	None	None	None
TVC	Storage time, storage temp	Air/MAP	Storage time, storage temp	NaCl, storage temp
H_2S-prod. bact	Storage time, storage temp, air/MAP	Storage time, storage temp, air/MAP, NaCl	Storage time, storage temp	Storage time, storage temp, NaCl
T_1	NaCl, air/MAP	None	None	None
T_2 mono	NA[a]	None	None	None
A_{21}	NaCl, air/MAP	Air/MAP	None	NaCl, storage temp
T_{21}	NaCl, air/MAP	Air/MAP	None	NaCl
A_{22}	NaCl, air/MAP	Air/MAP	None	NaCl, storage temp
T_{22}	None	Storage temp.	None	Storage temp

Significance was identified by Martens uncertainty test ($p<0.05$).
HS and LS refer to high salt and low salt loins respectively.
[a] NA = Not analyzed.

also showed that TVB-N and TMA formation was higher in brine injected loins than in brine immersed loins.

TVC were not significantly affected by any of the experimental design variables when all samples were analyzed together. A significant effect was found of storage time and temperature when each of the groups of the brined, brine injected, low salt and air-packed samples were analyzed separately. TVC counts in the samples stored at 0 °C and −2 °C were higher in the brine injected low salt samples than in their brined counterparts. The counts were on the other hand similar between the groups at −3.6 °C. In the high salt groups slightly higher counts were found in the brine injected samples than those brined and stored at −2 °C. Earlier studies have shown a positive effect of combining MAP and superchilling on decreased microbial growth in salmon fillets (Sivertsvik, Rosnes, & Kleiberg, 2003) as well as in cod products (Lauzon & Martinsdóttir, 2005; Wang, Tang, & Correia, 2000).

When all samples were analyzed together a significant effect of storage time and temperature, choice of packaging (air or MAP) and salt concentration was found on the growth of H_2S-producing bacteria. When the counts of H_2S-producing bacteria in 0 °C and −2 °C loins were studied significantly higher counts were found in low salt brine injected loins than in the low salt brined loins. This difference was however much smaller at −3.6 °C, but still followed the same pattern. This indicated that muscle brined by injection runs a higher risk of bacterial contamination during salting than muscle salted by brine immersion. Higher H_2S-counts were found in the air-packed high salt brined cod than in the low salt brined cod. In MA-packed loins higher TVC counts were on the other hand observed in the low salt brined samples than in the corresponding high salt samples. It is therefore obvious that MAP and brining had a significant lowering effect on the bacterial growth, especially on the H_2S-producing bacteria. This can possibly be explained by the lower pH values (pH 6.3–6.4) during early storage in these groups, since the growth of *S. putrefaciens* is highly pH sensitive (Gram & Huss, 1996). *S. putrefaciens* and *Photobacterium phosphoreum*, the dominant spoilage bacteria in air-packed and MAP cod loins respectively (Gram & Huss, 1996) are also known to be amongst the main bacteria, causing the reduction of trimethylamine oxide (TMAO) to TMA (Huss, 1995), describing the increase in TMA observed during storage.

3.2. NMR results

The transverse relaxation curves obtained were fitted with a bi-exponential function describing two water populations in most of the cod loins, resulting in a faster relaxation time T_{21} in the range from 38.2 to 85.9 ms and a slower relaxation time T_{22}, in the range of 85.5–196.4 ms, depending on the brining method and storage conditions. Although no quantitative information can be gained from the apparent populations (Belton, 1990) similar populations were associated with the two relaxation times regardless of the handling and storage chosen, except in the high salt brined cod. The size of the apparent water population A_{21} was in the range of 72.2–82.6% in the low salt brined samples and 66.1–77.0% in the low salt brine injected. However, substantial gelation was observed visually in the high salt brined cod groups. The high salt brined samples therefore distinguished themselves from the other samples, but a mono-exponential behavior was observed in these samples after day 13 of storage. This resulted in one large water population with a relaxation time ranging between 77.3 and 92.9 ms, dependent on the storage time and temperature, as well as choice of packaging (Table 2). The results therefore show that the salting method chosen had a large impact on the muscle quality and water distribution within the muscle, especially as the salt concentrations increased.

Several studies have been made on the water distribution of meat and fish, most of them reporting 2 or 3 populations (Aursand, Veliyulin & Erikson, 2008; Aursand et al., 2009; Bertram & Andersen, 2007; Bertram, Meyer, & Andersen, 2009; Erikson et al., 2004). However, Jensen, Guldager, & Jørgensen (2002) found four water populations with the transverse relaxation times 37, 56, 126 and 361 ms for minced cod by using the SLICING method, while Jepsen et al. (1999) found three exponential components of 33.8, 62.4 and 525.7 ms in frozen-thawed

cod by using an interpulse spacing of 500 μs. The measured relaxation times in this study are comparable with the two first relaxation times measured by Jepsen et al. (1999) as well as the two middle populations found by Jensen et al. (2002). Studies in pork have suggested the presence of three water populations at 1–3 ms (T_{2B}) probably corresponding to water closely associated with macromolecules, such as proteins, at 40–80 ms (T_{21}) corresponding to myofibrillar water and at 200–400 ms suggested to correspond to extra-myofibrillar water (Bertram & Andersen, 2007; Bertram et al., 2009). The most restricted population T_{2B} was not detected, since a long interpulse spacing of 500 μs was used in this study. However, the effects of salting and salting method on the muscle structure made exact interpretation of the data difficult. This includes the observed gelling properties observed visually in the high salt brined samples, thus differentiating them from the raw material and other samples in the study.

When the NMR parameters in the brined and brine injected loins on day 1 were compared, a significant difference was only found in the apparent water population distribution of the high salt loins (A_{21}) (Fig. 2). This large difference in the apparent water population A_{21}, associated with the shorter relaxation time (T_{21}), can be explained by the fact that the brined high salt samples showed strong gelling properties. Brining is known to cause proteins to coagulate and form a gel network, in which water can be entrapped. According to Nagashima & Suzuki (1981) most of the water in this gelled network of macromolecules is loosely bound. Fig. 2 also illustrates how longer relaxation times were achieved with added salt concentration, except in the more loosely bound relaxation time T_{22}, in which the opposite was observed, probably due to the gelling observed in this group. The increase in T_{21} with added salt concentrations is on the other hand in agreement with the studies of Aursand et al. (2009), which showed a shift towards longer T_{21} relaxation times and larger T_{21} population with the salt-induced swelling of myofibers in frozen-thawed salmon. The same trend was observed by Erikson et al. (2004) who hypothesized that this increase in water mobility was due to increased protein electrostatic repulsion as the salt content is increased, thus leading to increased myofilament spacing as described by Offer & Trinick (1983).

