University of Iceland
School of Health Sciences
Faculty of Food Science and Nutrition

Application of additives in chilled and frozen white fish fillets

- Effects on chemical and physicochemical properties –

Master Thesis in Food Science

By
Magnea Gudrun Karlsdottir

Reykjavík, September 2009

A thesis submitted in partial fulfilment of the requirements for the degree of

Master of Science in Food Science

Faculty of Food Science and Nutrition

School of Health Sciences

University of Iceland

Application of additives in chilled and frozen white fish fillets

- Effects on chemical and physicochemical properties –

Magnea Gudrun Karlsdottir

September 2009

Supervisors: Sigurjon Arason M.Sc., Gudjon Thorkelsson M.Sc. and

María Gudjonsdottir M.Sc.

DECLARATION

I hereby declare that this thesis is based on my own observations, is written by me and has not been in part or as a whole submitted before to a higher academic degree.

YFIRLÝSING

Hér með lýsi ég því yfir að ritgerð þessi er byggð á mínum eigin athugunum, er samin af mér og hefur hvorki að hluta né í heild verið lögð fram áður til hærri prófgráðu.

Reykjavík 15. september, 2009

_____

Magnea Guðrún Karlsdóttir

ABSTRACT

The main objective of the project was to study the influence of added ingredients, mainly functional fish proteins, on chemical and physicochemical properties of chilled and frozen whitefish fillets. Fresh saithe and cod fillets and light salted cod fillets were injected with fish protein solutions and compared with salt injected (1.5% and 4%) and untreated control fillets. The fillets were stored at +2°C and -24°C for various times. Evaluations were made on the effects on yield, water holding capacity, chemical composition and T_2 transversal relaxation time. Additions of fish proteins increased the weight gain considerably after injection of the fillets, compared with salt injected fillets, but varied with types of fish protein solutions. Adding fish protein solutions into cod fillets before chilled or frozen storage increased the storage yield and reduced drip loss of the fillets, compared with control and salt injected fillets. Addition of fish protein hydrolysate (FPH) and homogenized fish proteins (HFP) were particularly effective. These protein solutions showed also considerable improvement with regard to the total yield of the cod fillets. Addition of fish proteins and/or salt into fillets showed less effect on the water holding capacity of the muscle than expected. The most promising fish protein solutions to increase water holding capacity was the FPH solution. In summary, injection of protein solutions into cod and saithe fillets is an effective means to improve or stabilize the weight and WHC of the fillets, but more optimisation is needed with regards to different raw materials. The cod fillets showed better results than the saithe fillets. The saithe fillets seem to be more sensitive for this kind of treatment (injection and freezing) than cod fillets, but gaping is well known problem for saithe fillets. Addition of fish protein solutions into fish fillets is an option that is worth to take a look at.

ÁGRIP

Megin markmið verkefnisins var að rannsaka íblöndun hjálparefna, þá sér í lagi fisk próteina, og áhrif þeirra á efna- og eðliseiginleika kældra og frystra flaka. Fersk flök af ufsa og þorski og léttsöltuð flök af þorski voru sprautuð með nokkrum próteinblöndum og borin saman við ómeðhöndluð flök og flök sem voru sprautusöltuð (1.5% og 4%). Flökin voru síðan geymd við +2°C og -24°C í mislangan tíma. Þeir þættir sem voru skoðaðir voru nýting, vatnsheldni, efna samsetning og T_2 transversal relaxation tímar. Íblöndun próteinanna jók þyngdarupptöku við sprautun samanborið við saltsprautuð flök, en þó mismikið eftir próteintegund. Viðbættu próteinin höfðu einnig þau áhrif að nýting eftir geymslu jókst verulega og magn drips lækkaði samanborið við ómeðhöndluð og sprautusöltuð flök. Þau prótein sem höfðu hvað mest áhrif voru vatnsrofin fiskprótein (FPH) og himnusprengd fiskprótein (HFP), en þau gáfu einnig tiltölulega betri heildarnýtingu hjá þorsk flökunum. Íblöndun með próteinum og/eða salti hafði aftur á móti lítil áhrif á vatnheldni flakanna, en það var búist við því að vatnsheldnin yrði betri samanborið við ómeðhöndluð flök. Sú blanda sem hafði jákvæðustu áhrif á vatnsheldnina var FPH. Íblöndun á próteinum í ufsa og þorskflök hefur í heildina á litið jákvæð áhrif við að bæta stöðugleika og gæði flakanna, en þörf er á að þróa og besta íblöndunaraðferðirnar og hvaða íblöndunarefni er notað með tillit til hráefnisins. Þorskflökin sýndu betri niðurstöður samanborið við ufsaflökin. Ufsaflökin virðast vera mun viðkvæmari fyrir innsprautun og frystingu heldur en þorskflök, en los er þekkt vandamál hjá flökum úr ufsa. Íblöndun fiskpróteina er kostur sem vert er að skoða nánar með það að markmiði að auka nýtingu og verðmæti sjávarafurða.

ACKNOWLEDGEMENTS

This study was conducted at Matís ohf in Reykjavík and Iceprotein ehf in Sauðárkrókur, Iceland. Matís ohf, AVS R&D Fund of Ministry of Fisheries in Iceland (R 011-08), Technology development Fund, RANNIS – Icelandic Centre for Research (071321007) and Nordisk Innovation Center (NICe) (04252) funded this project.

I would like to thank my supervisors, Sigurjon Arason and committee members Gudjon Thorkelsson and María Gudjonsdottir for their knowledge and guidance through this study. I will also like to send my sincere thanks to Kristín A. Thorarinsdottir, Irek Klonowski and Sigurdur Hauksson for all their help and guidance. I also send my gratitude to the staff of the Chemical lab of the Matis laboratories and to the sensory panellists of Matis for their assistances.

At last I want to thank my lovely family for all the support and patience throughout my studies.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

A_1	Water population located intra-cellular
A_2	Water population located extra-cellular
CPMG	Carr-Purcell-Meiboom-Gill pulse sequence
DS	Number of dummy shots (echoes) between echo sampling
FPH	Fish protein hydrolysate
FPI	Fish protein isolate
FPS	Fish protein solution
HFP	Homogenized fish protein
LF NMR	Low Field Nuclear Magnetic Resonance
N	Number of points to be collected
NMR	Nuclear Magnetic Resonance
NS	Number of scans for signal averaging
PCA	Principal component analysis
QDA	Quantitive Descriptive Analysis
RD	Recycle Delay (s)
RG	Receiver Gain (dB)
T	Time (s)
T_2	Transverse relaxation time (s)
T_{21}	Shorter transverse relaxation time (s)
T_{22}	Longer transverse relaxation time (s)
WHC	Water Holding Capacity (%)
T	Interpulse spacing (s)

1 INTRODUCTION

In 2006, more than 110 million tonnes (77%) of the world fish productions was used for human consumption (Fao 2008). About 57 million tonnes were used for manufacturing products for direct human consumption. Up to 50-70% of the fish may end up as by-products as the yield in filleting operation is from 30-50% (Kristbergsson & Arason 2006). About 6 million tonnes of trimmings and by-products from fish processing are processed into fish meal and the rest is used in fish silage or discarded. Significant additional nutritional, economic and environmental value can be obtained by increasing the yield of raw material in fish filleting operation.

Fish fillets are very popular products that have been dominating the world fish market. The most important gadoids species, often referred to as round fish, include cod, haddock, saithe, and blue whiting, with cod being by far the most important, from an economical standpoint. Cod is either sold as frozen, salted, or fresh on ice. Although frozen fish only accounts for about 38% of the export volume, it represents, 50% of the value of exported fish products in Iceland (Kristbergsson & Arason 2006).

In recent years, the fish industry has placed emphasis on utilizing all the catch and as economical as possible. Most of the trimmings from filleting processing are utilized for mince production, e.g. backbones, flaps etc. It is common in the meat, poultry and fish industry to add up to 12% brine to modify both fresh products and further processed products. This is done to improve quality, firmness and juiciness and to increase yield. The brine is typically made up of water, salt, phosphates and sometimes other functional ingredients like whey and soy proteins (Xiong 2005; Thorkelsson *et al.* 2008). The use of functional proteins as additives in food products has increased over the last years. It is well established that addition of functional proteins can increase water- and fat binding properties of the products and improve texture and stability. Soy proteins have been used in the food industry to improve water binding and nutritional value. It is therefore of great interest to utilize fish protein as additive to increase quality and value of fish products.

The utilization of fish proteins as additives is promising with regard to improved yield of fish products. One comparison study where soy protein and fish proteins were injected into cod fillets, indicated that fish protein can have more impact to improve water holding capacity than soy protein (Thorarinsdottir *et al.* 2004). Protein addition can be carried out with multi-needle injection, but the pre-treatment can on the other hand vary. Processes have been developed where fish trimmings are reduced to micron sized particles and incorporated into traditional brines to create a homogenous suspension. Fish proteins products, which have undergone different isolation methods, are also commercial available as concentrated or dried products. The interest is increasing among the manufacturers to use fish proteins, but with difference methods, to improve yield and quality of the products.

2 LITERATURE REVIEW

2.1 The fish

2.1.1 Cod

Atlantic cod, *Gadus morhua*, is a well-known demersal fish belonging to the family Gadidae (Wikipedia 2009b). Cod occurs throughout the boreal region of the North Atlantic: in the west form North-Carolina to Labrador, around Iceland and Greenland, and in the Northeast from the Bay of Biscay up to Svalbard and Novaya Zemlya. In the North Sea, cod may be found from shallow coastal waters to the shelf edge (200 m depth) and even beyond. The cod can grow to two meter in length and weight up to 96 kg. It can live for 25 years and sexual maturity is generally attained between ages 2 to 4, but can be as late as 8 years in the northeast Arctic (Ices 2009a). Cod, growing up around south and west coast of Iceland, are mainly topical all their life. Spawning begins in March by the south coast and is over in the beginning of May. During spawning, the cod prefers sea temperatures of 5-7°C at 50-150 m depth. Spawning occurs close to the bottom or in mid water. The main feed of the cod is capelin, but he also eats shrimp and other marine animals (Jónsson 1992).

Since time immemorial, the cod has been one of the most important commercial fish species of the North Atlantic, and a crucial factor for the economy and in the politics of Iceland, Norway, Spain and Newfoundland. Originally fished by hook and line, cod is now mainly targeted by demersal trawl and gill nets, although it may be caught in virtually all demersal and pelagic fishing gears. Even when other species are the main target, a by-catch of cod is difficult to avoid. The total international catch from the whole North Atlantic peaked at about 4 million tonnes in 1968. In recent years cod stocks have declined everywhere and catches have been reduced to less than 1 million tonnes (Ices 2009b). The "Atlantic Cod" is labelled VU (vulnerable) on the IUCN (International Union for Conservation of Nature) red list of threatened species (Wikipedia 2009b).

2.1.2 Saithe

Saithe (*Pollachius virens*) belongs also to the gadoid family of cod-like fishes. Saithe is a semi-pelagic North Atlantic species that occupies the deeper waters over the shelf edge and beyond and, although frequently taken in bottom trawls, may form dense layers in mid water. The saithe are distributed in the western Atlantic from North Carolina to southwest Greenland, and in the eastern Atlantic from the Bay of Biscay to Iceland, Spitzbergen and the Barents Sea. (Ices 2009b). Around Iceland, saithe are mainly located in the warm sea off the S- and SW-coast.

The adult saithe shoals during the winter time for spawning, starting late January and ending in the middle of March. The saithe becomes sexually mature at the age of 4-7 and are by that time 60-80 cm long. The saithe feed is quite variable according to its size and area. Mature fish eats mostly krill, fry, capelin, herring and the bigger fish eats squid (Jónsson 1992).

2.1.3 Material diversity

It is well recognized that the composition of the fish varies with many factors such as species, sex, size, stage of sexual maturity, season and location of catching and onboard handling procedures (Botta *et al.* 1987a). Fresh cod-like fish is about 78-83% water; 16.5-21.7% protein and 0.1-0.8% fat (Sikorski 1995). Water-soluble materials are approximately 15-16% of the muscle dry matter, and are composed of e.g. amino acids, water soluble vitamins, minerals and nitrogen compounds (Dambergs 1964). Changes in water-, protein- and fat content caused by spawning and nutritional condition have great impact on the fish texture. During the summertime the fish regenerate its fat supplies and in the fall (October-November) the proportion of protein is maximised and the proportion of water-soluble material minimised (Dambergs 1964). Therefore, the fish condition is good in the fall and the muscles are firm, but during spawning or when feed accessibility is low the muscle is soft and watery with low fat content (Raversu & Krzynowek 1991). Botta *et al.* (1987b) showed that season significantly affected carbohydrate and protein content and method of catching (gillnet, handline, longline and trap) affected significantly caloric, moisture and protein content (Botta *et al.* 1987b). Thus, both time of season

and method of catching were important factors affecting the composition of Atlantic cod. Season of catch, method and location of catching has therefore great effects on yield and quality of fish products.

2.2 The fish muscle

There is some difference between the muscle function of fish and mammals. The fish body is supported by water and does therefore not require strong connective tissues to maintain and support the muscle. The fish lives in cold environment and therefore the fish proteins have properties different from warm blood animals. The structural arrangement is also different because of other kind of movements of the fish. The muscles of the tail and trunk consist of a series of muscle blocks called myotomes (Figure 2.1). The myotomes usually resemble a sideways letter "W". A connective tissue called myosepta separates the myotomes. A horizontal septum separates the myotomes into dorsal (top) myotomes and ventral (bottom) myotomes. (Seaworld 2007).

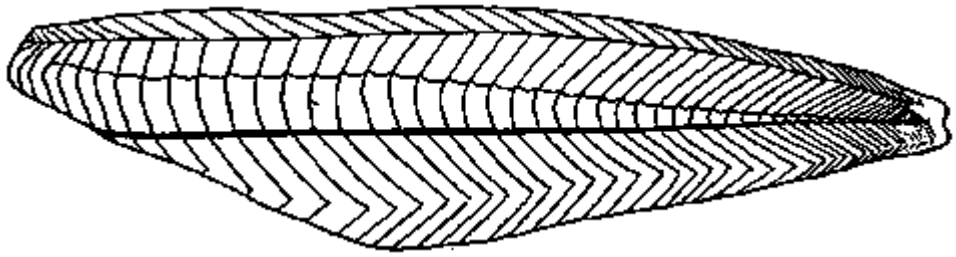

Figure 2.1. Muscle structure of fish (Murray & Burt 2001).

The myotomes are composed of long cylindrical cells named muscle fibres or myofibers (~50 µm in diameter). They form layers that lay parallel to the length of the fish and are bounded together and to the skeleton with connective tissues. Each fiber is filled with long cylindrical filamentous bundles called myofibrils which consist of mainly two different proteins, myosin and actin. A pattern of dark (A bands) and light (I bands) bands is caused by an ordered arrangement of specific protein filaments in repetitive structural units along the myofibril. Dark line, called the Z line, is located in the centre of each of the I bands. Each structural unit is the part of the fibril between two Z-lines and is called sarcomere (Figure 2.2), and it is

the basic contractile unit of the muscle. It contains two major filaments, one thick which is primarily composed of myosin molecules and one, composed of actin, troponin and tropomyosin molecules. The thin filaments do not overlap the thick filaments in the centre of the A band, this part called H band (a lighter zone). At the centre of the H band is a darker line that is termed the M line. The thin filaments extend outwards from the Z line but the thick filaments, extend from the centre of the sarcomere (M-line) towards the Z line. Those interact in muscle contraction and have a certain overlap in relaxed muscle. Cross-bridges are formed between myosin and actin filaments by binding of myosin heads to the actin during muscle contraction. Through ATP binding and hydrolysis, the myosin drives repeated cycles of interaction between the myosin heads and actin. The myosin heads binds to new sides, sliding the actin filament towards the M-line.