Table 2 shows that measured longitudinal relaxation times were in the range of 688–764 ms in the low salt brined cod samples, but 684–863 ms in the high salt brined samples when the whole storage time period and the various storage temperatures and packaging solutions were studied. Brine injected samples had on the other hand a longitudinal relaxation time ranging between 718–824 ms in the low salt samples and 780–812 ms in the high salt samples over the studied period. Chang, Hazlewood, & Woessner (1976) considered an increase in T_1 to reflect the loss of water structure (or order) imposed by macromolecules. Longer T_1 relaxation times observed in the brine injected loins therefore indicated that the muscle structure was affected more than by salting the loins by brining, probably due to cell puncturing and destruction during injection. This is in agreement with the increased drip observed in the brine injected loins compared to their brined counterparts.

The multivariate PLS1 models showed no significant effect of the experimental design variables on any of the NMR parameters when all samples were analyzed together (Table 3a). A significant effect of added salt was found when each of the brine injected; low salt and MA-packed samples were analyzed separately. The choice of packaging (air/MAP) showed a significant effect on the NMR variables when the low salt and the high salt samples were analyzed separately. An effect of storage temperature was also observed in the weighted water population distribution (A_{21} and A_{22}) and the longer relaxation time T_{22} in the MA-packed samples. In these samples the water population, relating to the slower relaxing component A_{22}, was larger in the MAP samples at −3.6 °C than at the other temperatures, indicating diffusion of water from the myofibers into the extracellular space, probably due to cellular damage and protein destruction caused by the partial freezing observed in the samples at this temperature. Lambelet, Revevey, Kaab, & Raemy (1995) showed that frozen storage of cod at −10 °C had a significant effect on the transitions related to myosin, as measured by Difference Scanning Calorimetry (DSC). These changes in myosin, which increased

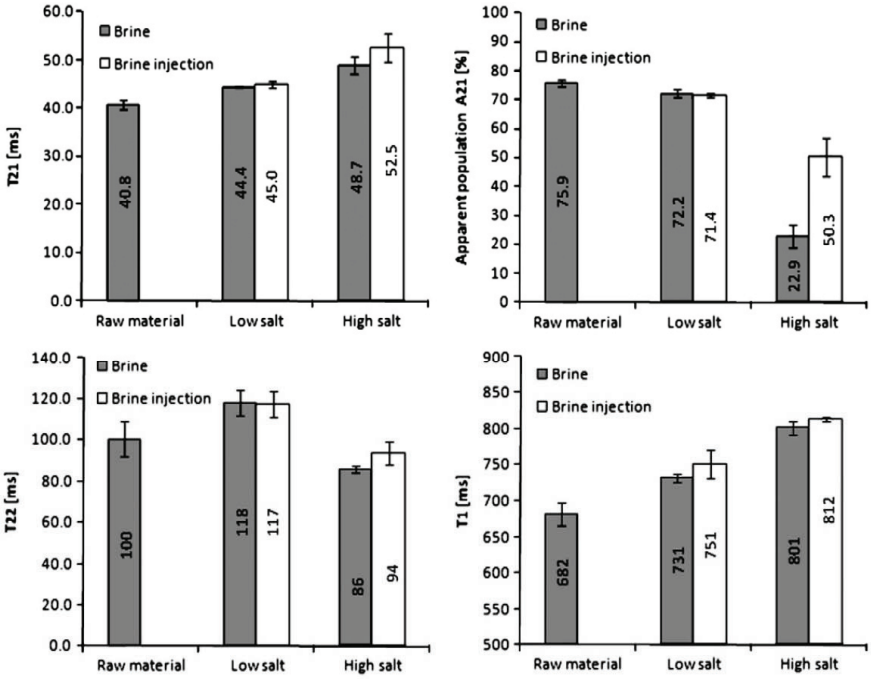

Fig. 2. Transversal (T_2) and longitudinal (T_1) relaxation time results on day 1 of the experiment.

Table 4
NMR parameters with significant correlation to quality parameters in the study.

Quality parameter	All samples	Brined (Br HS + LS)	Brine injected (BrInj LS + HS)	Low salt (Br LS + BrInj LS)	High salt (Br HS + BrInj HS)	Air	MAP
Moisture content	None	None	T_{22}	T_1, T_{21},T_{22}	None	T_1	None
Salt content (NaCl)	A_{21}/A_{22}, T_{21}	T_1, A_{21}/A_{22}	T_{21}, T_{22}	A_{21}/A_{22}	None	None	T_1, T_{21}, T_{22}
WHC	None	T_{21}	T_{21}, T_{22}	None	T_{21}, T_{22}	None	T_1, T_{21}, A_{21}/A_{22}
Drip loss	None	A_{21}/A_{22}	None	None	None	None	T_{21}
Cooking yield	None	None	T_{21}	T_1, T_{21}	None	None	T_1,A_{21}/A_{22}
pH	None	None	A_{21}/A_{22}, T_{22}	T_1, T_{21},T_{22}	None	None	None
H_2S-prod. bacteria	None	None	T_1,T_{21},T_{22}	None	None	T_1	None

No significant correlations were found between the NMR parameters and TVB-N, TMA or TVC respectively.
Significance was identified by Martens uncertainty test ($p<0.05$).
HS and LS refer to high salt and low salt loins respectively.

with storage time, was coupled to longer T_{22} values and decreased WHC, similar to the behavior observed in the samples stored at −3.6 °C in this study. Transitions associated with sarcoplasmic proteins and actin were on the other hand almost unaltered during the frozen storage at −10 °C. This indicates that the partial freezing in the MAP samples stored at −3.6 °C had a significant effect on the myosin structure.

To identify significant correlations between the NMR coefficients and the physicochemical and microbial parameters, similar PLS1 models, as were made for the experimental design variables, were made. The NMR parameters were set as the X-matrix and each physicochemical and microbial parameter as the Y-matrix. When all samples were analyzed together it was seen that only the salt content showed a significant correlation to the apparent water population distribution (A_{21} and A_{22}) and to the faster transversal relaxation time T_{21} (Table 4). When the samples were analyzed in smaller groups more significant correlations between the NMR parameters and other measured parameters were found, especially on the water properties, i.e. drip loss, cooking yield, moisture content and WHC. Increasing WHC was associated with longer T_{21} relaxation times in the brined, brine injected, high salt and MAP stored samples, indicating decreased restriction of the water due to the salting, regardless of salting method chosen. This is in agreement with the fact that the muscle swells when salt is added to the muscle, thus leading to improved WHC as reflected by higher T_{21} values. Jepsen et al. (1999) showed that the WHC can be predicted using low field NMR over a range of 30–90% in cod. As in the study of Jepsen et al. (1999) no significant correlation was found between the water content and the WHC, thus ruling out indirect correlations between the NMR parameters and moisture content through the measured WHC. Direct correlation between the NMR parameters and moisture content resulted in poor fits except in the brine injected samples, the low salt and air-stored samples. Andersen and Rinnan (2002) showed that good correlations to the moisture content can be achieved using the SLICING method to assess the sample amplitudes with the relaxation times. Increased drip loss with storage correlated to a smaller A_{22} found in the brined low salt samples compared to the brined high salt samples. A correlation between the drip loss and the shorter relaxation time T_{21} was also found in the MAP samples, where the T_{21} value reflected the difference in drip according to salt content in the brined samples (Table 3b). Strong correlations between the NMR parameters and muscle pH was also found in the brine injected samples and low salt samples, reflecting the effect of the storage conditions chosen. This correlation between the pH and the NMR parameters is in agreement with the study of Currie, Jordan, & Wolfe (1981) who demonstrated that changes in beef muscle water as measured with by longitudinal relaxation times T_1 were highly sensitive to changes in pH post mortem. Larsson & Tornberg (1988) also showed that T_2 values in the range of 70–180 ms, correlating to perimysial water in porcine meat correlated well with ultimate pH, indicating that pH can affect the pore size distribution within the muscle. Interestingly a significant correlation between the relaxation times and counts of H_2S-producing bacteria in the brine injected (T_1, T_{21} and T_{22}) and air-stored loins (T_1) was also found, reflecting the effect of the storage time and temperature. No significant correlations were found between the NMR parameters and TVB-N, TMA or total viable psychrotrophic counts (TVC).

4. Conclusions

The study confirmed that low field NMR is a valuable tool in the study of water in biological samples such as cod muscle.

Relaxation times were significantly affected by the salt concentration in the cod muscle, but longer relaxation times were observed with higher salt concentrations. Superchilling storage and MAP prolonged the shelf life of the cod loins, while brine injection had a negative effect on the shelf life. Too low temperatures should however be avoided since partial freezing was observed in the samples stored at −3.6 °C. Significant correlation was found between the relaxation times and water properties, such as drip loss, cooking yield, moisture content and WHC, but also to muscle pH and bacterial growth. It is therefore evident that low field NMR can be used to indicate fish quality deterioration where spoilage mechanisms affect the water properties of the muscle.

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Paper III

The effects of pre-salting methods on water distribution and protein denaturation of dry salted and rehydrated cod

A low-field NMR study

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The effects of pre-salting methods on water distribution and protein denaturation of dry salted and rehydrated cod – A low-field NMR study

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ABSTRACT

Low field Nuclear Magnetic Resonance (LF-NMR) relaxation time measurements were used to evaluate the effect of different pre-salting methods (brine injection of salt and/or phosphates followed by brining, solely brining, pickling and kench salting) on the protein denaturation and change in muscle properties during the production steps of dry salted cod fillets followed by rehydration. The NMR relaxation curves were affected by the salting method and represented well the structural differences between the salting methods at each processing step. Significant correlations were observed between the NMR relaxation parameters and all physicochemical quality properties measured, except the cooking yield, when samples from all processing stages were analyzed together. The longitudinal relaxation time T_1, and the faster relaxing transverse relaxation time T_{21} were shown to be especially sensitive to protein denaturation in the fillets. The water distribution indicated that the salting and rehydration processes changed the cells irreversibly. The study indicated that pre-brining by brine injection followed by brining, with low salt concentrations, led to the least protein denaturation during the dry salting and rehydration process.
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1. Introduction

Salting of fish is a traditional preservation method, which has been used for centuries. Dry salted Atlantic cod (*Gadus morhua*) is one of the largest export products from Iceland and Norway. The major markets are in Southern Europe and Latin America, where rehydrated cod is commonly used in bacalao dishes. During the production of dry salted cod the fish is filleted or butterfly split and then heavily salted. Traditionally the salting has been done by various combinations of pickling, brining and/or kench salting. In kench salting the fish is piled into stacks in alternating layers of fish and salt. The fish takes up salt while liquid diffusing from the muscle is allowed to drain away. In pickle salting a similar procedure is performed, but in closed vats. The liquid diffusing from the muscle during salting therefore forms a saturated brine solution as the salt dissolves (van Klaveren and Legrende, 1965). The use of lower salt concentrations has however been shown to lead to increased water holding capacity and higher yields (Offer and Trinick, 1983; Wilding et al., 1986; Slabyj et al., 1987; Barat et al., 2002). In recent years pre-salting by brining, followed by dry salting, has therefore become the most popular method in the production of heavily salted cod products and experiments with pre-salting by a combination of brine injection and immersion have recently been made. For brine pre-salted fish, the fillets are submerged in brine for 1–4 days before being stacked in alternating layers of salt, where the fish is kept for 10–13 days before packaging and export. The fish is rehydrated prior to consumption, but this step is usually performed by the consumer.

Low field Nuclear Magnetic Resonance (LF-NMR) proton relaxation behavior has been widely used to gain further insight into muscle behavior. The relaxation behavior observed corresponds to water and fat molecules in the muscle. However, in lean species such as cod, the fat content can be ignored. In meat and fish muscle at least two water populations can be identified. The interpretation of the relaxation times is however controversial. According to Bertram and Andersen (2007) and Bertram et al. (2009) three water populations were identified in pork muscle; the first with a fast relaxation time, T_{2B}, at 1–3 ms, suggested to correspond to water closely associated with macromolecules, the second relaxation time T_{21} at 40–80 ms, suggested to correspond to myofibrillar water and the slowest relaxation time T_{22} at 200–400 ms, believed to correspond to extra-myofibrillar water. Other studies have suggested that the shorter relaxation time T_{21} relates to water located within organized protein structures and the longer relaxation time T_{22} relates to water in the space between the myofibrils (Bertram et al., 2001; Erikson et al., 2004). The NMR parameters have been shown to correlate well with various physicochemical properties of meat and fish muscle. Andersen and Rinnan (2002) showed that

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good correlations between moisture content and the NMR relaxation times could be obtained using the SLICING method and according to Jepsen et al. (1999) the water holding capacity (WHC) can be predicted using LF-NMR within the range of 30–90%. Bertram et al. (2000) showed that T_2 relaxation times were an efficient method to determine the WHC in pork as well as indicate pH-induced structural changes occurring in the muscle postmortem.