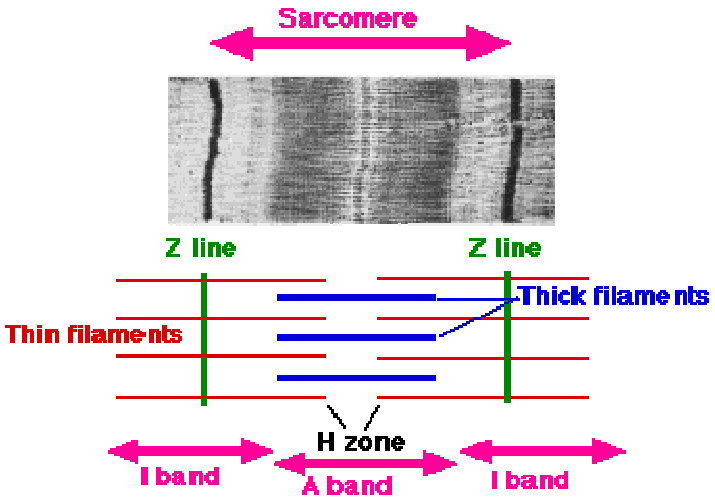

Figure 2.2. The structural unit, called a sarcomere (basic contractile unit) (Wikipedia 2009d).

2.3 Fish proteins

Proteins are one of the main constituent of fish and other marine species. The amount of protein in fishes are ~90% of the dry matter (16-20% of the total mass), but the protein proportion are similar to lean meat and fivefold more compared to dairy proteins. The amount of protein in fish are depended on species, feed availability, sexual maturity, spawning, season of catching and processing methods (Thorkelsson & Gunnlaugsdóttir 2005). The protein combinations in the fish muscle are also variable due to muscle type. Fish have three major types of muscle: smooth

(involuntary), cardiac (heart), and striated (skeletal) which are dominating in the majority of the fish.

All proteins, including those from fish, are chains of amino acids linked together to make one long molecule (Voet & Voet 2004). There are about twenty types of amino acids and certain of them are essential in the human diet for the maintenance of good health.

Muscle proteins can be dividing in to three groups based on their solubility in aqueous solution, but they are: Myofibrillar protein, Sarcoplasmic protein and Stromal protein.

2.3.1 Myofibrillar proteins

Myofibrillar proteins account for 65-75% of the total muscle protein and are the principal structural and functional components of muscle-based foods. They are responsible for muscle contraction in live fish and are also important for the proteins physiological properties in food systems (stabilisation of emulsions, binding of water and lipids and formation of gel structure) (Lanier 1986). However, in order for the myofibrillar proteins to exhibit desirable processing properties effectively a relatively high salt concentration (1.8-3.5% sodium chloride) is required. The high salt is necessary for solubilisation or partial extraction of the myofibrillar proteins (Thorkelsson 2007). About twenty different types of myofibrillar proteins are known. Actin, myosin, tropomyosin, troponin and actinin are all myofibrillar proteins.

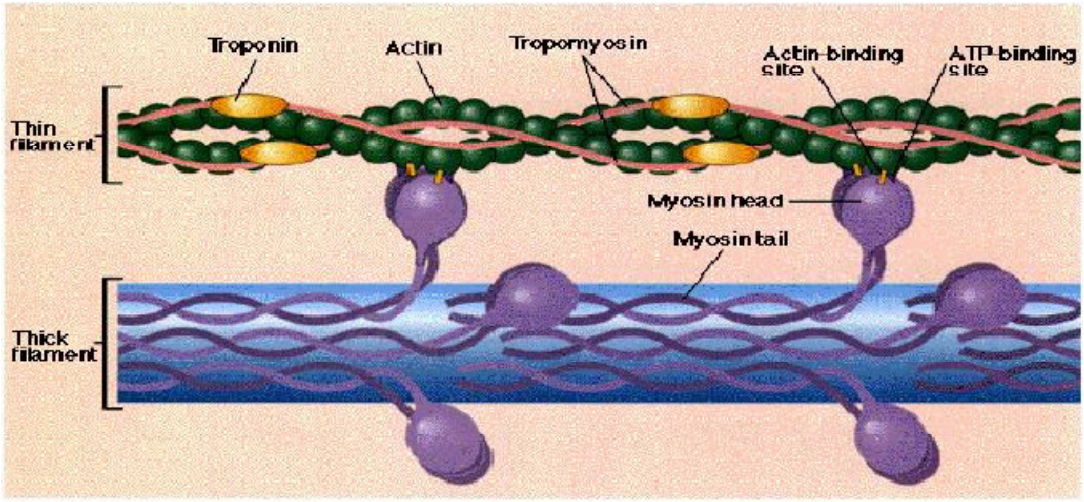

Figure 2.3. Thick (myosin) and thin (actin) filaments, the main components of the sarcomere. (http://www.sci.sdsu.edu/Faculty/Paul.Paolini/ppp/lecture18, as cited by Thorkelsson, 2007)

Myosin is the major component of the thick filaments. It has a high content of glutamic and aspartic acids and of dibasic amino acids, is highly charged and has some affinity for calcium and magnesium ions. Myosin comprises about the 50-60% of the myofibrillar contractile proteins. The myosin molecule is very long (140 x 2 nm) and is an elongated protein molecule with a molecular weight of about 240 kDa. It is made of two big units (heavy chains) and four smaller units (light chains). Each heavy chain consists of a globular head region and a long α-helix tail (Belitz *et al.* 2004). The α-helix tails twist around each other in a coiled-coil structure to form a dimer, and remain together because of non-covalent bonds (An *et al.* 1996). The head is responsible for the ATPase activity and the ability to interact with actin. Each heavy chain consists of approximately 2000 amino acids and forms together the head- and tail regions. The four light chains, two on each head, associate with the neck of each head region to from the complete myosin II molecule (Wikipedia 2009c). 400 myosin molecules from thick filament where the tails twine and the heads turn out. The head region, that the heavy chain forms have ATPase activity (Belitz *et al.* 2004). The SH1 (Cys-707) and SH2 (Cys-697) groups are the two most reactive cysteines on the S1 (subfragment 1) myosin head. They are located on the opposite ends of a short α-helix in the catalytic domain of the myosin head and are separated from one another (Figure 2.4). This helix is believed to play a key role in the conformational changes that occur in the myosin head during the force generation coupled to ATP hydrolysis (Bobkova *et al.* 1999).

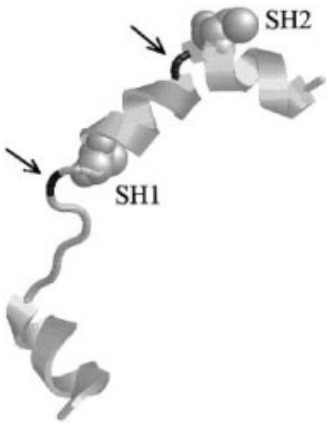

Figure 2.4. Representation of the SH1-SH2 helix based on the crystal structure of myosin subfragment 1 (Bobkova *et al.* 1999).

Actin is the major component of the thin filament and it comprises about 15-30% of the myofibrillar protein of the muscle. The monomeric form of the protein is a globular molecule (G-actin). In the presence of ATP and magnesium or neutral salt, it forms a long double helical structure, termed fibrous actin (F-actin) (Wikipedia 2009a). Troponin and tropomyosin are two other major components of the thin filaments.

2.3.2 Sarcoplasmic proteins

The sarcoplasmic protein account for 20-30% of total protein in fish muscle (Thorkelsson 2007). They contain at least 50-100 different proteins like myogen, myoalbumin and myoglobulin. They have various functions in live fishes, e.g. as enzymes, control osmosis, as "antifreezer" and serve key roles in several biochemical reactions. They are often termed "soluble protein" because they are easily soluble in weak salt solutions (ionic strength ~0.06). They can have direct or indirect effects on the quality of fresh and processed fish products (i.e. colour, taste, texture and nutritional value). Sarcoplasmic proteins do not form gels and have very low water holding capacity, and they can have negative influence on myofibrillar protein gel formation (disturb myosin cross-links) (Thorkelsson 2007).

2.3.3 Stromal protein

Stromal proteins account for 2-3% of the total protein in fish. They are insoluble proteins, such as titin and nebulin, but the main components are collagen (connective tissue) and elastin. They are partial soluble in salt solutions but have low emulsifying and binding properties. Titin is a long, rather insoluble, high molecular weight protein, which composes 8-10% of the total amount. It holds thick filaments in lateral register and regulates elasticity and stiffness of the muscle. Nebulin constitutes about 3-4% of the myofibrillar proteins. It is a long, very insoluble molecule and it is thought to sustain stability of the thin filament.

The connective tissue consists of various fibres, several different cell types, and amorphous ground substances (carbohydrates, proteins and lipids), but collagen is the principal component. The amount is about 3% in cod-like species. The collagen of fish is about ten times less than of red meats and the hydroxyproline is also less in the muscle and the skin of the fish. The fish collagen also shows a wider range of composition. Seasonal changes occur in the connective tissue; it thickens during the periods of sexual maturation and becomes thinner during the intensive-feeding season. It is generally more easily solubilised than those of mammals are and normal cooking processes destroy it. Due to that, the connective tissue in fish is relatively unimportant in the consideration of textural properties of fish as food (Hultin 1976).

2.3.4 Protein denaturation

A great proportion of utilized fish are cold adjusted or poikilothermic. Poikilothermic properties of fish protein make them more sensitive towards heat and consequently higher tendency to denaturate with increased temperature (Kristinsson & Rasco 2000a). In addition to temperature (heating/freezing), there are many other factors that actuate protein denaturation e.g. mechanical treatment, pressure and radiation (Guðmundsdóttir 2005). Protein denaturation results e.g. in lower solubility, changes of water binding properties, increased viscosity, interruption of S-S bounds and therefore altered taste and lower nutritional value. The protein-water binding and water holding capacity has a great impact on the food texture. Increased temperature denaturates proteins, causes damage of collagen and the structure of the

fish muscle. Increased temperature leads therefore to lower solubility and less water holding capacity. Myofibrillar protein from cold sea fishes has more tendencies to denaturate compared with protein from warm sea fishes. Acidity (pH) also effects the T value (the temperature needed to denaturate 50% of the protein) of myofibrillar proteins and has been recorded 29-35°C at pH 7 and 11-27°C at pH 5.5 (Kristinsson & Rasco 2000b).

Myofibrillar protein from fish has been much studied. Park and Lanier (1989) studied the behaviour of myosin and actin from tilapia during surimi processing. Their results indicated that the denaturation temperature of myosin is relatively stable at 58.7±0.5°C during reduction and later washing before surimi production (Park & Lanier 1989). On the other hand, the stability of actin decreased at same conditions. It is also known that myosin is more sensitive towards denaturation during frozen storage and dehydration (Hastings *et al.* 1985).
Thorarinsdottir *et al.* (2002) studied the effects of salt-curing, drying and rehydration on muscle proteins in cod during the processing of heavily salted cod or "bacalhau". The salting process significantly decreased the heat stability of both myosin and actin. The protein denatured at lower temperatures and with less energy input. The conformational stability of myosin and actin was less than in the fresh material. The results showed also that the myosin heavy chain (MHC) was cleaved into smaller sub fragments in the salting process with the two heavy meromyosin fractions (HMM S1 and S2) and the light meromyosin (LLM) fraction being the most abundant. Actin was less affected than myosin (Thorarinsdottir *et al.* 2002).

2.3.5 Protein solubility

The solubility of proteins is very important property for the food industry. Solubility is equilibrium between protein-protein and protein-solvent bonds, where hydrophobic and ionic effects are in equilibrium. Protein solubility is variable and is influenced by the number of polar and apolar groups and their arrangement along the molecule. Generally, proteins are soluble only in strongly polar solvents such as water (H_2O), glycerol ($C_3H_5(OH)_3$), formamide (CH_3NO), dimethylformamide (C_3H_7NO) or formic acid (HCOOH). In less polar solvent such as ethanol, proteins

are rarely noticeably soluble. The solubility in water is dependent on pH and salt concentration (Belitz *et al.* 2004).

The solubility of myosin and actin in fish increase with increased salt concentration and reach it maximum at 5% concentration (ionic strength of 0.8). The solubility of these proteins then increases again if the salt and the soluble protein (sarcoplasmic protein) are removed and are fully soluble in pure water (Pétursson 2006).

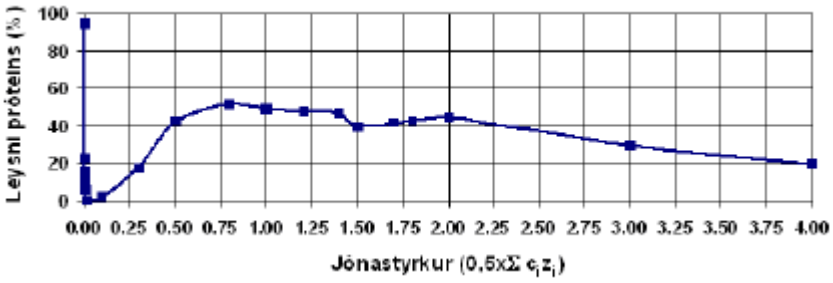

Figure 2.5. The effects of ionic strength on protein solubility (Protein solubility (%) vs. Ionic strength). (Pétursson, 2006).

Intact fish myofibrillar proteins are quite insoluble in water over a wide pH range. Smaller peptides produced by hydrolysis have more of the hydrophilic polar amino acid side groups exposed and can bind more readily to water than the intact protein can (Kristinsson & Rasco 2000a). High solubility over a wide range of pH is important for many food applications as it influences other functional properties, such as emulsifying and foaming properties.

2.4 Water in fish muscle

The main constituent of fish flesh is water, which usually accounts for 60-80% of the weight of a fresh white fish fillet. Water plays a central role in the quality changes occurring in fish muscle during storage and processing (Murray & Burt 2001). Water influences quality attributes such as appearance, texture and storage ability. Additionally, the ratio of water accessible for microbes, enzymes and moulds, i.e. water activity is important with regard to storage life of the products. The water content in fish muscles can be separated into three different populations according to the mobility and how tight the water molecules are bound to the muscle structure,

termed tightly bound, bound and loosely bound water. About 5-15% of the moisture content is water that is not available for chemical reactions and is very difficult to remove from proteins. The water molecules are located inside the proteins and form a strongly bound monolayer. This tightly bound water is very difficult to remove and does not freeze at -40°C (at least not all). Bound water accounts for 15-25% of the moister content. It corresponds to additional layers of water which are bound to the monolayer through hydrogen bonds and can take part in chemical reactions. It is located inside the muscle cells and is retained there with capillary force. Part of this water can be removed. Loosely bound water is located between cells and is easily removed or lost. The water content can be from 35% and it relates to condensation of water in capillaries and pores of the material (Guðmundsdóttir 2005; Thorkelsson 2007).

The water in fresh fish muscle is tightly bound to the proteins in the structure in such a way that it cannot readily be expelled even under high pressure. After prolonged chilled or frozen storage, however, the proteins are less able to retain all the water, and some of it, containing dissolved substances, is lost as drip (Murray & Burt 2001). Water can be added during processing, resulting in a swelling of the muscle if the ionic strength or pH in the muscle is changed by adding e.g. salt to the products. The inter filament spaces are dependent on pH, ionic strength, osmotic pressure and state of rigor. After slaughter, the myofibril is thought to expand or shrink laterally affecting the volume available for water. During rigor, glycolysis leads to formation of lactic acid and the pH is lowered (Figure 2.6). ATP is hydrolysed and since the ATP is necessary to remain relaxed state, this leads to irreversible cross bridges between actin and myosin. The formation of the actomyosin complex results in shrinkage of the muscle fibrils and swelling of the muscle is resisted by the links between myosin and actin in the rigor state. Swelling or shrinkage of myofibrils or muscle fibres alters the distribution of water within the muscle but does not necessarily change the total volume. If the environment is changed, by e.g. increased ionic strength, it can lead to increased or decreased spacing between the filaments depending on the concentration of added salts. This can lead to changes in the distribution of water. More water may be located extra-cellular, between the muscle fibres (Offer & Knight 1988).

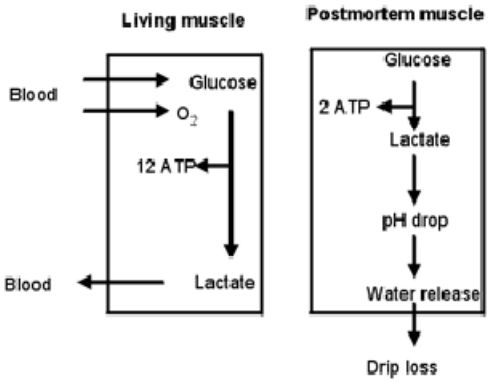

Figure 2.6. Mean difference between living and post-mortem muscle (Toldrá 2003).