The aim of the present study was to follow the structural changes in cod muscle occurring during dry salting and rehydration, as affected by various pre-salting methods, by means of LF-NMR relaxation time measurements. The NMR parameters obtained were compared to physicochemical measurements of moisture, salt, protein and non-protein nitrogen content of the muscle, as well as WHC and muscle pH as presented by Thorarinsdottir et al. (2010) for validation. The NMR parameters were then compared to electrophoresis (SDS–PAGE) and differential scanning calorimetry (DSC) measurements, as presented by Thorarinsdottir et al. (2011). The aim was also to show the applicability of LF-NMR in optimization of quality control within the dry salting process.

2. Materials and methods

2.1. Experimental design

Wild Atlantic Cod (*G. morhua*) was caught by long-line in February 2006. The fish was bled and gutted on board and stored in tubs on flake ice until processed 3–4 days post catch. The fish was beheaded and filleted at the processing plant. The fillets were divided into five groups of 45–50 fillets and each fillet was identified with a numbered plastic tag. Brine injections of salt (IS) and a combination of salt and phosphates (ISP) were performed in a FOMACO FMG 64/256F (FOMACO Food Machinery Company, Koga, Denmark) injection machine, using an injection pressure of 1.1 bar. The injected fillets were immersed in brine for 2 days (12% salt) in a 1:1 fish-to-brine ratio. Fillets from an additional group were immersed in brine for 2 days without prior injection (brined, Br). The fourth pre-salting method was pickling (Pi), where fillets were covered by alternating layers of fish and salt in closed tubs for 3 days, causing liquid extracted from the muscle due to the strong salting to form a saturated brine. After pre-salting, fillets from all groups were dry salted for 22–23 days at 3–5 °C by stacking them in alternating layers in open tubs. The liquid extracted from the flesh by the salt was allowed to drain away. Cod kench salted for 26 days was held as a reference group (Ke). Commercial coarse salt from Tunisia and the Bahamas were used for pre-salting and dry salting, respectively. A mixture of sodium and potassium pyrophosphates and sodium and potassium tripolyphosphates (Carnal 2110, CFB Budenheim, Budenheim, Germany) was used in the phosphate injected group.

2.2. Low-field NMR measurements

A low field Bruker mq 20 benchtop NMR analyzer (Bruker Optics GmbH, Rheinstetten, Germany) with 20 MHz and 0.47 T magnetic field was used for measurements of proton longitudinal (T_1) and transversal (T_2) relaxation times. Samples were taken from the middle part of the fillet and successively minced and placed in 10 mm sample tubes. Six replicates were made from each sample group and all measurements were performed at 2 °C. The sample compartment was cooled to the desired temperature by pumping compressed air through liquid nitrogen and finally through the sample compartment. The flow and temperature was controlled by a variable temperature control unit (BTV3000, Bruker Optic GmbH, Rheinstetten, Germany). The Receiver Gain (RG) was set to 70 dB, the Receiver Delay (RD) was 4 s, the Number of Scans (NS) was 4 and no dummy shots were used. Longitudinal relaxation times were measured with an Inversion Recovery (IR) pulse sequence measuring 30 points. Transverse relaxation times were measured with a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (Carr and Purcell, 1954; Meiboom and Gill, 1958), with an interpulse spacing τ of 1 ms and the number of collected echoes was 200.

The NMR data was collected with the Bruker Minispec software and successively maximum normalized to allow comparison of samples with different size and water content. This was done by giving the strongest echo signal a value of 100 and scaling the following echo signals accordingly. Transversal relaxation data was fitted to a multi-exponential curve by using the Low-field NMR toolbox for Matlab (The Mathworks Inc. Natric, MA), as described by Pedersen et al. (2002). Residual analysis of the exponential fittings indicated that two exponentials were sufficient to describe the system for all samples. A bi-exponential fitting thus resulted in two water populations A_{21} and A_{22}, with corresponding relaxation times T_{21} and T_{22}. The weighted amount of water in each population compared to the total amount of observed water ($A_{21} + A_{22}$) was calculated. Longitudinal relaxation times (spin–lattice), measured with an Inversion Recovery (IR) pulse sequence, were fitted with a mono-exponential function.

2.3. Physicochemical reference measurements

All fillets were weighed individually before salting as well as after each processing step. The total processing yield was found by comparing the weight after each processing step to the original weight prior to salting. The cooking yield of the rehydrated fillets was estimated by steam cooking middle pieces from three fillets from each group for 12 min at 95–100 °C in a Convostar steam oven (Convotherm, Elektrogeräte GmbH, Eglfing, Germany). The samples were weighed and cooked on a grid whereby the water extruded from the muscle during cooking could leak away. Samples were allowed to cool down to 25 °C before being weighed again (Mettler Toledo SB 16001 DR, ±0.01 g, Mettler Instruments AB, Greifensee, Switzerland).

The moisture content was measured by drying 5 g of minced muscle mixed with sand in a ceramic bowl for 4 h at 103 ± 2 °C. The moisture content was based on the weight differences before and after the drying of three replicates for each sample (ISO-6496, 1999). The salt content was measured with the Volhard Titrino method (AOAC, 2000) and the total protein content was obtained from the total nitrogen content (TN * 6.25) and analyzed with the Kjeldahl method (ISO-5983, 2005). Trichloroacetic acid (TCA)-soluble nitrogen was measured to estimate amounts of non-protein nitrogen by the Kjeldahl method as described in Thorarinsdottir et al. (2004). Water holding capacity (WHC) was determined with the centrifugal method described by Eide et al. (1982). Approximately 2 g of the samples were weighed precisely into a vial and centrifuged (Sorvall RC-5B, Dupoint Company, USA) at 210g (1300 rpm) and temperatures in the range of 2–5 °C for 5 min. The WHC (%) is calculated as the ratio of water in the sample after centrifugation to water in the sample before centrifugation. Results are presented as an average of three measurements. pH measurements were performed with a pH electrode (SE 104 Mettler Toledo GmbH, Greifensee, die Schweiz) connected to a Knick pH meter (Portames 913 pH, Knick, Berlin, Germany). The electrode was immersed in the minced samples at 20 ± 2 °C. The results from the physicochemical and yield measurements were described by Thorarinsdottir et al. (2010).

Measurements and results from electrophoresis (SDS–PAGE) and differential scanning calorimetry (DSC) analysis in the fillets

were described by Thorarinsdottir et al. (2011). Water activity of the muscle was obtained by placing approximately 3 g of minced muscle sample a plastic sample beaker and measured in a Novasina Aw-center (Novasina AG, Axair Ltd., Pfäffiko, Switzerland) at 25 °C. The water activity measurements were done in triplicate.

2.4. Data analysis

Statistical analysis and plotting of figures was performed in Microsoft Excel 2007 (Microsoft Corporation, US). A two tail *t*-test, assuming unequal variances, as used to distinguish between significant NMR-variables within each processing step.

Principal component analysis (PCA) was performed on all data using Unscrambler® (Version 9.8, CAMO ASA, Trondheim, Norway) to identify similarities and differences between samples. Firstly a PCA was made with maximum normalized raw T_2 relaxation data. Secondly a weighted PCA of the fitted NMR parameters and physicochemical quality parameters of the muscle, as presented by Thorarinsdottir et al. (2010), was made. All variables in the latter PCA were weighted with the inverse of the standard deviation to correct for different scales of the variables and the data was centered prior to analysis. Individual Partial Least Square Regression (PLS1) models, with Martens Uncertainty Test (Martens and Martens, 2001) were then made to identify the significant effect of the NMR parameters on each physicochemical quality parameter. The obtained NMR parameters were set as the *X*-matrix while each individual physicochemical quality parameter was set as the *Y*-matrix. All models were fully cross validated. All significant levels were set to $P < 0.05$.

3. Results and discussion

To analyze the overall variation in the NMR T_2 relaxation data a principal component analysis (PCA) was performed on the raw transverse relaxation data. The relaxation curves were maximum normalized to only take into account the water distribution, while ruling out effects from the absolute water content. Fig. 1 shows the PCA results for the normalized transversal relaxation decays of all samples in the study. The results show the difference between the samples at each salting step. The T_2 data reflects similar relaxation behavior in the pre-salted fillets and the rehydrated fillets, with the exception of the pickled fillets after the pre-salting step, which showed similar relaxation behavior as the dry salted fillets.

Bi-exponential fitting of the transverse relaxation data resulted in the observation of two water populations in the range of 23.1–94.2 ms (T_{21}), possibly associated with myofibrillar water and water within the protein structure, and 126–210 ms (T_{22}), possibly associated with extra-myofibrillar water, in the samples at all processing stages (Table 1). This is in agreement with Aursand et al. (2008) who reported T_{21} and T_{22} values for fresh and brined cod in the range of 40–60 ms and 150–400 ms, respectively. Their study did on the other hand not include dry salted cod as presented here, explaining the deviation from their values. A tri-exponential fit was also tried but this gave a general over-fit of the data. In occasional samples a third population with a relaxation time >400 ms was observed. This water is thought to correspond to water extruded from the muscle due to handling, such as mincing of the samples. The relative amount of water in this population was however very small (<0.2%) and this water population was therefore ignored in the overall analysis. It should also be noted that

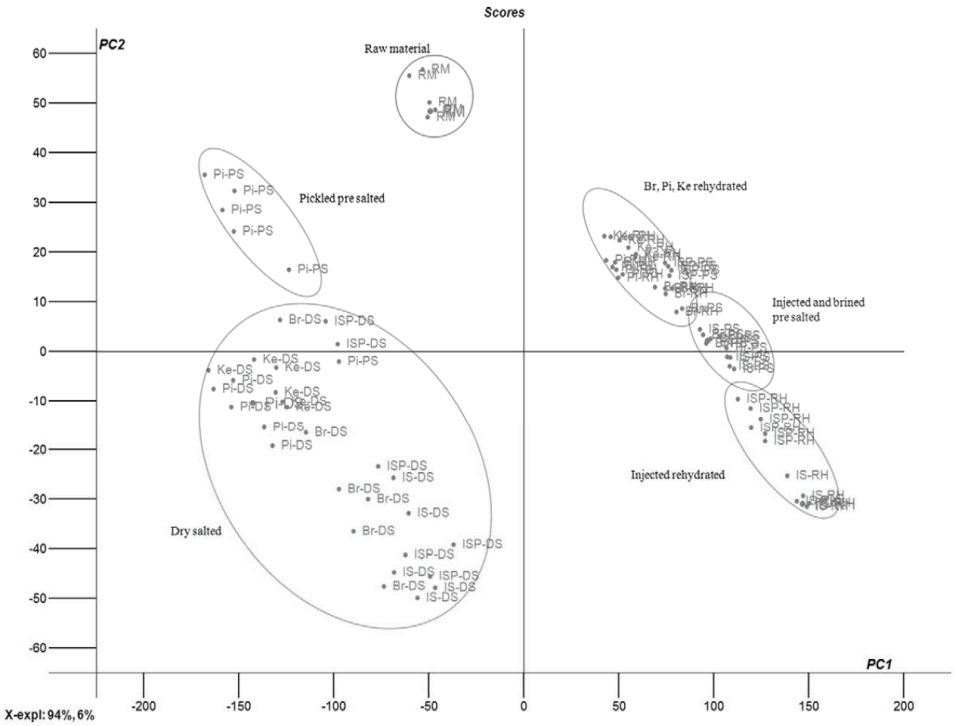

Fig. 1. PCA plot of maximum normalized raw T_2 data of all samples at all salting stages. Explained variances by PC1 and PC2 were 94% and 6%, respectively. RM indicates the raw material, while PS indicates samples after the pre-salting step, DS samples after the dry salting step and RH after the rehydration step. IS refers to salt injected samples, ISP to salt and phosphate injected samples, Br to brined samples, Pi to pickled salted samples and Ke to kench salted samples.

Table 1
LF-NMR relaxation measurement and water activity results in cod muscle throughout various dry salting methods and rehydration.