2.4.1 Water holding capacity (WHC) of fish muscle

Water holding capacity (WHC) is a general term referring to the ability of a defined sample to retain intrinsic or extrinsic fluids under specified conditions (Fennema 1990), i.e. the properties of the myofibrillar protein to retain the natural water and thereby maintain the textural-, chemical- and physiochemical properties of the fish muscle during processing. The concept has also been used for the muscle property to retain added water (Brown 1986). The muscle WHC depends mainly on the conditions of the myofibrillar proteins, the space between filaments and how much water is located intracellular.

The factors that influence the water holding capacity of muscle tissue can be categorised as internal or external factors. Internal factors are e.g. species, age, size, muscle type, amount of intra muscular fat and muscle tissue condition *post mortem*. External factors are e.g. feeding patterns, season and location of catching and handling post slaughter. Changes in chemical composition during processing are also important, especially in processes like salting (Fennema 1990). Wagenknect *et al.*, (1975) (as cited by (Thorarinsdottir *et al.*, 2001)) studied changes in water holding capacity of fish muscle (*Gadus callarias*), with regard to *rigor mortis*. The water holding capacity reached it maximum in the *rigor mortis* process. Shorter *rigor mortis* periods leading to less decrease in pH due to catching method (trawl), resulted in increased water binding. Sesonal difference is on the *rigor mortis* process, the condition of the muscles, decrease in pH during *rigor mortis* and water holding capacity (Wagenknecht & Tuelsner 1975).

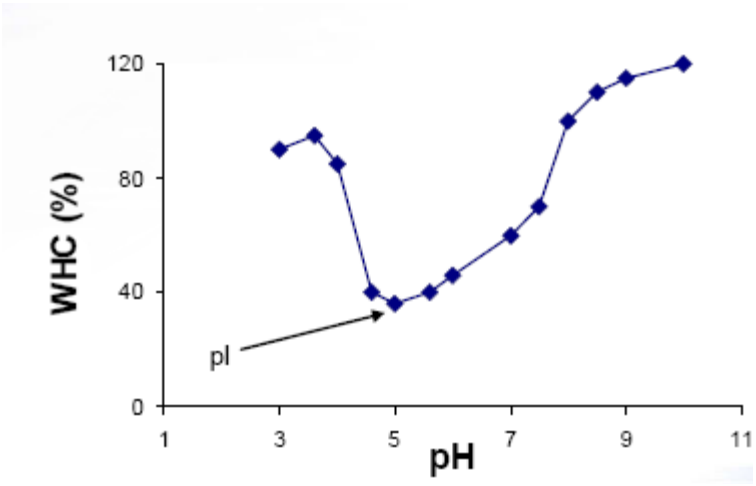

Figure 2.7. The effect of pH on water holding capacity (Thorkelsson 2007).

Salting has great impact on the water holding capacity, but depending on the salting methods, combination of the salt and the salt concentration (Fennema 1990). At very low concentrations (0-0.1M) the salt screen the protein charges which leads to decrease filament spacing and causes lateral shrinkage of the fibre. At salt concentration greater than 0.1M, spacing of filaments increases with increasing ionic strength due to the binding of anions to filaments. The protein can also be partial denaturated. Increase in salt concentration up to certain level leads to increase in water holding capacity (Fennema 1990). With increased salt concentration, the proteins continue to denaturate which leads to loss in water holding capacity. A denatured protein has approximately 10% higher water binding capacity than the native protein but when it is allowed to aggregate, a loss in water holding capacity can be observed due to protein-protein interactions (Damodaran 1996). When the concentration reaches 4.5M the muscle shrinks (salting-out effects) (Offer & Knight 1988).

2.5 Fish by-products

In the fish industry, definition of by-products varies with fish species as well as both the harvesting and processing methods used. The general understanding of by-products when considering round fish such as cod is that the main body flesh constituting the fillets will be considered to be the main product, but the head, backbones, trimmings, skin and guts constitute what is generally thought of as by-

products (Kristbergsson & Arason 2006) (Table 2.1). The decreased harvest of white fish for filleting production has resulted in increased fish material and production cost. Full utilization of fish by-products is therefore not only important but profitable as well. Amount of products prepared from by-products are limited and the value is generally low. Products like protein isolate and hydrolysate, surimi and gelatine are produced for human consumption from fish by-products, but some of them go to animal feed or are discarded. Protein isolates and hydrolysates are usually dried powders or sometimes concentrates, while surimi and mince block are frozen products. The value of these products is generally low compared with the fillets. See further discussion about products from by-raw material in paragraph 2.7.3.3.

Table 2.1. By-products from the fishing industry in Iceland (tons). (Statice 2008; Statice 2009).

Product	2007	2008
Trimmings	21,819	22,117
Heads	2,398	2,574
Roe	2,256	3,337
Mince[1]	4,233	4,340
Meal	1,141	574

2.6 Injection

The use of new marinade technologies and ingredients for use with poultry, meat and seafood is expanding. Several different methods have been used to add additives into muscles, e.g.:

- ✓ Blend minced muscle with additives
- ✓ Soaking muscles or fillets into solution of additives
- ✓ Vacuum tumbling
- ✓ Injection of additives into muscle or fillets

Addition of additives into muscle can be done with direct injection carried out by multi needle systems. Choosing the right multi needle system is very important when a process is organised, because the system can control the production success.

[1] Exported frozen fish mince

The aim with injection is to obtain a homogenous distribution of brine in the muscle in a short time and minimize the variation in injection percentage due to size or thickness of the product. Salt injection shows more uniform distribution of salt within fillets compared with dry salted fillets, but lower salt concentration (Rörå *et al.* 2004). The distribution of brine affects, among other things, yield and appearance. Therefore a precisely setting is important to produce products of the same quality[2]. Disadvantages are risk of microbial contamination and damages of the muscle structure (Foster 2004; Pellegrini 2009).

Injection of brine into muscles is both applied to meat- and fish products. Multi needle systems for meat- and fish products are quite similar, but it has to be considered that fish products have more sensitive muscle structure than meat products.

2.6.1 Basic information and equipment

The multi needle systems can be divided in two main categories: Low pressure injectors and high pressure injectors (spray system). Low pressure injectors deposit the brine during a needle stroke through the muscle, through 2-4 holes (>1 mm diameter) per needle. Low pressure equipment is usually used for fish products since they are more sensitive against pressure than meat. On the other hand, high pressure injectors release a measured dosage of brine after the needles have penetrated the muscle and are stopped. This system has 11-14 holes (0.6 mm diameter) which are distributed at different heights. Figure 2.8 shows the main difference between low- and high pressure injectors and the distribution of the brine.

[2] www.metalquimia.com

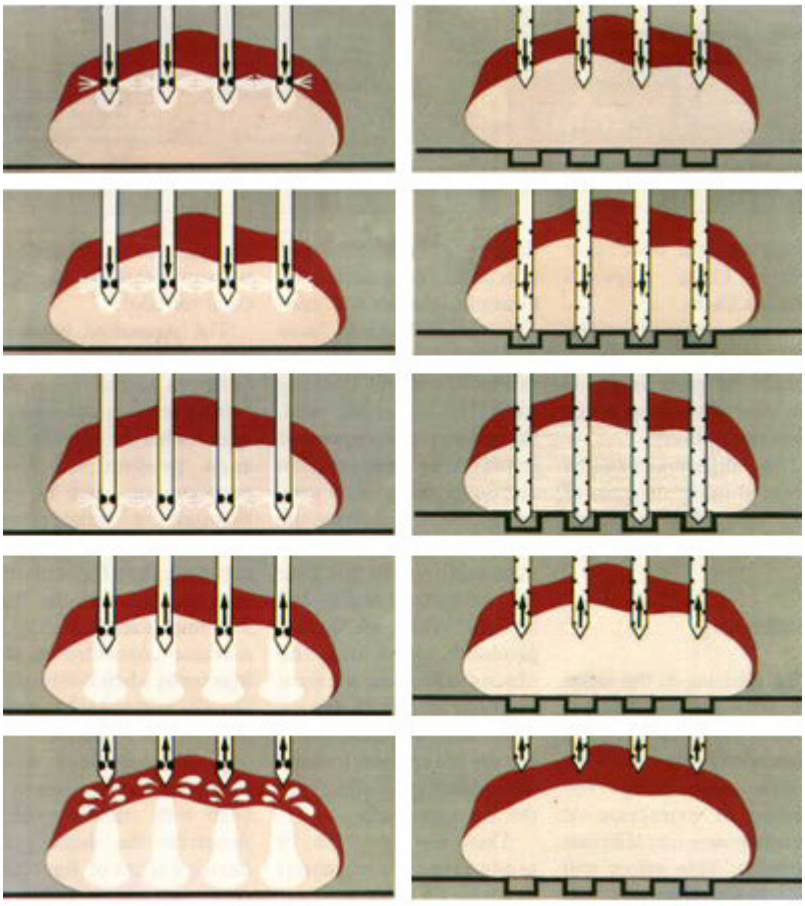

Figure 2.8. Left: Low pressure injection system. Right: High pressure injection system (spray system)[3].

Injection is a fast method to incorporate salt into fish fillets. The rate of uptakes of salt in the fillets and distribution depends on the method used, species, fillet size and thickness, brine concentration, brining time, ratio between fish and salt and a number of intrinsic muscle factors (e.g. chemical composition, muscle structure and rigor condition). (Birkeland *et al.* 2003). Most of the low pressure injectors available are equipped with continuous brine pumps, propelling the brine as a continuous jet into the muscle tissue through holes in the needle tip. The pressure inside the brine circuit does normally not exceed 4 bars (0.4 MPa), since a high pressure may cause damage to the muscle structure. Injection pressure influences the brine flow-rate through the needles during injection. The brine flow-rate influences the amount of brine injected into the amount of brine injected into the muscle tissue per injection and consequently, the total amount of brine injected (Freixenet 1993) (as cited by Birkeland *et al.* 2003).

[3] www.metalquimia.com

The needle size is also important factor when fish products are injected, because the fish muscle is very sensitive compared with meat muscle. The most suitable needle sizes for the fish industry are 1.5-2.0 mm in diameter[4]. Selections of needles are based on:

- ✓ The brine viscosity
- ✓ Final product
- ✓ The amount of brine injected per product

The needle speed can be controlled. The residence time of the needles within in the fillets during injections depends on both the needle speed and the fillet thickness. If fillet thickness is equal for all the fillets, the needle speed can be thought of as a direct expression of the residence time, and subsequently an expression of the amount of brine injected into the muscle tissue per injection. Thus, at a needle speed of 0.1 m/s the total amount of brine injected into the fillet should be twice as high compared with that at a needle speed of 0.2 m/s, given that the muscle tissue absorbs all the liquid. In other words, the weight gain should be less when the needle speed is increased (Birkeland *et al.* 2003). The needle speed may influence the distribution of brine in the areas of the muscle tissue close to the needles and the proportions of brine pocket formation in the fillets. When injection is carried out with higher needle speed, formation of smaller brine pocket containing less free brine could be expected, due to decreased residence time of the needles. Therefore, less brine becomes available for diffusion into the surrounding muscle tissue during further processing. A possible effect may be a less homogenous distribution of brine in the muscle tissue, and a less uniform salt content of the fillet. It is important to have a uniform distribution of brine in the muscle tissue to reduce the minimum time required for the brine to migrate to the non-injected areas (Freixenet 1993).

High pressure injection (HPI) is a new injection method which use small diameter, high-velocity liquid jets to instantly penetrate food without using needles or other contacting equipment. Pressure injection burst with 6.9-69 MPa into the food products surface to force the fluids inside them. This process is alternative to method such as needle injection and soaking. High pressure injection has been used to add

[4] www.townsendeng.com, Townsend Engineering B.V, Holland

moisture, oil, colour, spices, salt, enzymes and preservatives into meat, poultry, cheese, fruits, vegetables and other soft food. One of the major advantages of HPI compared to other methods is versatility. Nearly any shape or product thickness can be injected by making simple adjustments on a computer touch screen. Other advantages include less cross contamination and homogenised fluid distribution. High-velocity liquid jets have been used both outside and inside the food industry to cut and cut up hard material such as stone and ice, to clean processing equipment, to inject fluids into soft material, and to measure physical properties. Applications in food processing include curing of fresh pork, injection of brine into fish, injection of enzymes, fatty acids, salt, water and other liquids into cheese, poultry and beef. (Hansen 2005).

2.6.2 Applications and benefits of injection

Birkeland *et al.* (2003) studied, among other things, the effects of needle speed, injection pressure and number of repeated injections on post-salting and post-smoking yield and severity of muscle gaping in smoked Atlantic salmon fillets after salt injection. The results indicated that injection pressure affects the brine distribution in the muscle. It was concluded that increased injection pressure may result in a more homogenous distribution of the brine in the muscle tissue and a more uniform salt content in the fillets. In this trial, increased injection pressure increased the post-smoking yield (total yield), but had on the other hand negative effects on quality and appearance of the fillets. An increased severity of fillet gaping was observed when the brine injection pressure was increased. Increased injection pressure can also cause variegated stains on the muscle tissue (Freixenet 1993, as cited by Birkeland *et al.* 2003).

Freixenet (1993) showed that the brine distribution improved by increasing the needle density during injection. Where the fish muscle is relatively sensitive, it can be concluded that numbers of injection could have negative impact on fillets appearance and quality. Birkeland *et al.* (2003) compared fillet which had been injected once and twice. They concluded that the number of injection was an important process parameter for yield, but increasing the number of injections was likely to increase homogeneity of salt distribution in the fillets. The results indicated

that the number of injection did not influence the fillet gapping score, like increased injection pressure did. Birkeland *et al.* (2007) study confirmed these results and showed that twice injected fillets gained more weight compared to once injected fillets and numbers of injection had no influence on the fillets appearance (gaping) (Birkeland *et al.* 2007).

The injection direction can be controlled, i.e. the brine deposit while the needles are going inside (IN) the muscle or deposit when the needles are going in and then again when they are going out of the muscle (IN/OUT). The IN/OUT direction increase the amount of brine injected into the muscle per injection. This trend was also reflected by Birkeland *et al.* (2003) results, which showed that fillets injected with IN/OUT direction gained more weight, but had on the other hand similar post-smoking yield (total yield) compared with fillets injected with IN direction. The injection direction may also influence the extent of brine pocket formation and brine leakage.

Injection of brine into muscles is both applied on meat- and fish products. Materials used for injection are e.g. salt, phosphate and binding agents. Functional proteins have also been used to increase the stability during frozen storage, e.g. by injecting soy proteins into the muscle (Thorarinsdottir *et al.* 2004). To be able to inject the protein into muscle they have to be mixed into brine solution. Combination of the brine is depended e.g. on the final product and what effects are desired to gain. Injection leads to weight gain of the product, but the composition of the brine effects how well the muscle can hold the brine. Salt concentration is very important factor considering protein denaturation and the water holding capacity.

2.7 Ingredients in the fish industry

More and more meat and seafood products are enhanced with various ingredients to improve the functional properties and sensory attributes of products (Strategro 2005; Kim & Park 2006; Lynch 2006; Strategro 2006; Jóhannsson *et al.* 2009). In the following paragraphs some common ingredients are discussed.

2.7.1 Salt

Salting has been used in fish processing to, among other things, increase protein stability during frozen storage (Thorarinsdottir *et al.* 2001). Other aim of salting is to decrease water content and increase salt content and therefore lead to lower water activity. This results in less water for the microbes to utilize and therefore longer shelf life. Addition of salt or other additives reduce drip loss, but they also minimise loss of water-soluble materials such as protein, vitamins, minerals and flavourings. The salt-injection technique is increasingly gaining popularity due to its time saving potential and the higher processing yield obtained when compared to conventional salt-curing methods. The salt-injection method has shown higher yield compared to untreated fillets, but the fillets are on the other hand more sensitive against contamination and temperature changes (Matís 2009).

Salt uptake occurs due to osmotic pressure because of different salt concentration in the fish muscle an in the brine. Protein change during salting, but the changes depend on composition and concentration of the salt. At the beginning of salting process the muscle swells, binds water at the same time as the salt content increases. At low salt concentration, the muscle swells and space for water increases. Protein-water bounds become stronger and more water is bounded to the muscle. The maximum water binding is considered to be at 5-6% salt concentration. Higher salt concentration in the muscle (>10%), leads to protein denaturation, the muscle stops swelling and loses its water binding ability (Thorarinsdottir *et al.* 2001).

2.7.2 Polyphosphates

Polyphosphate are additives that are widely used to aid processing or to improve eating quality of many foods, particularly meat and fish products. Phosphates are present normally in all living things and are an essential component of our diet. Phosphate is a salt of phosphoric acid. When a number of simple phosphate units are linked to form a more complex structure, this is known as a polyphosphate. The phosphates used in foods may be simple phosphates, pyrophosphates containing two phosphate units, tripolyphosphates containing three units, or polyphosphates containing more than three phosphate units (Aitken 2001).

The use of polyphosphates have been claimed to have many benefits. The main value of polyphosphates lies in improving the retention of water by the protein in fish. Polyphosphate treatment of fish before freezing or chilling often reduces the amount of thaw drip. Addition of polyphosphates to fish products can reduce the drip loss if fish stored under MAP (modified atmosphere packaging) (Alvarez *et al.* 1996), inhibit the growth of bacteria in fish stored at ice (Kim *et al.* 1995) and retard the oxidation of unsattured fatty acid in seafood products (Dziezak 1990). Increased water retention ability by the phosphates is achieved through muscle fiber expansion (swelling) caused by electrostatic repulsions, which allows more water to be immobilized for the myofibril lattices (Offer & Knight 1988). Inhibition of oxidative changes may be through the chelation of pro-oxidative metal ions by phosphates (Matlock *et al.* 1984). The effectiveness of phosphates on functional properties of meat products depends on the type of phosphate, the amount used, and the specific food products (Lindsey 1996).

Masniyom et.al. (2005) studied the effect of phosphate on seabass slices stored under MAP. Pre-treatment with sodium pyrophosphate resulted in the delayed protein denaturation and increase in water uptake ability. Masniyom et.al. (2005) concluded that the effective retardation of microbiological, chemical, and sensory deterioration of seabass slices stored under MAP could be achieved by pre-treatment with pyrophosphate (Masniyom *et al.* 2005).

Another study, where phosphate were added to salted cod fillets, showed poorer quality compared with untreated fillets (Thorarinsdottir *et al.*, 2001).

2.7.3 Functional proteins

There appears to be an increased interest in utilizing natural and organic ingredient alternatives to chemical ingredients, such as polyphosphate, in food processing. Proteins are widely used as ingredients in processed food, e.g. minced and emulsified products. The addition of functional proteins to muscle food has also been practiced and is well established in the meat industry, being used e.g. as fat- and water-binding agents.

The methods used to add proteins to food are few and depend on the final product and the form of the ingredient. They can be added dry (dried protein powder) or as protein gel (jelly addition), pre-emulsions (water and fat are mixed together to prepare stabilized solution with the protein), but also by injection or brining or with combinations of these methods. With injection, the protein flow into e.g. fish fillets is much faster compared with the other methods (Thorkelsson 2007).

Commercially available proteins are obtained both from plant and animal sources. The functionality of added protein is dependent on their origin, molecular structure, method of isolation, various modifications of the isolated proteins, and their interactions with other ingredients in the food system. Proteins are added to food for various reasons, e.g. to improve water- and fat-binding properties, viscosity, gelation, nutritional value, emulsification and foaming properties (Thorarinsdottir *et al.* 2004).

2.7.3.1 Soy proteins

The leading and dominating vegetable based protein is soy. Much work has been done with processing of different soy protein products but they cover a wide range of functional properties. Soy protein isolates are 90% pure proteins and are considered to improve "meat properties", and are used in processing of fish- and poultry muscles. It has been shown that soy protein isolates may be used in emulsified fish products to improve water- and fat-binding properties of products in which the functional properties of proteins in the raw material have been weakened by frozen storage. Karmas and other showed that by adding soy protein isolate to fish improved water-binding during cooking (Karmas & Turk 1976). Other study have shown that addition of soy protein isolate to red hake fillets improved water binding and retarded freeze-induced texture changes (Bigelow & Lee 2007).

2.7.3.2 Dairy proteins

Dairy proteins have been used in food products for over 50 years. The main advantages of diary proteins are e.g. the white colour, none or minimal taste, good emulsifying properties and stability during frozen storage. The main diary proteins are casein and whey. They are leading animal-based proteins followed by gelatine and dried egg whites. The main applications of whey proteins are in bakery products, soups, etc., but they have also good water- and fat binding ability.

Karmas *et al.* (1976) studied water binding of cooked fish in combination with various proteins. Whey protein concentrate and caseinate increased the water binding of cooked fish compared with untreated fish. Swartz (1983) showed that injection of whey protein concentrate increased yield of halibut without appreciably diminish protein level (Swartz 1983).

2.7.3.3 Marine proteins

Great additional economic, nutritional and environmental values can be obtained by increasing the yield of raw material in fish filleting operation. Researches of marine peptides have shown that they, like dairy and soy peptides, have bioactivities which make them a very interesting alternative for the food industry. Fish proteins are considered high biological value protein or a "complete" protein because all nine essential amino acids are presented in these proteins (Insel *et al.* 2004; Protein 2007). Fish proteins have therefore high nutrition value and are easily absorbed in the human body (Nettlon 1985). Experiments with animals have among other things showed that fish protein seems to inhibit obesity linked insulin immunity in muscle, but serious insulin immunity can cause diabetes II. It has also been shown that fish protein can potentially lower the blood pressure (Geirsdóttir 2006).

Productions of fish protein functional proteins are still in its infancy (Giese 1994). Little has been published on the functional characteristics of fish proteins added to food system, but there is a great interest to utilize them for miscellaneous productions.

The main purpose of producing marine proteins is to increase the yield and to increase the value of unutilized products, of certain species or of by-raw material from processing lines. The interest to utilized by-raw material instead of discarding it has grown over the years. It is an exciting and promising advantage to isolate and modify muscle proteins that are already in by-raw material and use them as functional additive in food systems. It is important for the fish industry to develop methods that are more economically profitable than discarding the by-raw material. Two traditional methods have been used for processing of marine proteins, i.e. surimi production and fishmeal processing for animal feed. Production of fish proteins for addition into food has shown limited success. Today great developments are in process where the main aim is it develop new applications for fish proteins and increase their value. That has been the aim of the Iceprotein ehf.[5], Icelandic company based on processing of proteins from fish raw material.

Using fish proteins as additives can improve water holding capacity, decrease drip loss and therefore improve total yield. Observations of added fish proteins are promising, but it must be kept in mind that the particular process used for protein isolation affects their functional properties and must be controlled with respect to the desired characteristics of the isolated proteins (Bárzana & García-Garibay 1994).

Innovative technologies used in improving yield in fish filleting operation are using raw material from by-products like cut off and backbones of the same species. This applies both to the SuspenTec® system[6] (often referred as Cozzini) and acid and alkali extraction (pH-shift) of fish proteins (Kelleher & Hultin 1999). The SuspenTec® process is an automated method of reducing fish trimmings at low temperatures (-4—6°C) to micron-sized particles and incorporating them into traditional brines to create homogeneous suspensions. The controlled temperature ensures efficient protein binding and dispersal of suspension into the muscle product. Other methods where reduced fish trimmings are injected into muscle products have been established. Jacquier *et al.* (2000) established method were reduced fish is reduced to particles (<1 mm) which are incorporated into dilute brine. Moisture before injection is 85%-90% and the salt concentration in the suspension is 4%-8%.

[5] www.marifunc.org
[6] www.suspentec.com

This is injected into fish portions which at the instant of injection are at -2°C to -8°C, whilst the suspension is at -14°C to -4°C (Jacquier *et al.* 2000). Simon *et al.* (1981) also established method where minced fish muscles are injected into fillets or loins, giving weight gain of 8-50% (Simon *et al.* 1981). The pH-shift protein isolate can be added to fresh or frozen seafood of the same species by needle injection into fillets, soaking, or vacuum tumbling. Proteus[7] has developed and holds patent for the production process *NutraPure* which comprehend processing and treatment on proteins from animal muscle cut-offs (US: 6,005,073; US: 6,288,216). Proteus has also develop process to utilize protein for injection into fresh fish in the purpose to lower bacterial count, increase protein content and to increase shelf life (Griffin 2005a). These proteins have also been used to coat food products but the proteins hold that ability to swallow water and hold it at high temperature. When products are fried, the proteins prevent the fat to be absorbed into the product and keep the moister inside. The fried products contain ~50% less fat and are juicier (Griffin 2005b; Kelleher 2005).

The pH shift methods (Figure 2.9) involves solubilising muscle proteins by subjecting diluted, finely homogenized fish meat to either very low pH (~2.5-3) or a very high pH (~10.8-11.2) at low temperature. Solids such as bones, scales, neutral fat and disrupted cellular lipid membranes are then removed by centrifugation and the soluble protein is precipitated by adjusting the pH to the isoelectric point of the myofibrillar proteins to give a protein isolate (Kristinsson *et al.* 2006). Injection of brine with protein isolates or homogenized muscle have increased weight gain in cod and haddock fillets 5-20% and also increased cooking yield. There are indications that protein isolates give higher cooking yield and microbiologically more stable products than products with injected fish mince (Valsdóttir *et al.* 2006).

[7] www.proteusindustries.com

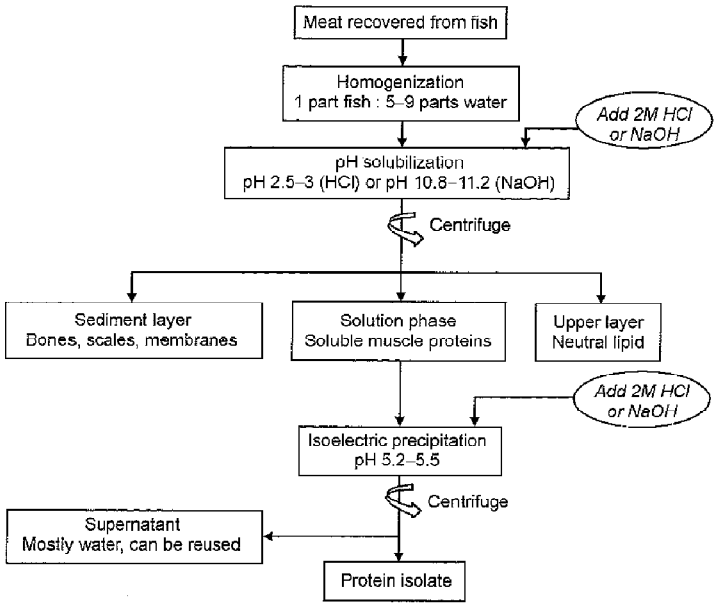

Figure 2.9. Schematic representation of the acid and alkaline processes used in the production of fish protein isolates. The process involves solubilising muscle proteins at low or high pH, separating them from undesirable muscle components via centrifugation and recovery of the protein of interest by isoelectric precipitation. The final protein isolate can then be used directly, or stabilised with cryoprotectants and frozen until used. (Adapted from Kelleher & Hultin, 2000)

Fish protein hydrolysates (FPH) are produced industrially by chemical and enzymatic processes, but chemical hydrolysis is used more commonly. Biological processes using added enzymes (Figure 2.10) are employed more frequently, and enzyme hydrolysis holds the most promise for the future because it results in products of high functionality and nutritive value (Kristinsson & Rasco 2000b; Slizyte *et al.* 2005). FPH have good solubility over a wide range of ionic strength and pH and usually tolerate strong heat without precipitation. FPH have good functional properties and con contribute to water holding, texture, gelling, whipping and emulsification properties when added to food (Dauksas *et al.* 2005). Some studies have shown that FPH can contribute to increased water holding capacity in food formulation (Shahidi *et al.* 1995) and addition of FPH from salmon reduced water loss after freezing (Kristinsson & Rasco 2000a).

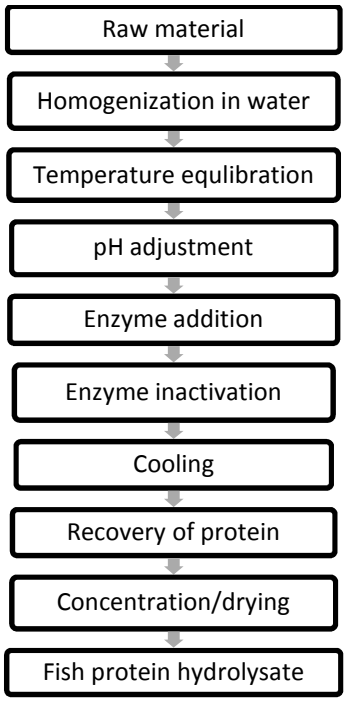

Figure 2.10. Outline of the main step in the production of fish protein hydrolysates (Adapted from (Kristinsson 2006).

Fish skin, which is a major by-product of the fish processing industry, causing waste and pollution, can provide a valuable source of gelatine (Karim & Bhat 2009). Gelatin is a fibrous protein that is extracted from collagen, which is the principal constituent of animal skin, bone, and connective tissue. The quality of gelatine for particular application largely depends on its physicochemical properties, which are greatly influenced by both the species and tissue from which it is extracted and the method of extraction (Hao *et al.* 2009). Interest in fish gelatines as ingredients has increased due to good gel forming ability and it can be used as binding agent in food formulations. In recent years, fish skin had been reported as a new alternative source of producing gelatine (Guðmundsson & Hafsteinsson 1997). This is partly due to the requirements for kosher and halal food products and no risk of outbreaks of Bovine Spongiform Encephalopathy (BSE, "mad cow disease") in products containing ingredients derived from mammals by various types of rendering processes (Zhou *et al.* 2006).

Methods to produce valuable protein products from by-raw material are in continuous development. Thorarinsdottir *et al.* (2009) developed a process for homogenisation of fish mince solution (HFP) for injection in fish products. This is a

new method which has never been applied before and no patents are listed. The process was well suited for preparing a solution which had the right particle size, viscosity, water holding properties and stability for injection. The yield and stability of the protein injected fillets was increased compared with untreated fillets and fillets injected with pure salt brine. Freezing reduced water holding capacity but the yield was still higher than of untreated fillets, both after thawing and cooking (Thorarinsdottir *et al.* 2009).

2.7.4 Other additives

In addition to using salt, phosphate and functional protein to increase the yield of seafood and meat, other ingredients can be used such as starches, hydrocolloids, enzymes, MSG (mono sodium glutamate) etc.

Starches and maltodextrins are glucose polymers that are found in maize, oats, rice, potato, etc. Their effects vary according to origin, induced modification, condition of use and the nature of the product to which they are added (Colmenero 1996). Starches can offer a range of benefits e.g. increased yield and high water binding capacity[8] and as fat-replacer. They can be applied both to meat and fish products. Addition of corn starch to surimi, from tropical fish, increased its firmness and toughness (Gopakumar *et al.* 1992). Murphy and others showed also that starch was effective in improving the functional properties of surimi from Atlantic whiting (Murphy *et al.* 2005).

Dietary fibres have also been studied. Sánchez-Alonso *et al.* (2007) added white grape dietary fibre concentrate (WGDF) to minced fish muscle of horse mackerel (*Trachurus trachurus*). The main feature of interest of WGDF is that it a natural product containing high concentrations of dietary fibre. The results indicated that WGDF had good functional properties, high water and oil retention capacity, and considerable swelling properties. Water retention was significantly enhanced when WGDF was added, and the cooking yield improved (Sánchez-Alonso *et al.* 2007).

[8] www.avebe.com

2.8 Freezing and frozen storage

Freezing is one of the most common procedures applied nowadays to preserve quality and increase shelf life of fish products and other food products (Matís 2009). The main purpose of freezing is to stop microbial growth and decrease enzymatic activity and therefore prevent or slow food damage.

During freezing, microbial growth is stopped and certain chemical- and physiochemical changes are slowed down, but other unfavourable changes can occur. Water in fish products starts usually to freeze right below freezing point but complete freezing depends on the content of dissolved matter in cell water. Fish is considered frozen when a big part (50% or more) of the water content has turned into ice. During ice formation, the proportion of dissolved matter increases in the water that is still unfrozen. Most of the water freezes from 0°C to -5°C, but below -10°C only small part can be frozen. At -24°C, most of the ice formation is complete. It is therefore very important that the final temperature of fish during freezing is the same (or very close) to the storage temperature.