	Group name	Water activity	T_1 (ms)	A_{21} (%)	T_{21} (ms)	A_{22} (%)	T_{22} (ms)
Raw material	RM	$0.994 \pm 0.008^{*}$	597 ± 5	$94.0 \pm 1.9^{*}$	51.3 ± 1.1	$6.0 \pm 1.9^{*}$	$197 \pm 14^{*}$
Pre-salting	IS–PS	0.952 ± 0.011^{a}	703 ± 24^{a}	90.8 ± 4.6^{a}	90.3 ± 10.2^{ab}	9.2 ± 4.6^{a}	$185 \pm 19^{a*}$
	ISP–PS	0.958 ± 0.007^{a}	688 ± 14^{a}	$95.9 \pm 0.3^{b*}$	86.4 ± 1.1^{a}	$4.1 \pm 0.3^{b*}$	233 ± 8^{b}
	Br–PS	$0.978 \pm 0.017^{a*}$	661 ± 15^{b}	$95.0 \pm 1.5^{b*}$	94.2 ± 1.4^{b}	$5.0 \pm 1.5^{b*}$	$210 \pm 28^{ab*}$
	Pi–PS	0.740 ± 0.010	296 ± 6^{c}	$85.9 \pm 6.0^{a\dagger}$	30.9 ± 2.2^{c}	$14.1 \pm 6.0^{a\dagger}$	138 ± 36^{c}
Dry salting	IS–DS	0.649 ± 0.061^{b}	319 ± 16^{de}	79.1 ± 11.7^{cd}	29.1 ± 0.4^{d}	20.9 ± 11.7^{cd}	239 ± 23^{d}
	ISP–DS	0.685 ± 0.032^{b}	325 ± 6^{d}	75.4 ± 3.3^{c}	29.8 ± 1.4^{d}	24.6 ± 3.3^{c}	$209 \pm 36de^{*}$
	Br–DS	0.720 ± 0.004^{b}	304 ± 12^{e}	75.7 ± 2.7^{c}	26.3 ± 1.3^{e}	24.3 ± 2.7^{c}	$196 \pm 28^{ef*}$
	Pi–DS	0.722 ± 0.007^{b}	271 ± 8^{f}	$81.2 \pm 1.3d^{\dagger}$	23.1 ± 0.5^{f}	$18.8 \pm 1.3d^{\dagger}$	170 ± 14^{fg}
	Ke–DS	0.719 ± 0.008^{b}	270 ± 13^{f}	80.1 ± 1.3^{d}	24.1 ± 0.9^{g}	19.9 ± 1.3^{d}	160 ± 11^{g}
Rehydration	IS–RH	0.973 ± 0.008^{c}	669 ± 7^{g}	73.2 ± 2.1^{e}	93.4 ± 1.0^{h}	26.8 ± 2.1^{e}	$187 \pm 9^{i*}$
	ISP–RH	0.981 ± 0.013^{c}	651 ± 19^{g}	70.6 ± 2.7^{e}	89.7 ± 2.4^{i}	29.4 ± 2.7^{e}	158 ± 7^{j}
	Br–RH	0.989 ± 0.008^{c}	576 ± 9^{h}	66.0 ± 2.1^{f}	78.0 ± 1.3^{j}	34.0 ± 2.1^{f}	126 ± 2^{k}
	Pi–RH	0.984 ± 0.010^{c}	553 ± 8^{i}	78.0 ± 1.0^{g}	70.2 ± 0.7^{k}	22.0 ± 1.0^{g}	152 ± 4^{j}
	Ke–RH	0.988 ± 0.007^{c}	560 ± 6^{i}	73.1 ± 4.2^{e}	72.0 ± 2.4^{k}	26.9 ± 4.2^{e}	128 ± 6^{k}

RM refers to raw material, IS to salt injected pre-salted fillets, ISP to salt and phosphate injected fillets, Br to brined pre-salted fillets, Pi to pickle pre-salted fillets and Ke to kench salted fillets.
Same letters a, b, c, ... in a column indicate insignificant differences within each salting step (pre-salting (PS), dry salting (DS) and rehydration (RH)).
* Indicates insignificant difference compared to the raw material.
† Indicates insignificant difference within each salting method between salting steps.

according to Bertram et al. (2001), mincing of pork muscle leads to no significant changes in the T_2 relaxation times compared to the intact muscle.

The faster relaxing water population A_{21}, corresponding to the faster relaxation time T_{21}, was dominant (66.0–95.9%) in the samples at all processing stages. This is in agreement with Andersen and Rinnan (2002) who observed two water populations with T_2 values of 50 and 94 ms in cod muscle after 5–7 days of ice storage, where the shortest relaxation time was dominating in the head region (loin), while the longer relaxation time was primarily observed in the tail region. This was thought to correspond to smaller muscle cells and fibers in the tail region, affecting the water distribution within the muscle. A significant decrease was observed in the A_{21} population in the salt injected (IS) and the pickled salted cod after the pre-salting step compared to the raw material. These groups also showed a significant lower amount of water in the A_{21} population compared to the salt and phosphate injected (ISP) and brined cod. This can be explained by the higher salt content in the salt injected (6.2%) and pickled (19.2%) compared to the ISP (5.8%) and the brined (5.1%) fillets (Thorarinsdottir et al. (2010), coupled with salting-in effects and swelling of the muscle in the salt concentrations up to approximately 6%, while the higher salt concentrations lead to salting-out effects (Offer and Knight, 1988). After the dry salting step a significant decrease was observed in the A_{21} populations, corresponding to an increase in the more mobile A_{22} population. This is in agreement with Thorarinsdottir (2010) who showed that the intercellular spacing increased, while the cross sectional area of the cells decreased during the dry salting step of fillets. Thorarinsdottir (2010) also reported larger intercellular spaces in brine pre-salted cod compared to the fillets pre-salted by a combination of brine injection and brining. This trend was seen in the A_{22} population in the present study, although the difference after dry salting was only significant between the fillets injected with both salt and phosphates (ISP) and the brined fillets. A significantly higher A_{21} population was observed in the pickled and kench salted fillets than in the other groups after the dry salting step in agreement with higher WHC observed in these groups after dry salting (Thorarinsdottir et al., 2010). In the rehydrated fillets significant difference was found in the A_{21} population between the salt brined and pickled fillets compared to the other treatments. Thorarinsdottir (2010) observed swelling of the fibers at rehydration to a similar cross sectional area as in the raw material, although the shape of the cells seemed irreversibly altered. The general decrease observed in the A_{21} population of the fillets between the processing steps indicates that the water distribution cannot be explained by a simple inter myofibrillar and extra myofibrillar model, but rather with a model associating A_{21} and the corresponding relaxation time T_{21} to water in close association with muscle proteins as suggested by Bertram et al. (2001) and Erikson et al. (2004). Furthermore this decrease in the water population between the processing steps suggests that the salting process has an irreversible effect on the water distribution of the fillet.

The faster relaxation time, T_{21}, was significantly longer in the injected and brined groups after the pre-salting step compared to the raw material, indicating muscle swelling due to the added salt (Offer and Knight, 1988; Offer and Trinick, 1983). The pickled samples gave on the other hand a shorter relaxation time, similar to the relaxation times seen after the dry salting step, which is in agreement with the measured salt content of the samples. After dry salting, all groups had a salt content in the range from 20.4% to 21.4% (Thorarinsdottir et al., 2010), leading to a decrease in the T_{21} relaxation times from 86.4 to 94.2 ms in the brine injected and brined fillets after the pre-salting step to a range of 23.1–29.8 ms in the dry salted fillets. Significantly higher T_{21} relaxation times were observed in the brine injected samples compared to the other pre-salting treatments after the dry salting and rehydration steps, indicating less protein denaturation in the brine injected fillets. This is in agreement with differential scanning calorimetry (DSC) and electrophoresis (SDS–PAGE) results, which indicated less protein aggregation, mainly in the heavy myosin chain, in the brine injected fillets compared to fillets of other treatments, especially the pickled and kench salted groups (Thorarinsdottir et al., 2011). The study also showed that although a lower average salt content was observed in the brined samples than in the brine injected samples, the initial salt concentration of the superficial parts of the fillet was higher during in the brined fillets, giving rise to more myosin aggregation in these fillets, explaining the differences observed in the T_{21} relaxation times between the fillets pre-salted by solely brining or brine injection followed by brining. Aursand et al. (2009) also showed by ^{23}Na magnetic resonance imaging (MRI) that the salt distribution after brining of salmon (*Salmo salar*) was inhomogeneous, with highest salt content near the fillet surface and with a gradual decrease inward in the muscle tissue.