Freezing affects the physical properties of the fish muscle but usually has a small effect on taste, odour and appearance (Arason & Ásgeirsson 1984). During freezing, certain chemical- and physical changes are slowed down. Frozen fish muscle has lower quality compared with fresh fish, for example stiffer muscle and is less juicy (Mackie 1993). The structure of the fresh fish muscle changes during freezing and frozen storage. The muscle cells shrink, causing liquid leak out of the cells to the inter-cellular space (Bello *et al.* 1981; Hurling & Mcarthur 1996). Is has been shown that ice crystals are formed within the muscle cells and between them during freezing. The location and size of the ice crystals are dependent of the freezing conditions (Howgate 1979). The freezing rate is therefore very important factor (Figure 2.11). During slow freezing (less than 0.3 cm/h), big ice crystals are formed outside the muscle cells and extract water from the cells causing ice crystal formation between the cells and disrupting them. This decreases the fish muscle water binding ability which can lead to lower quality and yield when thawed. The main reason for these changes on water binding ability is due to the instability of myosin. It is believed that myosin is denatured during freezing which leads to less water binding

capacity. During fast freezing, the water within the muscle cells is frozen before it can leak out which leads to formation of small ice crystals inside and outside of the cells. Fast freezing leads therefore to minimum changes to texture (Arason & Stefánsson 1999; Belitz *et al.* 2004).

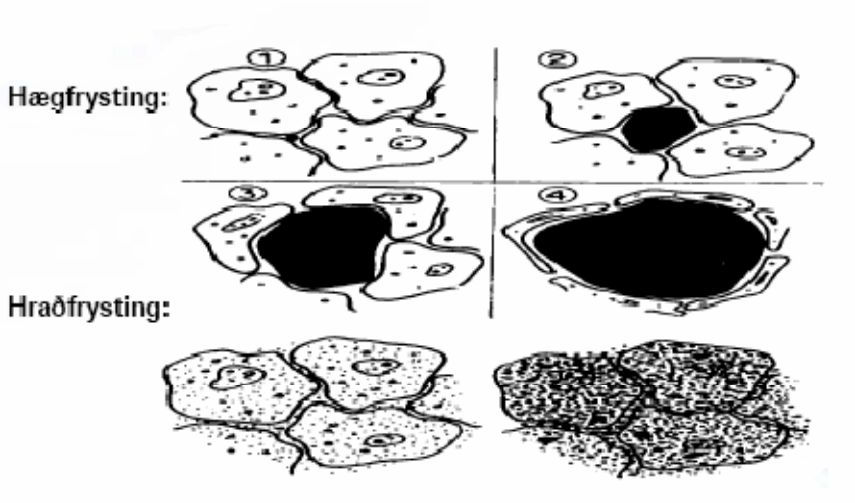

Figure 2.11. Ice crystal formation within fish muscles during slow (upper) and fast (lower) freezing.

The quality of fish proteins decreases during frozen storage (faster at -4°C than at -18°C). The fish protein properties change during frozen storage and they are partially denaturated which can lead to water leakage (drip) when thawed. In general, the drip loss is about 3-5% (Jul 1984) but can be up to 15% (Cormier & Leger 1987). The water content in fish is relatively high (78-83%), therefore a high drip loss has negative effect on yield. Freezing is not thought to affect the nutritional value of fish although proteins are partially denaturated during freezing,

2.9 NMR measurements

Nuclear Magnetic Resonance (NMR) spectroscopy has received a lot of attention recently as an important tool in food research. This technique has many applications and possibilities for food research and processing. Over the past fifty years NMR has become the preeminent technique for determining the structure of organic and inorganic compounds and for analyzing large molecules such as protein. The use of NMR in food analysis is mainly for determining fat-, protein- and water content.

Three decades ago, is was demonstrated that pulsed low-field NMR relaxation techniques (LF-NMR) can provide information about the state of water in muscle tissue, as relaxation data were found to indicate the existence more than one type of water state in muscle tissue (Betram *et al.* 2001). The LF NMR applications spans from reviewing texture properties, sensory features, water contents and distributions and fat content and properties (Lastein 2002). This is a fast and accurate method, in comparison with chemical methods, and is non-destructive because no solvents are used in the sample preparation. The technique measures the mobility of protons and can therefore be used to measure the change of states of water and fat as well as its quantity at various temperatures (Betram *et al.* 2004a; Betram *et al.* 2004b). It has also been established that LF NMR is more accurate for evaluating water holding capacity (WHC) and water content in meat (Bröndum *et al.* 2000).

Lambelet and others studied how the fish flesh was affected by heat and pressure treatment as well as after frozen storage using water proton transverse relaxation times (T_2) obtained from LF NMR measurements (Lambelet *et al.* 1995). Transversal relaxation data from muscular tissues can be decomposed into various water population having different T_2 values depending on the mobility (environment) of water protons. T_{21} population is suggested to correspond with water located within highly organized myofibrillar protein matrix including actin and myosin filament structures (Betram *et al.* 2001).

Lambelet *et al.* (1995) found that the extra water component arose when protein denatures either due to pressure, heat or subzero temperatures (cold induced protein denaturation) and that the magnitude correlated with the temperature of the heat treatment and the duration of frozen storage. Steen and Lembelet (1997) found and resolved the first (fastest relaxing component) two components which remained steady throughout the experiment and correlated the then third component to sensory texture parameters and objective mechanical and chemical parameters. They also found that the slowest relaxing component (third) increased with frozen storage time and temperature of heat treatment (Steen & Lambelet 1997). It is seen that LF NMR may be used for prediction of the water holding capacity which were found to be correlated with the quantity of the third and slowest relaxing component (Jespen *et*

al. 1999). The changes in water holding capacity is due to protein denaturation which in terms also are correlated to changes in sensory parameters and texture.

The results from Erikson *et al.* (2004) indicated that NMR can be used as useful tools to evaluate and optimize fish processing unit operations (Erikson *et al.* 2004). In Iceland (Matís ohf.) the LF NMR has been used to study water properties of cod during chilled storage, salting and drying. Gudjonsdottir *et al.* (2009) studied the difference in farmed and wild cod muscle as indicated by low field relaxation measurements and how these results could be related to more traditional measurements of physical, chemical and sensory analysis. The results indicated, among other thing, that the technique can be used for traceability, with the purpose of distinguishing between wild and farmed cod (Gudjonsdottir *et al.* 2009).

2.10 Study objectives

The main objectives of this project were to investigate the effects of added functional fish proteins on chemical and physicochemical properties of chilled and frozen fish fillets. The aim was to maintain or increase yield of the fillets and thereby increase quality and value. Homogenized fish protein solutions from fish trimmings and other commercial available fish protein products were used as ingredients, parallel with salt. The results may indicate how the added ingredients might improve the stability and total yield of fish products without having negative effects on taste or texture. The results may also indicate how the added ingredients might compensate for fluctuations in the raw material. The main variables evaluated were fillet yield, drip loss, water holding capacity, cooking yield, T_2 transversal relaxation time measurements, and chemical analysis.

3 MATERIALS AND METHODS

Several injection studies were performed on whitefish fillets. Fresh saithe and cod fillets and light salted cod fillets were injected with fish protein solutions and compared with salt injected (1.5% and 4%) and untreated control fillets (Table 3.1). The fillets were stored +2°C and -24°C for various times. Parameters evaluated for the fillets were weight gain, yield after storage, drip loss, cooking yield, water holding capacity, transversal relaxation time T_2 with LF-NMR, chemical composition and sensory evaluation (Table 3.2).

The chemical- and functional properties of the fish proteins were also evaluated. Parameters evaluated were colour, weight loss, viscosity, acidity, chemical composition and protein pattern.

Table 3.1. Experimental design and sampling

Material	Group/marker	Injection brine #1	Injection brine #2	Storage	Sampling	Date of trial
Fresh cod fillets	F0 (control)	--	--	Frozen	After 1 month	13.10.08
	F0-c	--	--	Chilled	After 1, 3, 5 and 10 days	13.10.08
	F1	1.5% Salt	--	Frozen	After 1 month	13.10.08
	F1-c	1.5% Salt	--	Chilled	After 1, 3, 5 and 10 days	13.10.08
	F2	HFP	--	Frozen	After 1 month	13.10.08
	F2-c			Chilled	After 1, 3, 5 and 10 days	13.10.08
	F3	FPI	--	Frozen	After 1 month	30.06.07
	F4	FPH	--	Frozen	After 1 month	30.06.07
Light salted cod fillets	L0 (control)	--	--	Frozen	After 1 month	23.06.08
	L1	HFP	--	Frozen	After 1 month	23.06.08
	L2	FPI	--	Frozen	After 1 month	30.06.07
	L3	FPH	--	Frozen	After 1 month	30.06.07
Fresh saithe fillets	S0 (control)	--	--	Frozen	After 1 week and 1 month	08.05.09
	S0-c	--	--	Chilled	After 4 days	08.05.09
	S1	4.0% Salt	--	Frozen	After 1 week and 1 month	08.05.09
	S1-c	4.0% Salt	--	Chilled	After 4 days	08.05.09
	S2(a)	4.0% Salt	HFP(a)	Frozen	After 1 week and 1 month	08.05.09
	S2(a)-c	4.0% Salt	HFP(a)	Chilled	After 4 days	08.05.09
	S2(b)	4.0% Salt	HFP(b)	Frozen	After 1 week and 1 month	08.05.09
	S2(b)-c	4.0% Salt	HFP(b)	Chilled	After 4 days	08.05.09
	S3	4.0% Salt	2% Gelatine	Frozen	After 1 week and 1 month	08.05.09
	S3-c	4.0% Salt	2% Gelatine	Chilled	After 4 days	08.05.09
	S4	4.0% Salt	HFP(a) + 2% gelatine	Frozen	After 1 week and 1 month	08.05.09
	S4-c	4.0% Salt	HFP(a) + 2% gelatine	Chilled	After 4 days	08.05.09
	S5	FPH	--	Frozen	After 1 week and 1 month	08.05.09
	S5-c	FPH	--	Chilled	After 4 days	08.05.09

Table 3.2 Sampling and analysis. (d5=day 5).

Group/ marker	Weight gain	Drip loss	Storage yield	Cooking yield	Total yield	WHC	T_2	Chemical content	Sensory analysis
F0 (control)	x	x	x	x	x	X	x	X	
F0-c	x	x	x	xd5	xd5	xd5		xd5	xd5
F1	x	x	x	x	x	X		X	
F1-c	x	x	x	xd5	xd5	xd5		xd5	xd5
F2	x	x	x	x	x	X		X	
F2-c	x	x	x	xd5	xd5	xd5		xd5	xd5
F3	x	x	x	x	x	x	x	X	
F4	x	x	x	x	x	x	x	X	
L0 (control)	x	x	x	x	x	x		X	
L1	x	x	x	x	x	x		X	
L2	x	x	x	x	x	x		X	
L3	x	x	x	x	x	x		X	
S0 (control)	x	x	x	x	x	x	x	X	
S0-c	x	x	x	x	x	x	x	X	
S1	x	x	x	x	x	x	x	X	
S1-c	x	x	x	x	x	x	x	X	
S2	x	x	x	x	x	x	x	X	
S2-c	x	x	x	x	x	x	x	X	
S3	x	x	x	x	x	x	x	X	
S3-c	x	x	x	x	x	x	x	X	
S4	x	x	x	x	x	x	x	X	
S4-c	x	x	x	x	x	x	x	X	
S5	x	x	x	x	x	x	x	X	
S5-c	x	x	x	x	x	x	x	X	

3.1 Material

3.1.1 Protein products

A few types of protein products were studied in this project and used for injection into fish fillets. Fish protein solutions (HFP), made from homogenized fish mince, were produced for fillets injection. Other protein products were obtained from commercial producers: Fish protein isolate (FPI) was obtained from Iceprotein ehf. (Sauðárkrókur, Iceland), fish protein hydrolysate (FPH) from Højmarklaboratoriet a.s

(Maripep C, Hoejmark, Denmark) and dried collagen peptides (low molecular weight fish gelatine) from Faroe Marine Biotech (Faroe Islands).

3.1.1.1 Preparation of solutions for injection

All solutions were prepared by using tap water (0-1°C). The protein concentration of the HFP, FPH, FPI and gelatine solutions was 3%, 14%, 3% and 2% (w/w), respectively. The protein products were used as recommended by the producers. Therefore the protein content and the pH value of the solutions were not altered. Viscosity of the solutions for injection was a limiting factor with regard to protein concentration.

Homogenized fish proteins (HFP) were produced according to a specific continuous process. Approximately 4 part of cold water (0-1°C) was added to 1 part of fresh mince from saithe/cod cut-offs. After infusion of water and mince, the solutions were sieved (1000 µm) to dispose of insoluble and undesired material. It was then homogenized at about 3000 psi by a special homogenizer and directly injected into fillets using a multi needle injector.

Three types of HFP were produced (HFP, HFP(a) and HFP(b)), from three types of by-raw material. The HFP used for injection into fresh and light salted cod fillets was produced from cod mince made of cut-offs and frames (by-raw material) after filleting. This solution contained also 1.5% (w/w) salt. The HFP(a) and HFP(b) were used for injection into fresh skinless saithe fillets. HFP(a) was produced from fresh saithe mince made of all cut-offs and frames (by-raw material) after filleting. HFP(b) was produced from frozen saithe mince made of cut-offs from skinless fillets (flaps and backbones). No salt was added to both HFP(a) and HFP(b). The protein concentrations of all the HFP solutions were set at 3% (w/w).

Fish protein hydrolysate (FPH) is obtained by hydrolysis of specific parts from cod. It is composed of polypeptides and free amino acids and is sold as a functional ingredient to the food industry. It is known to have antioxidant, emulsifying and freeze stabilizing properties. The FPH concentrate was diluted with cold water (0-1°C) just before injection, with the ratio between FPH/Water 1:3, as recommended

by the producer. It was also important to stir the FPH concentrate before dilution to prevent precipitation. Due to strong foaming ability after dilution, 0.03% (w/v) of anti-foaming agent (AFEK-FDV2K-25) was added to the solution.

Fish protein isolate (FPI) was provided by Iceprotein ehf. (Sauðárkrókur, Iceland). The fish proteins were purified from fresh filleting by-products or headed and gutted by-catches using the pH-shift extraction method, based on the solubility of myofibrillar and sarcoplasmic proteins at extreme acid and alkaline pH. Fish muscle was minced, prewashed with water and centrifuged ($3000 \times g$, 10 min). The resulting pellet was dissolved in water (1:6, w: v) and pH was adjusted with NaOH 1 M at 10.8, to solubilize myofibrillar and sarcoplasmic proteins. Proteins were then efficiently separated from lipids, membranes, skin and bones by centrifugation ($3000 \times g$, 10 min). Two phases were obtained: a solid phase containing membranes, skin and bones (pellet) and a soluble phase containing solubilized proteins. The soluble phase was carefully collected and pH was adjusted to 5.6 with HCl 1 M, to precipitate proteins, which were collected by centrifugation ($3000 \times g$, 10 min).

The dried fish collagen peptide (Gelatine) was dissolved in cold water and diluted to set the gelatine concentration at 2% (w/w) prior to injection. When the gelatine was used combined with HFP, the gelatine concentration was also set at 2% (w/w).

Salt brines were prepared from food grade pure dried vacuum salt (>99.9% NaCl) with purity of 99.9%. In the injection trials, two concentrations of salt brines were used, 1.5% and 4% (w/w).

3.1.2 Fish fillets for injections

All the injections experiments were performed at Iceprotein ehf. (Sauðárkrókur, Iceland). The raw material was cod (*Gadus morhua*) and saithe (*Pollachius virens*) fillets, provided by FISK Seafood hf. (Sauðárkrókur, Iceland). The cod fillets were fresh and light salted with skin on. Light salted cod fillets were first injected with 1.2% salt brine and then brined in the same salt brine for approx. 48 h before protein injection. The weight gain that occurs during light salting (injection with pure salt

brine and brining) were not taken into calculations. The saithe fillets were fresh and skinless.

3.2 Analytical methods for protein solutions

In order to examine chemical- and physiochemical characteristics, and other properties of the protein products, specific measurements were performed exclusively on the protein products and not on the injected fillets.

3.2.1 Weight loss (%)

Weight loss (%) was determined by centrifuging 2 g of the fish protein solutions using Biofuge Stratos; Heraeus Instruments (GmbH&Co., Hanau, Germany), the same procedure as for water holding capacity measurements. Temperature was set at 5°C, speed 1350 rpm and the time was 5 min. The difference in weight of the samples before and after centrifugation was noted.

3.2.2 Viscosity

The **Brabender** viscosity of the fish protein solutions was determined using a Brabender® Viscograph E coaxial viscometer (Brabender® OHG, Duisburg, Germany). The Brabender® Viscograph E enables automatic analysis on samples where the material can be studied on a wide temperature scale and the effects of heating and cooling can be analyzed. It is a rotational viscometer comprised of an electronic measuring system, sample bowl (with 8 protruding pins in it), and a seven pin stirrer. A computer is connected to the device to enable visual inspection of the progress of the analysis and input of test parameters. This instrument measures a resistance of the sample against flow. It is assumed that this resistance is proportional to the viscosity of the sample. The device does this by measuring torque acting on pins that are in contact with the sample. At the same time the measuring bowl rotates and the temperature can be increased or decreased. The Brabender® Viscograph E gives the torque or viscosity results in the form of Brabender® Units.

Approximately 450 g of fish protein solution was placed in the measuring bowl of the device. Starting temperature was 5˚C, heating rate 1.5˚C/min, and maximum temperature 45˚C with a holding time 3 minutes, then cooling rate of 1.5˚C/min to 5˚C. Measuring cartridge was 700 cmg (0.7 Nm) and speed of the bowl 7 rpm. The measurements were done in duplicate. The temperature of the sample should be 0 to 2˚C at the beginning of measurement. Samples viscosities were recorded from 5˚C to 45˚C and again after cooling to 15˚C.

The viscosity was also analysed by using **Bohlin** BV88 viscometer (Bohlin Instruments, England). A beaker containing 200 mL of fish protein solution was put inside a 500 mL beaker containing crushed ice to control temperature. The instrument cylinder was immersed into the solution. The viscosity of the sample was recorded after 20 seconds of operating instrument at 5 to 7°C, speed setting 6, system switch 6. Measurements were done in triplicate. Viscosity was reported as Pascal.

3.2.3 Acidity (pH)

The pH of the fish protein solutions was measured by inserting a combined electrode (SE 104 – Mettler Toledo, Knick, Berlin, Germany), connected to a portable pH meter (Portamess 913, Knick, Berlin, Germany), directly into the solutions.

3.2.4 Colour

Colour was measured with Minolta CR-400 Chroma meter (Minolta Camera Co., Ltd., Osaka, Japan) using the CIE Lab scale, with L^* (black 0 to light 100), a^* (red 60 to green -60) and b^* (yellow 60 to blue -60) to measure lightness, redness and yellowness. Whiteness was calculated according to the methods of (Park 1994):

$$\boldsymbol{Whiteness} = L^* - 3b^*$$

The instrument was calibrated against a white standard at the same light conditions and temperature (20°C). The analysis was performed five times on each sample.

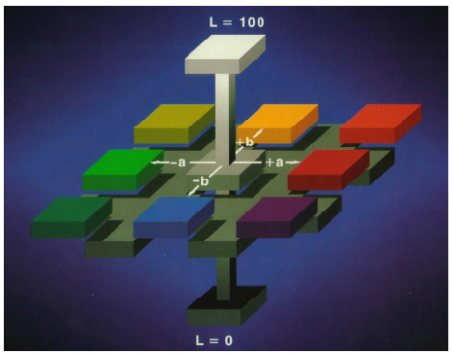

Figure 3.1 Visual division of the CIE Lab scale.

3.2.5 Determination of molecular weight using SDS-PAGE

Protein patterns of the protein products were analysed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970), using 10% separating gel and 4% stacking gel. The samples were dissolved in distilled water and diluted as needed. The protein solutions (10 µL) were mixed with SDS loading buffer (3µL) and heated at 100°C for 5 min. The load volume was 13 µL in all lines. Standards from New England Biolabs were used to identify the protein fractions with molecular masses ranging from 2-212 kDa. The samples were run at 20 mA for approximately 2 h. Protein bands were stained with Coomassie Brilliant Blue R-250, and then destained according to the method of Fairbanks and others (1971). (Laemmli 1970; Fairbanks *et al.* 1971).

3.3 Analytical methods for injected fillets

3.3.1 Process and sampling

The experimental layouts are shown in Table 3.1. Fillets were injected with salt and fish protein brines by using an automatic brine injection system (Dorit INJECT-O-MAT, PSM-42F-30I, Auburn NSW, Australia) with 1 bar pressure. The injection system was equipped with 42 needles in two rows. The needles were 4 mm in diameter and the radius around each needle was 1 cm. The needles were open in two directions. Fillets were injected once or twice. The fresh cod fillets were injected once with 1.5% salt brine or fish protein solutions. The light salted cod fillets were injected once with protein solutions (except the pre-treatment, salt injection and

brining). Most of the fresh skinless saithe fillets were injected twice, first with 4% salt and then with the protein solutions. This was done instead of increase the salt concentration of the protein solutions, because increased ionic strength can reduce solubility of the myofibrillar protein selectively.

The temperature of the injections solutions and the processing room where the injection took place was 5°C and 16°C, respectively. After injection, fillets were placed carefully on a grid for approx. 15 min to drain off excess solution liquid. The fillets were chilled (+2°C) and/or frozen (-24°C) and stored for various times prior analysis at -24°C. Freezing was carried out in freezer compartment at -24°C (in FISK Seafood hf. fish factory, Sauðárkrókur, Iceland). The fillets were placed on iron pans and covered with plastic film, and stored for 4 days in the freezer compartment prior transport to Reykjavík. The fillets were packed and stored in expended polystyrene boxes with plastic film on the bottom. Thawing was carried out at +2°C for approximately 36 h. Each fillet was identified with a numbered plastic tag and weighed before and after injection, frozen, after thawing and after chilling. Before analysis, fillets were skinned by hand and minced in a mixer (Braun Electronic, type 4262, Kronberg, Germany). Parameters evaluated were weight gain, yield after storage, drip loss, cooking yield, water holding capacity, transversal relaxation time T_2 with LF-NMR, chemical composition and sensory evaluation (Table 3.2).

3.3.2 Determination of weight gain after injection

Fillets were weighed raw and 15 minutes after injection and the weight gain of the fillets was calculated with respect to the weight of the raw fillets. Values less than 100% indicated that fillets had lost weight; while values over 100% indicate that fillets had gained weight. The weight gain of light salted cod fillets, that occurs during light salting (injection with pure salt brine and brining), were not taken into the calculation. The light salted fillets (before protein injection) were therefore "defined" as raw material, to obtain the additional effects of injected protein solutions.

$$\textbf{\textit{Weight gain}}\ (\%) = \frac{g\ injected\ fillets}{g\ raw\ fillets} \times 100$$

3.3.3 Determination of drip loss during storage

Drip loss was expressed as weight reduction during storage. Drip loss of chilled fillets was determined based on weight after injection.

$$\textbf{Drip loss}(\%) = \frac{g\ fillets\ before\ storage - g\ fillets\ after\ storage}{g\ fillets\ before\ storage} \times 100$$

Drip loss of fillets after frozen storage was determined on weight of frozen fillets. Frozen fillets were placed on a plastic racks with plastic film on the top to prevent drying of the fillets and thawed at +2°C for approx. 36 h.

$$\textbf{Drip loss}(\%) = \frac{g\ frozen\ fillets - g\ thawed\ fillets}{g\ frozen\ fillets} \times 100$$

3.3.4 Determination of yield after storage

The yield of the chilled and/or thawed fillets after storage was determined by the observed changes in weight with respect to the weight of the raw fillets.

$$\textbf{Yield after storage}\ (\%) = \frac{g\ fillets\ after\ storage}{g\ raw\ fillets} \times 100$$

3.3.5 Determination of cooking yield

For evaluation of cooking yield, each fillet (n=3) was cut in approx. 50 g pieces. Cooking yield was determined by steam cooking the pieces at 95°C to 100°C for 8 min in a Convostar oven (Convotherm, Elektrogeräte GmbH, Eglfing, Germany). After the cooking period, the pieces were cooled down to room temperature (25°C) for 15 min before weighing for cooking yield determination. The yield after cooking (%) was calculated as the weight of the cooked pieces in contrast with the weight before cooking.

$$\textbf{Cooking yield}\ (\%) = \frac{g\ cooked\ pieces}{g\ pieces\ before\ cooking} \times 100$$

3.3.6 Determination of total yield

Evaluation of total yield of the fillets was determined by multiplying the yield after each processing step (injection, storage) and the cooking yield.

$$\textbf{Total yield}\ (\%) = \frac{g\ fillets\ after\ storage}{g\ raw\ fillets} \times \frac{g\ fillets\ before\ cooking}{g\ fillets\ after\ cooking} \times 100$$

3.3.7 Determination of water-holding capacity

Water holding capacity (WHC) was determined by a centrifugation method (Eide *et al.* 1982). The fillets (n=3) were coarsely minced in a mixer (Braun Electronic, Type 4262, Kronberg, Germany) for approximately 15 s at speed 5. Specific sample glasses made of plexiglass were used for this measurement. The glasses had membrane (100 µm) on the bottom and were 6.2 cm in height, hand inner diameter of 1.9 cm and outer diameter of 2.5 cm. The sample glasses were put into holsters for certain size of rotor (Heraeus #3335) for appropriate centrifuge (Biofuge Stratas, Thermo electron corporation, Germany). Glass balls were put in the bottom of the holsters.

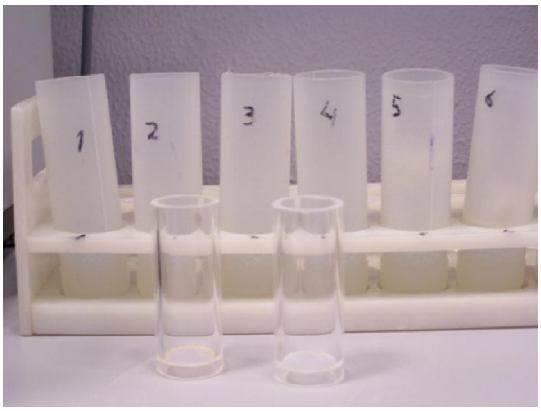

Figure 3.2 The sample glasses for determination of WHC and holsters for Heraeus rotor í Biofuge Stratas centrifuge.

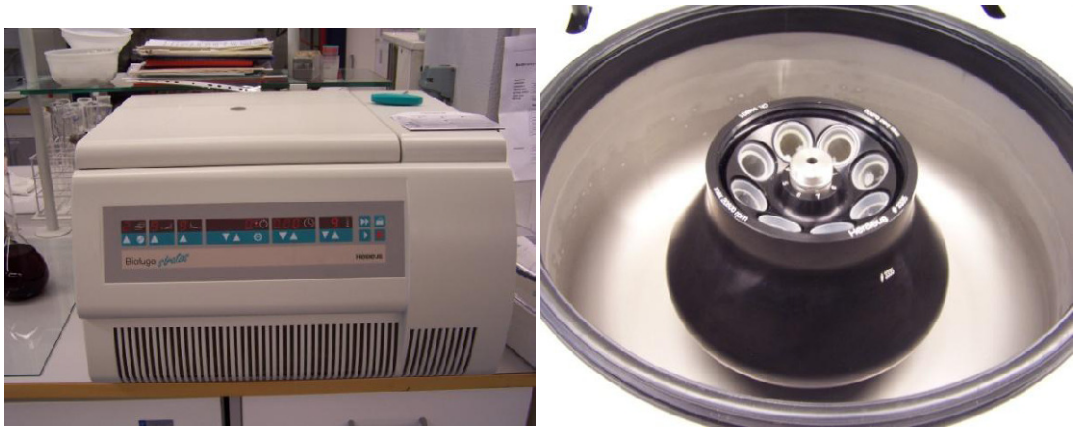

Figure 3.3 Left: The centrifuge (Biofuge Stratas, Thermo electrion corporation, Germany) for determination of WHC. Right: Rotor for Biofuge Stratas centrifuge.

Approximately 2 g of the minced fish muscle was weighed accurately into the sample glasses and immediately centrifuged at 1350 rpm (210 x g) for 5 min, with temperature maintained at 4°C. The weight loss after centrifugation was divided by the water content of the fillet and expressed as %WHC.

$$\boldsymbol{WHC}(\%) = \frac{[\%Water * g\ sample] - [g\ weight\ loss\ ]}{\%Water * g\ sample} \times 100$$

where the weight loss is defined as:

$$\boldsymbol{Weight\ loss}(\%) = \frac{g\ Weight\ loss\ in\ centrifuge}{g\ orginal\ sample\ weight}$$

3.3.8 Water and protein yield after processing

The yield with respect to protein and water content was calculated to observe changes in these factors during processing. The water and protein yields were calculated as the quantity of water and protein after chilling/thawing divided by the original quantity in the raw material.

$$\boldsymbol{Water\ yield}(\%) = \frac{\%Water\ in\ chilled\ or\ frozen\ fillets}{\%Water\ in\ fresh\ fillets} \times weight\ yield$$

$$\boldsymbol{Protein\ yield}(\%) = \frac{\%Protein\ in\ chilled\ or\ frozen\ fillets}{\%Protein\ in\ fresh\ fillets} \times weight\ yield$$

3.3.9 T_2 transversal relaxation time measurements

The transverse relaxation time, T_2, was measured with CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence. The data was processed with a bi-exponential fit, giving various relaxation times, characteristic for the different water population, i.e. water tightly bound to the muscle structure and free water (less tightly bound water). Fitting the absolute value of the CPMG is shown in following equation:

$$\boldsymbol{Signal} = A_{21} \exp\left(-t/T_{21}\right) + A_{22} \exp\left(-t/T_{22}\right)$$

Where T_{21} and T_{22} were the relaxation components, and A_{21} and A_{22} were the corresponding amplitudes. Since the absolute relaxation amplitudes are proportional to the amount of sample (or water) present, the relative amplitudes within the samples were used. T_{21} population were calculated as:

$$\boldsymbol{A_1 norm} = \frac{A_{21}}{A_{21} + A_{22}}$$

and T_{22} population as:

$$\boldsymbol{A_2 norm} = \frac{A_{22}}{A_{21} + A_{22}}$$

Four parallel samples from each group were averaged. The measurement settings for the T_2 measurement can be viewed in Table 3.3.

Table 3.3. Transverse relaxation time settings

NS	16
RD [s]	10
RG [dB]	70
DS	0
Detection mode	Magnitude
Bandwidth	Narrow
τ [ms]	0.25
N	8100

3.3.10 Sensory analysis

It was of great interest to investigate the effects of brine injection on sensory quality of cooked fillets.

Table 3.4 Sensory attributes and theirs definition for QDA analyses for cod.

Sensory attributes	Scale (0-100)	Definition
Odour		
Sweet	none | strong	Sweet odour
Shellfish	none | strong	Fresh odour
Meat	none | strong	Like boiled meat
Vanilla/boiled milk	none | strong	Vanilla, sweet boiled milk
Potatoes	none | strong	Warm, hole, boiled potatoes
Frozen	none | strong	Odour from refrigerator/freezer
Table cloth	none | strong	Dirty, moist table cloth
TMA	none | strong	TMA, amine, stockfish
Sour	none | strong	Sour milk, acetic acid
Sulphur	none | strong	Sulphur, match, boiled kale
Appearance		
Colour	light | dark	Light: white colour. Dark: yellow, brown, grey
Appearance	homogenise | heterogenic	e.g. spots
Precipitation	none | lot	White precipitation
Flakes	none | lot	Fish sample turns to flakes when pressed with fork
Taste		
Salt	none | strong	Salt taste
Metallic	none | strong	Typical metallic taste of fresh cod
Sweet	none | strong	Typical sweet taste of fresh cooked cod
Meat	none | strong	Like boiled meat
Frozen	none | strong	Refrigerator, frozen storage
Pungent	none | strong	Bitter taste
Sour	none | strong	Damage sour
TMA	none | strong	TMA, stockfish
Off	none | strong	Off-flavour
Texture		
Soft	rigid | soft	First bite
Juicy	dry | juicy	Dry: extract saliva from mouth
Tender	chewy | tender	When chewed for short period
Mushy	low | high	Mush, porridge
Meat	low | high	Like meat texture
clammy	low | high	Clammy (dry red wine, tannin)
rubbery	low | high	Rubber, springy

Fresh cod fillets after chilled storage were evaluated. Prior quantitative descriptive analysis (QDA) evaluation the fillets were kept at chilled storage for 5 days. The samples were heated, 30-40 g for each panellist, at 95-100°C in a pre-warmed oven (Convostherm, Convostar, Germany) with air circulation and steam for 6 minutes in aluminium boxes. Only the loins from the fillets were used for the sensory evaluation. The samples were served warm for the sensory panel. Each sample was

coded with composite of 3 numbers that did not indicate treatment or any other information. Each panellist evaluated 3 samples in each session and each sample was evaluated in duplicate. Samples were evaluated by the QDA method. The method assumes detailed description of a product, such as odour, flavour, appearance and texture (Stone & Sidel 1985). Eight panellists of the Matís sensory panel participated in the sensory evaluation. They were all trained according to international standards (ISO, 1993). The QDA sensory attributes used have been defined in former trials (Table 3.4) (Thorkelsdottir *et al.* 2005).