The less protein aggregation observed in the injected fillets thus indicates that a more homogeneous salt distribution can be obtained by brine injection followed by brining than with pre-salting by brining alone.

According to Erikson et al. (2004) the slower relaxing component, T_{22} was not affected by the dry salting and rehydration process. This trend was observed in the salt injected (IS) fillets when the raw material and rehydrated samples were compared. Other groups did on the other hand show significant differences in the T_{22} values between the processing steps, as well as between the treatments within each salting step. In general higher T_{22} values were observed in the brine injected and brined fillets than in the pickled and kench salted fillets, although not significant at all salting stages.

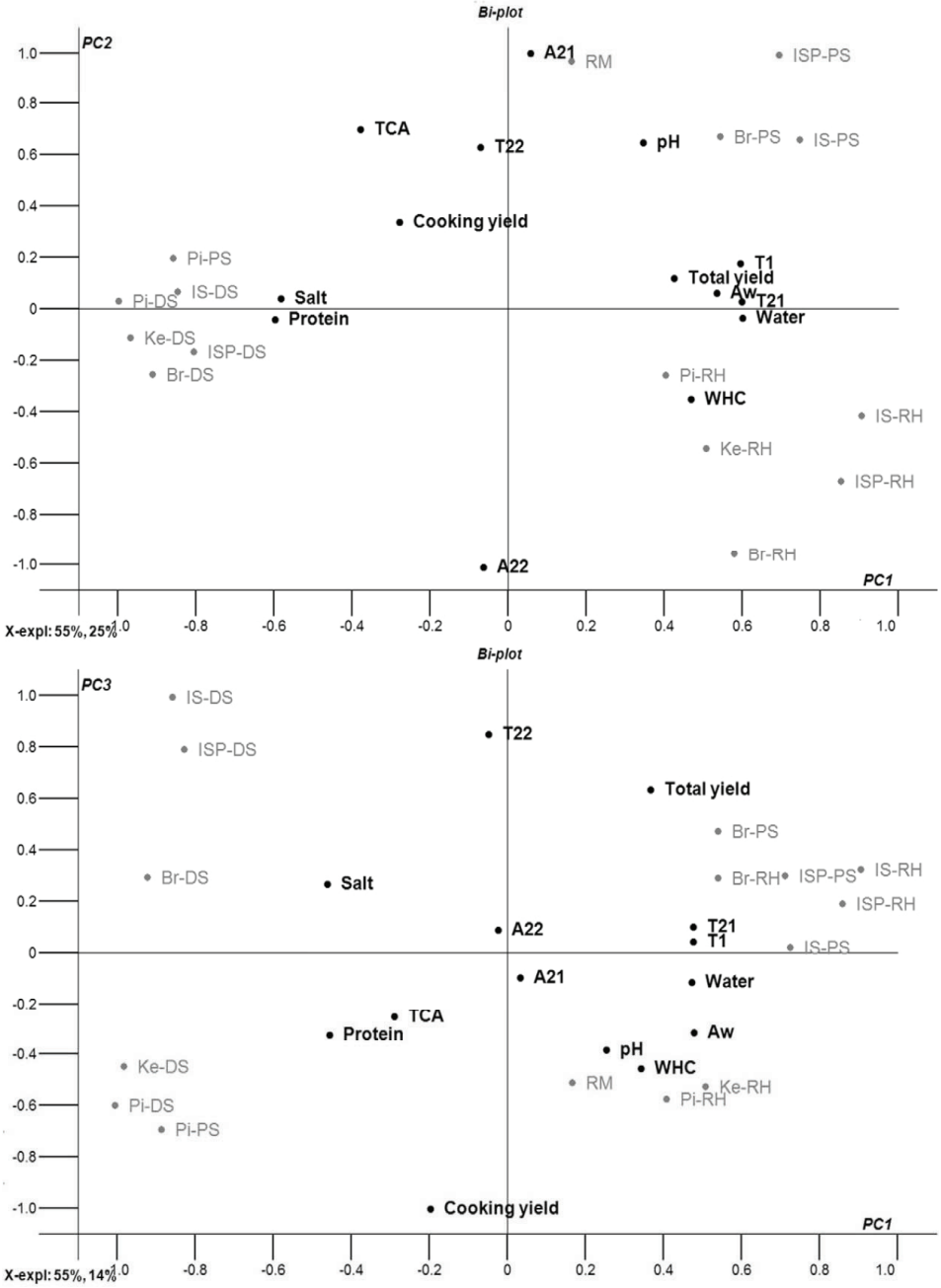

Fig. 2. Weighed PCA bi-plot of scores and loadings for the first three principal components. All samples and chemical and NMR parameters were used. PC1 describes 55% of the variation, PC2 describes 25% and PC3 14%. RM indicates the raw material, while PS indicates samples after the pre-salting step, DS samples after the dry salting step and RH after the rehydration step. IS refers to salt injected samples, ISP to salt and phosphate injected samples, Br to brined samples, Pi to pickled salted samples and Ke to kench salted samples.

Similar behavior, as for the T_{21} relaxation time, was observed for the longitudinal relaxation time, T_1 in all samples. Longer T_1 values were observed in the brine injected and brined fillets after the pre-salting step, compared to the raw material, indicating an alteration of water–macromolecular interaction or restructuring of tissue water (Currie et al., 1981). After the dry salting step, a decrease was observed in the T_1 values, indicating more restriction of the water protons in the dry salted fillets. The rehydration furthermore led to an increase in the T_1 values, coupled with the increased water uptake and retention during the rehydration. A significant difference could be seen in the T_1 values of the brine injected and brined fillets compared to the pickled and kench salted fillets at all processing stages.