3.4 Chemical analysis

3.4.1 Water content

The water content was determined by accurately weighing out 5 g of sample (minced fish or brine solutions) in a ceramic bowl with sand. The sample was then mixed to the sand and dried in oven at 102-104 °C for 4 h. The water content was based on weight differences before and after drying (ISO, 1983).

3.4.2 Salt content

The salt content was determined by the method of Volhard according to AOAC 937.18 (2000). Approximately 5 g of sample (minced fish or brine solutions) were weighed into 250 mL plastic bottles and then 200 mL of distilled water was added. The bottles were then shaken for 45 minutes in an electric shaker. After sedimentation 20 mL of the solution were pipette into a 100 mL beaker along with 20 mL of HNO_3 solution. The solution was then titrated with 0.1 N $AgNO_3$ in a 716 DMS Titrino device.

3.4.3 Protein content

The total protein contents of the fish muscle or brine solutions were estimated by Kjeldahl method (ISO, 1997) with the aid of a Digestion System 40 (Tecator AB, Hoganas, Sweden) and calculated using total nitrogen (N) x 6.25.

3.5 Data analysis

Statistical analyses were performed by Microsoft Office Excel 2007 (Microsoft Inc, Redmond, Wash., U.S.A.) and SigmaStat 3.5 (Dundas Software Ltd., GmbH, Germany). Student *t*-test and one way ANOVA were performed on the means of values. The significance level was $p<0.05$.

Results from NMR measurements were obtained from Bruker Minispec software (Bruker Optics, GmbH, Germany).

The sensory software Fizz (Biosystems, 9, rue des Mardors, F-21560 Couternon, France) was used for experiment design and for performance of the sensory evaluation.

Multivariate analysis of the data (principal component analysis) was conducted with the statistical program LatentiX (Latent5 Aps, 2200 Copenhagen N, Denmark) and Unscrambler (Version 9.8, CAMO ASA, Oslo, Norway). Principal component analysis is a statistical tool, for identifying relationships in complex analytical data by comparing data in more than 1 dimension. The main objective is to detect structure in the relationship between measured parameters and experimental factors. It has been used to transform a number of possibly correlated variables in to a (smaller) number of uncorrelated variables called principal components. The 1st component explains as much of the variability in the data as possible, then the 2nd component will account for as much of the remaining variability as possible, and so forth. PCA was performed with chemical (water, salt and protein content) and physiochemical (drip loss, storage yield, WHC, cooking yield and T_2 transversal relaxation times) parameters.

Pearson´s correlation analysis was performed to observe correlation between measured variables. The correlation between two variables reflects the degree to which the variables are related. The results are listed in Appendix G.

4 RESULTS

4.1 Protein product studies

Chemical composition (Table 4.1) and quality properties (Table 4.2) of the fish protein solutions (FPS) were measured. Chemical compositions of the raw material used in the FSP are listed in Table F.1 (Appendix F). The FPH contained the lowest level of water and had therefore higher protein content. FPH had also more amount of salt (3.6%±0.1%) than the other samples. Other FPS had similar chemical composition. This difference in chemical composition might have had an impact on the FPS properties and their influence on the fish muscle.

Table 4.1 Chemical composition of injection solutions (FPS). (n=3)

Injection brines	Water (%)	Protein (%)	Salt (%)
HFP	95.4 ± 0.4	3.0 ± 0.4	1.5 ± 0.1
HFP(a)	97.9 ± 1.2	2.1 ± 0.4	<0.1
HFP(b)	97.6 ± 1.2	2.4 ± 0.4	<0.1
HFP(a)+Gelatine	96.0 ± 1.2	4.0 ± 0.4	<0.1
FPI	95.5 ± 0.4	3.1 ± 0.4	1.4 ± 0.1
FPH	82.6 ± 0.4	14.2 ± 0.4	3.6 ± 0.1
Gelatine	98.1 ± 0.4	1.9 ± 0.4	<0.1
Salt brine (for cod)	98.7 ± 0.1	--	1.3 ± 0.1
Salt brine (for saithe)	94.4 ± 0.3	--	3.6 ± 0.3

The FPS gave different results in the "quality properties measurements". The FPI had low weight loss, high viscosity and a high pH value compared with the other FPS. According to Fennema (1990), the mean isoelectric point (pI) of the myofibrillar proteins are about pH 5-6. Minimum water holding capacity, swelling and protein solubility of meat has been observed around the pI, but it increases again with either decreasing or increasing pH value.

The SDS-PAGE results showed how the fish protein solutions differ in protein composition. The FPH and the gelatine solutions contained much smaller protein

units while the other fish protein solutions contained much larger protein units and even myofibrils. The results from the SDS-PAGE are shown in Appendix F.

Table 4.2. Quality properties of the fish protein solutions (FPS). (average ± stdv. of n=3 samples). [a-b]) Values with the same superscript letter within a column are not significantly different (p>0.05).

Sample	Weight loss (%)	Viscosity (BU)[1]	Viscosity (Pascal)[2]	pH
HFP	95.2 ± 1.5[a]	42.0 ± 2.0[a]	--	6.9 ± 0.1[a]
HFP(a)	95.9 ± 0.5[a]	43.5 ± 2.0[a]	0.079 ± 0.003[ab]	7.1 ± 0.1[ab]
HFP(b)	95.2 ± 0.8[a]	37.0 ± 2.0	0.057 ± 0.007[ab]	6.85 ± 0.1[a]
HFP(a)+*Gelatine*	95.8 ± 0.5[a]	43.5 ± 2.0[a]	0.079 ± 0.005[ab]	6.61 ± 0.1[a]
FPI	88.5 ± 2.9	46.5 ± 2.1	0.181 ± 0.004[b]	9.28 ± 0.1
FPH	94.8 ± 2.6[a]	0.0	0.024 ± 0.000[a]	7.67 ± 0.1[b]
Gelatine	--	0.0	0.0	5.51 ± 0.1

[1]At +5°C
[2]At +5 to +7°C

4.2 Injection studies

4.2.1 Weight gain after injection

Weight gain of fillets after injection(s) is shown in Figure 4.1 and Table A.1 (Appendix A). The injection of FPS resulted in 5.1% to 16.1% weight gain. Fresh cod fillets and fresh skinless saithe fillets gained more weight when injected with different protein solutions compared to light salted cod fillets. The most effective protein solution with respect to weight gain was HFP, both for fresh cod fillets and fresh satihe fillets, 16.1% and 15.7%, respectively.

Both fresh and light salted cod fillets injected with FPI gained more weight than fillets injected with FPH (P<0.05). Light salted cod fillets injected with FPH gained the lowest weight (p<0.05) compared with the other fillets while FPI and HFP gave similar effects on the light salted cod fillets.

The fresh saithe fillets gained approximately 5% weight when injected first with 4% salt brine and then again gained weight of 8-10% when injected with the fish protein

solutions. Fresh saithe fillets injected first with 4% salt and then with HFP(a) and HFP(b) gained most weight while fillets injected only with 4% salt gained the lowest weight (p<0.05). Addition of gelatin, alone and combined with protein solution (HFP(a)), had no additional effects on the weight gain compared to fillets injected only with salt and fillets injected with HFP(a).

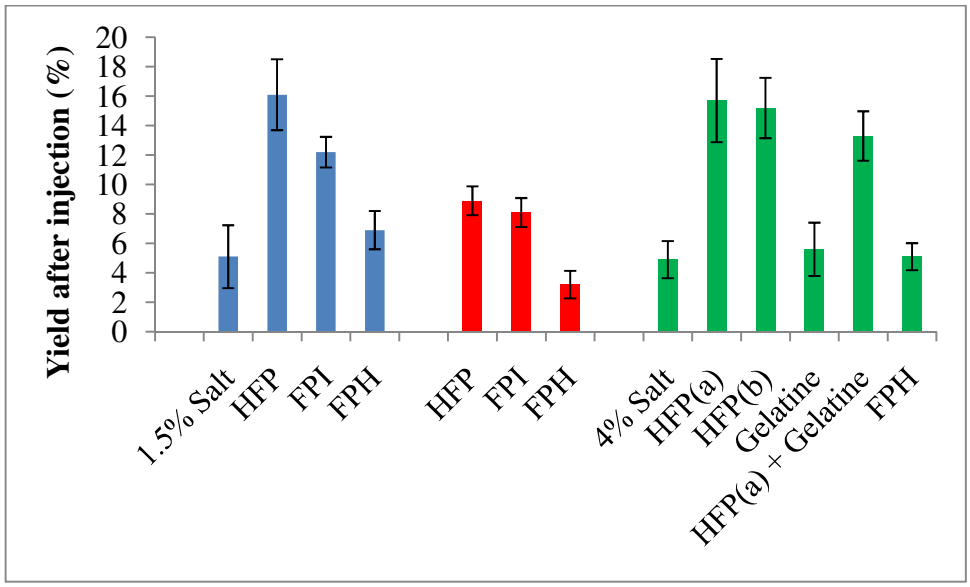

Figure 4.1. Weight gain (%) after injection(s) of fresh cod fillets (n=20), light salted cod fillets (n=20) and fresh skinless saithe fillets (n=26). Saithe fillets treated with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine. Statistical difference is listed in details in Appendix A.

4.2.2 Drip loss after storage

Drip loss of fresh cod fillets after various storage times at +2°C is shown in Figure 4.2. Fillets injected with HFP gave on all sampling days the lowest value of drip loss (p<0.05). The difference between control fillets and fillets injected with 1.5% salt was on the other had not significant. The difference in drip loss within each group (Control, Salt and HFP) during the storage time was not significant (p>0.05).

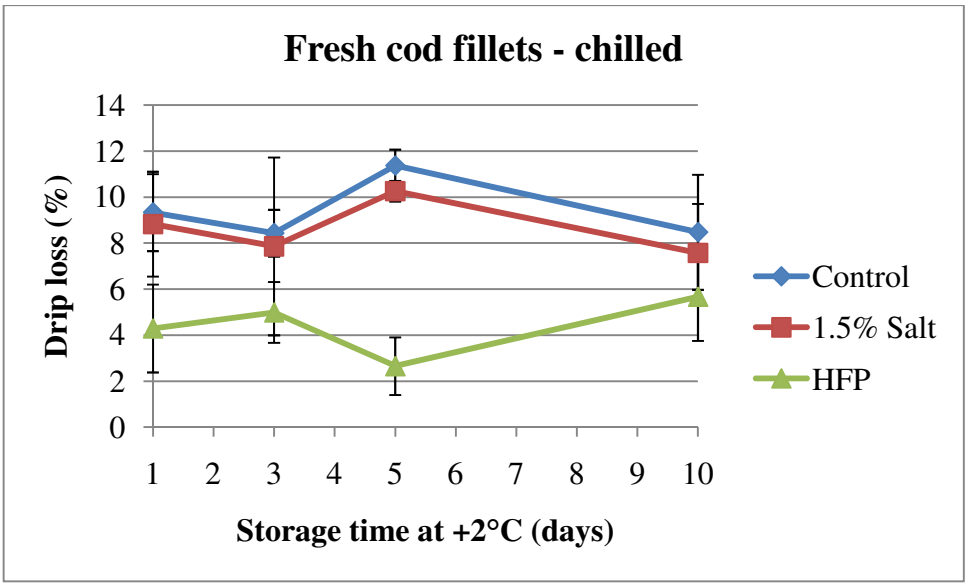

Figure 4.2. Drip loss (%) of fresh cod fillets after various times at chilled storage (+2°C) (n=6 per group).

The drip loss (thaw drip) of fresh and light salted cod fillets after 1 month of frozen storage is shown in Figure 4.3 and Table A.6 (Appendix A). Injection of salt or fish protein solutions into fresh cod fillets reduced drip loss compared with control fillets. The least effect on drip loss was obtained from FPI while the most effect was obtained from FPH. The same trend was shown for the light salted cod fillets. Addition of FPH into light salted cod fillets resulted in lower drip compared to the control group (p<0.05).

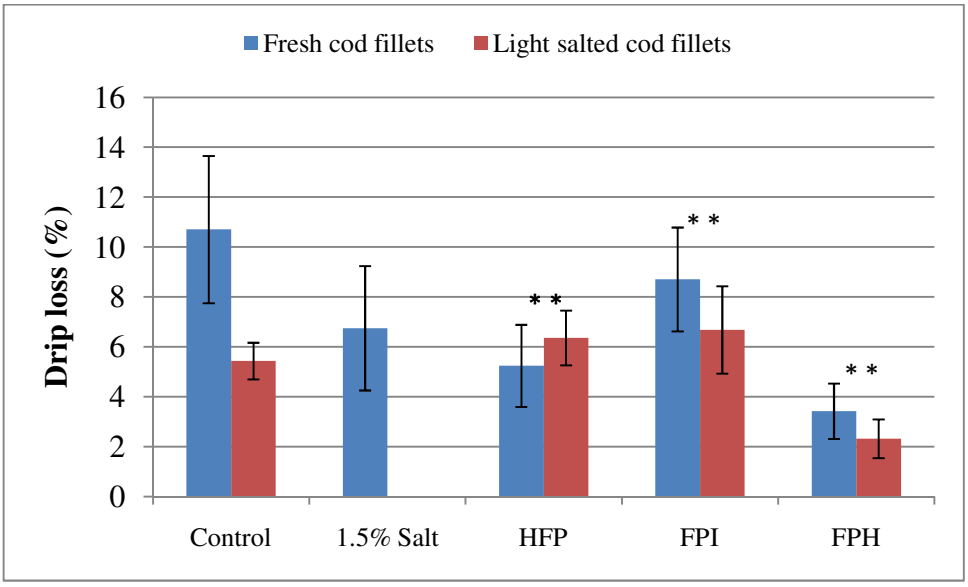

Figure 4.3. Drip loss (%) of fresh and light salted cod fillets (n=10) after 1 month of frozen storage. *) Asterisk denotes no significant difference between values of fresh and light salted cod fillets within each treatment group (p>0.05). Statistical difference is listed in details in Appendix A.

The drip loss of fresh skinless saithe fillets after 4 days storage at +2°C and 1 week and 1 month storage at -24°C is shown in Figure 4.4 and Table A.7 (Appendix A). Addition of salt and/or fish protein solutions into fillets did not decrease the drip loss compared with control fillets. Drip loss of most of the fillets increased during frozen storage. Control fillets and fillets injected only with FPH showed no significant difference in drip loss during storage.

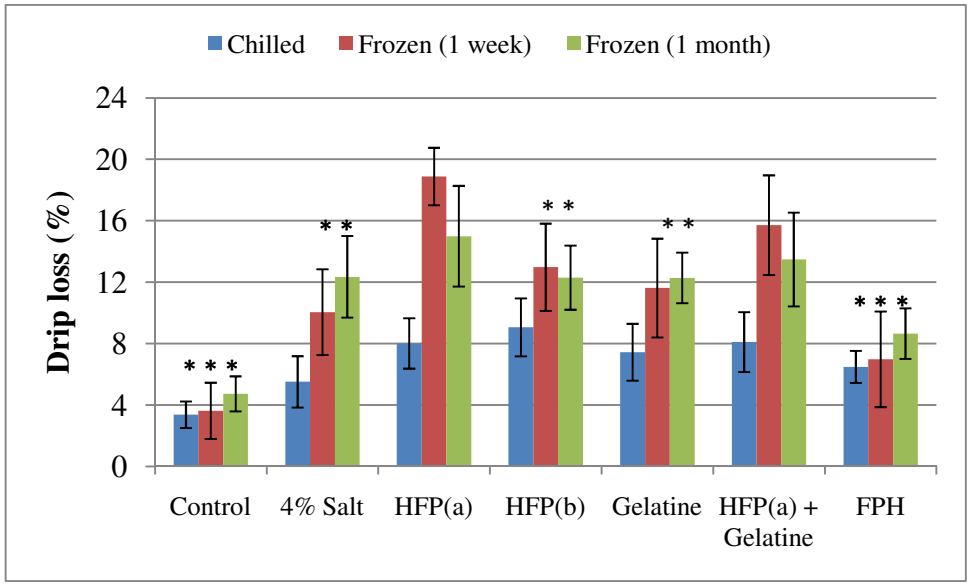

Figure 4.4 Drip loss (%) of the fresh skinless saithe fillets after chilled (n=6) and frozen storage (1 week and 1 month) (n=10). Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine. *) Asterisk denotes no significant difference between values (chilled, frozen 1 week and frozen 1 month) within each treatment group (p>0.05). Statistical difference is listed in details in Appendix A.