NMR is a fast and non-destructive analytical method, which can in many cases replace the more time consuming and often sample destructive physicochemical methods if strong correlations between the NMR parameters and relative physicochemical parameter is found. To analyze the relationship between the NMR relaxation parameters and various physicochemical quality factors, such as moisture, salt, and protein content, and WHC. A weighted PCA of the fitted NMR relaxation parameters and the physicochemical parameters reported by Thorarinsdottir et al. (2010) was made (Fig. 2). This comparison of the NMR data to known and acknowledged methods was done to give a clearer understanding of the dynamics of the salting processes and how NMR can be interpreted with regard to muscle and water behavior. The PCA bi-plots of the first three PCs, describing 94% of the total variation between the samples, showed a similar trend in the sample groupings as observed in the NMR T_2 relaxation PCAs in Fig. 1, but here illustrating an overall view of the physicochemical and NMR relaxation characteristics of the samples. The raw material, rehydrated samples and fillets after the pre-salting stage, except the pickled fillets, were characterized by a high water content, WHC and water activity, coupled with long T_1 and T_{21} relaxation times. On the other hand, dry salted fillets and pickled fillets after the pre-salting step had characteristic higher salt and protein contents. In order to find which NMR parameters represented these physicochemical properties significantly ($P < 0.05$), individual Partial Least Square (PLS1) models were made with the NMR parameters as the *X*-matrix and each physicochemical quality parameter as the *Y*-parameter (Table 2). When all samples were analyzed, significant correlations were found between the various NMR parameters and all physicochemical quality parameters, except the cooking yield. However, all NMR parameters contributed significantly to the estimation of the cooking yield in the rehydrated samples.

Table 2
NMR relaxation parameters with significant effect on physicochemical quality parameters reported by Thorarinsdottir et al. (2010).

Quality parameter	All samples[a]	Pre-salted	Dry salted	Rehydrated
Moisture content	T_1, T_{21}	None	T_1, T_{22}	T_1, T_{21}, T_{22}, A_{21}/A_{22}
Salt content	T_1, T_{21}	None	None	None
Protein content	T_1, T_{21}, T_{22}, A_{21}/A_{22}	None	T_1, T_{21}	T_1, T_{21}, T_{22}, A_{21}/A_{22}
TCA	T_1, T_{21}, A_{21}/A_{22}	None	T_{22}	T_1, T_{21}
WHC	T_1, T_{21}, T_{22}	NA	T_1, T_{21}	None
pH	T_1, A_{21}/A_{22}	None	None	A_{21}/A_{22}
Cooking yield	None	NA	NA	T_1, T_{21}, T_{22}, A_{21}/A_{22}
Total yield	T_1, T_{21}, T_{22}	None	None	T_1, T_{21}
Water activity	T_1, T_{21}, T_{22}	None	T_1, A_{21}/A_{22}	A_{21}/A_{22}

NA = not analyzed.
[a] All samples include raw material and samples from each salting method at each processing step (pre-salted, dry salted and rehydrated).

Surprisingly no significant correlations were found between the NMR parameters and the physicochemical parameters after the pre-salting step. This is probably caused by the smaller variation in the physicochemical properties observed in the fillets after the pre-salting step compared to the physicochemical state of the muscle after the other steps.

The T_1 and T_{21} relaxation times contributed significantly to the prediction of the moisture content when all samples were analyzed together, as well as in the dry salted and rehydrated samples. In addition, T_{22} and the water distribution between the populations A_{21} and A_{22} correlated well to the moisture content in the rehydrated samples. This is in agreement with the observations of Andersen and Rinnan (2002) who found good correlations between moisture content and relaxation times as achieved by using the SLICING method.

Even better correlations were observed for the protein content of the samples, but all NMR parameters gave significant correlation to the protein content when all samples were analyzed together and when the rehydrated samples were analyzed solely. The T_1 and T_{21} turned out to be the most sensitive to protein changes and denaturation of the NMR parameters. All NMR parameters, except T_{22}, showed significant correlation to the non-protein nitrogen content, as estimated by measurements of trichloroacetic acid (TCA)-soluble nitrogen. The non-protein nitrogen generally includes various nitrogenous compounds, such as free amino acids, peptides, trimethylamine oxide (TMAO), and trimethylamine (TMA) (Velankar and Govindan, 1958). According to Gudmundsdottir (1995) the amount of free amino acids and peptides increase, due to proteolytic degradation of proteins, during salting. This degradation of the protein was reflected in the correlation of the non-protein content to the obtained relaxation parameters.

The relaxation times T_1, T_{21} and T_{22} showed significant correlations to the WHC, total yield and the water activity of the muscle, when fillets from all processing stages were analyzed together. Longer relaxation times were observed in the injected and brined pre-salted samples compared to the raw material, indicating higher water retention and swelling of the muscle. According to Erikson et al. (2004) practically all the water was retained in the muscle after the brining step of fresh and frozen-thawed cod. The muscle swelling caused by the salt addition was reflected in longer T_{21} values as also observed in the present study. Significantly shorter T_1 and T_{21} relaxation times were observed in the pickled and kench salted fillets compared to fillets from the other groups at all processing stages, indicating lower water mobility and increased protein denaturation in the pickled and kench salted fillets.

Strong correlations were then observed between the muscle pH during the salting and rehydration process and the T_1 relaxation time and the transversal water distribution (A_{21}/A_{22}), respectively. Currie et al. (1981) demonstrated that the longitudinal relaxation time T_1 was highly sensitive to postmortem pH changes in beef. Larsson and Tornberg (1988) found a correlation between T_{22} values and the ultimate pH, indicating that the pH can affect the pore size distribution within the muscle. Although no strong correlation was found to the T_{22} relaxation time itself was found in this study, the pH affected the water distribution in agreement with Larsson and Tornberg (1988).

4. Conclusions

The study showed that different salting methods, during the production of heavy salted cod, have a significant effect on the distribution of water in the muscle. Injection of salt and phosphates did however not have a significant effect on the water distribution compared to injection of solely salt. The NMR parameters indicated that the proteins were the most denatured in the pickled and kench salted fillets, while the brine injected fillets endured the

least denaturation through the salting and rehydration process. This observation was well in agreement with differential scanning calorimetry (DSC) and electrophoresis results of the fillets. Brine injection followed by brining, with low salt concentrations, is therefore the recommended pre-salting method in the production of dry salted cod, resulting in a product with an even salt concentration through the fillet. When samples of all processing stages were analyzed together strong correlations were found between various NMR parameters to all physicochemical quality parameters measured, except the cooking yield. However all NMR parameters represented the cooking yield in the rehydrated fillets. The longitudinal relaxation time T_1 and the faster transverse relaxation time T_{21} turned out to be highly sensitive to the protein denaturation and physicochemical changes observed in the muscle during the salting processes and can serve as good indicators of these changes during processing. It is therefore clear that NMR serves an important role in the understanding of muscle behavior during processing and can be used further for quality management within the food processing industry, such as in the production of dry salted cod.

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Paper IV

Injection of fish protein solutions to fresh saithe (*Pollachius virens*) fillets studied by low field Nuclear Magnetic Resonance and physicochemical measurements

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Paper V

Shrimp processing assessed by low field Nuclear Magnetic Resonance, Near Infrared spectroscopy and physicochemical measurements – The effect of polyphosphate content and length of pre-brining on shrimp muscle

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