4.2.3 Yield (%) after storage

Injection with fish protein solution (HFP) increased the yield of fresh cod fillets after chilled and frozen storage (Figure 4.5 and Figure 4.6) compared with control fillets and fillets injected with 1.5% salt brine. Control fillets showed at all times the lowest values of storage yield. The storage yield of fillets with HFP were lower after 10 days storage at +2°C compared with day 1, but were still significantly higher (p<0.05) than the other fillets. The difference in storage yield between the control fillets and fillets injected with 1.5% salt during the storage time was not significant (p>0.05).

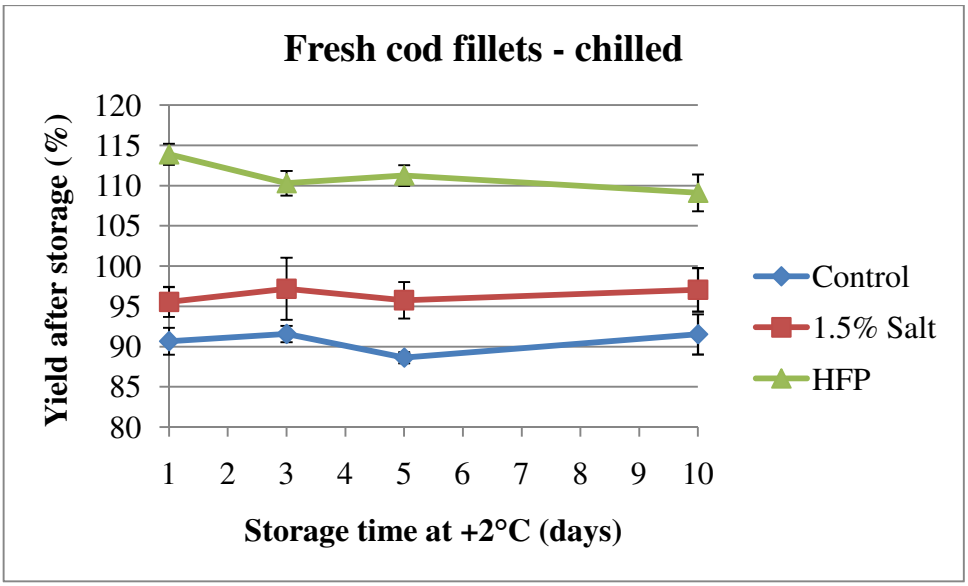

Figure 4.5 Yield (%) of fresh cod fillets (n=6) after various times at chilled storage (+2°C). Statistical difference is listed in details in Appendix A.

The yield of fresh and light salted cod fillets after 1 month at frozen storage is shown in Figure 4.6 and Table A.4 (Appendix A). The lowest value of yield (p<0.05) after frozen storage of the fresh cod fillets were obtained for the control fillets. The fresh cod fillets injected with FPI and FPH showed the highest (p<0.05) yield after 1 month at frozen storage compared with other fillets. Addition of HFP into the fresh cod fillets resulted also in higher storage yield compared with control fillets and fillets injected with 1.5% salt. The fish protein solutions also increased the yield of light salted cod fillets after 1 month of frozen storage compared with control fillets. The highest value of storage yield was obtained for fillets injected with HFP (p<0.05). In contrast to fresh cod fillets, addition of FPH into light salted cod fillets did not improve the storage yield compared to the control fillets (p>0.05). Comparison of the fresh and light salted cod fillets showed significant difference between the control fillets and fillets injected with HFP where the light salted fillets gave higher yield after frozen storage. On the other hand, fresh cod fillets with FPH showed significant higher yield than light salted cod fillets with the same additive. No significant difference was found between the fresh and the light salted cod fillets with added FPI.

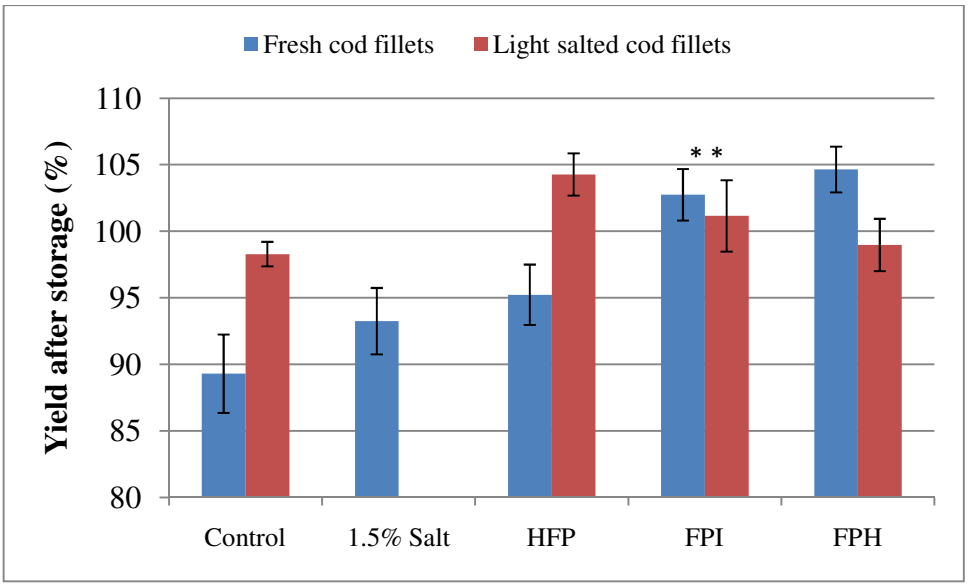

Figure 4.6. Yield (%) of fresh and light salted cod fillets after 1 month of frozen storage (thawed) (n=10). *) Asterisk denotes no significant difference between values of fresh and light salted cod fillets within each treatment group (p>0.05). Statistical difference is listed in details in Appendix A.

Addition of fish protein solutions into fresh skinless saithe fillets did not show a decisive impact as was seen for the fresh cod fillets. The values of storage yield were significant different (p<0.05) between the groups of chilled saithe fillets (Figure 4.7 and Table A.5). The highest values were obtained for the fillets treated with HFP (HFP(a), HFP(b) and HFP(a) + Gelatine). Addition of gelatin combined with salt resulted in increased yield compared to the control group (p<0.05), but the effect were similar when salt was used alone. Addition of gelatin, combined with HFP(a), had no additional improvement on the storage yield. The values of storage yield after one week and one month of frozen storage were also significant different (p<0.05) between the groups. The difference was on the other hand not significant when treated fillets were compared to the control fillets. The highest value of yield after one month of frozen storage was obtained for fillets injected with HFP(b) and the lowest for fillets injected only with 4% salt brine (p<0.05). The storage yield of all the groups decreased after one week of frozen storage compared to after chilled storage (p<0.05), except for fillets injected only with FPH. The difference in storage yield after one week and one month of frozen storage was on the other hand not significant, except for the control fillets and fillets injected only with FPH.

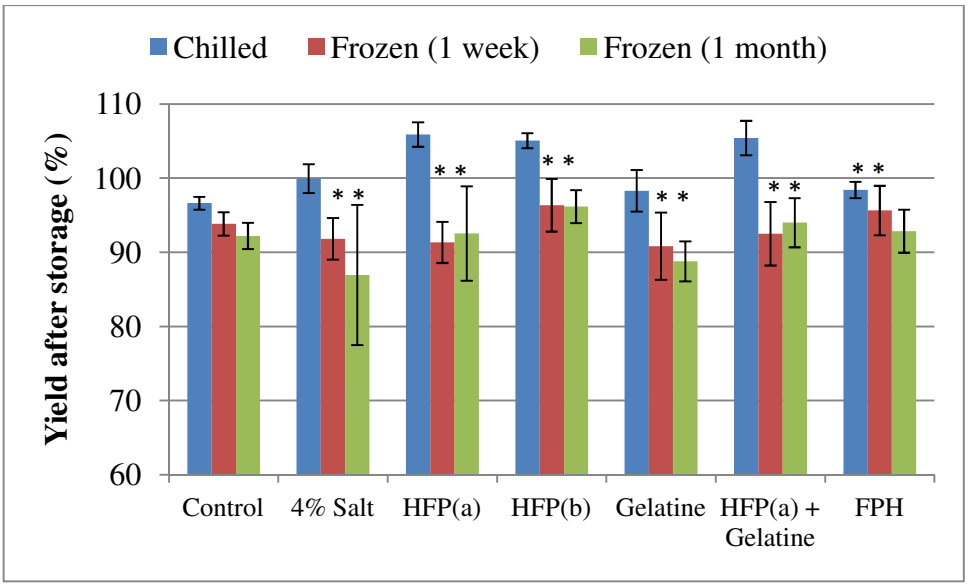

Figure 4.7. Yield (%) of fresh skinless saithe fillets after 5 days of chilled storage (n=6); after 1 week and 1 month of frozen storage (thawed) (n=10). Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a) + Gelatine were first injected with 4% salt brine. *) Asterisk denotes no significant difference between chilled, frozen 1 week and frozen 1 month fillets within each treatment group (p>0.05). Statistical difference is listed in details in Appendix A.

4.2.4 Cooking yield (%)

The cooking yield of fresh and light salted cod fillets is shown in Figure 4.8 (and Table A.8). Addition of the HFP fish protein solution into chilled fresh cod fillets showed no improvement on yield after cooking compared with the control fillets. Comparison between groups of fresh cod fillets after frozen storage showed only significant difference between control fillets and fillets with added FPH (p<0.05), where FPH had the weakest effect on cooking yield. Within the light salted fillets, FPH had on the other hand stronger effects on yield after cooking compared with other treated fillets. The weakest impact on cooking yield of light salted cod fillets were obtained from FPI. When the groups of fresh and light salted cod fillets after frozen storage are compared, no significant differences were found between the control fillets and between the fillets injected with HFP. Overall, addition of fish protein solutions into fresh and light salted cod fillets showed none or very small improvement on yield after cooking compared with control fillets and/or fillets injected with 1.5% salt brine.

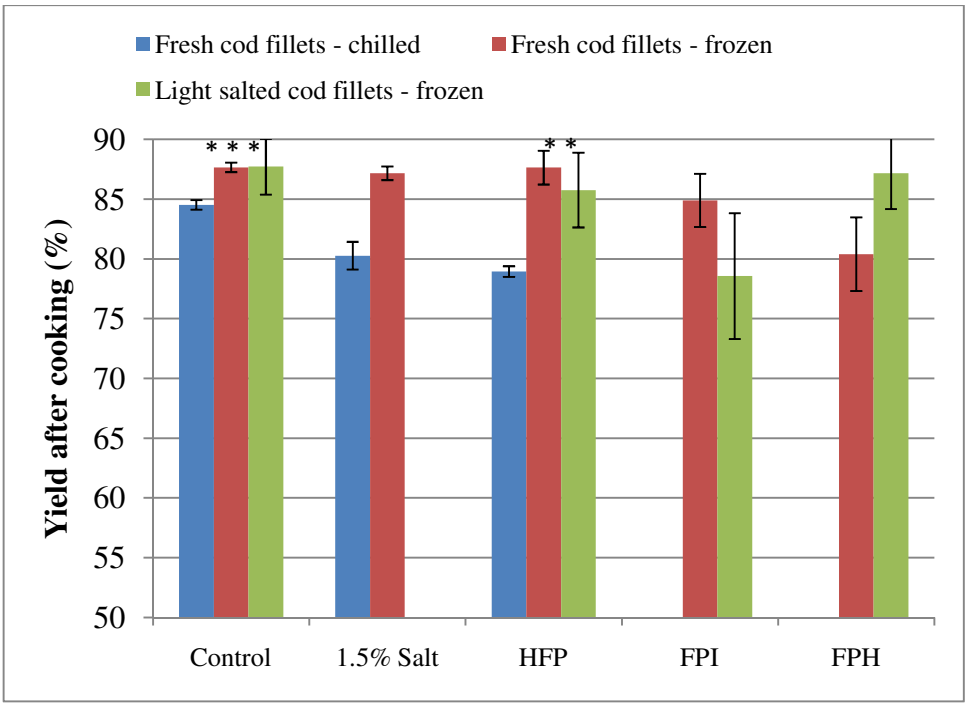

Figure 4.8. Cooking yield (%) of fresh cod fillets after 5 days of chilled storage and fresh and light salted cod fillets after 1 month of frozen storage (n=3). *) Asterisk denotes no significant difference between values of fresh cod fillets (chilled and/or frozen) and light salted cod fillets within each treatment group (p>0.05). Statistical difference is listed in details in Appendix A.

The highest values of cooking yield for the chilled saithe fillets (p<0.05) were obtained for fillets injected only with 4% salt brine and only with FPH (Figure 4.9 and Table A.9). After 1 week of frozen storage, the highest values were obtained for fillets injected with 4% salt, with HFP(a)+Gelatine and with FPH (p<0.05). The same trend was shown after one month of frozen storage. The values of cooking yield decreased significant (p<0.05) for the control fillets and fillets injected only with 4% salt after frozen storage (1 week and 1 month). On the other hand, the cooking yield for fillets with added HFP(b) and HFP(a)+Gelatine increased significantly (p<0.05) after frozen storage. Addition of protein solutions into fresh saithe fillets showed overall no additional improvement in cooking yield above salt, but less deterioration after frozen storage which can be an advantage.

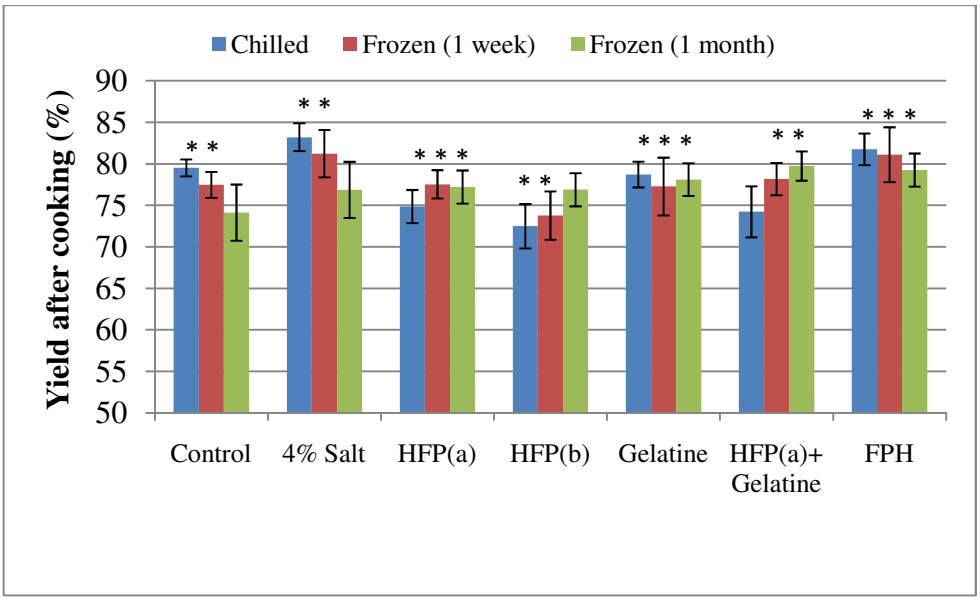

Figure 4.9. Cooking yield (%) of fresh skinless saithe fillets after 4 days of chilled storage; after 1 week and 1 month of frozen storage (thawed) (n=3). Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a) + Gelatine were first injected with 4% salt brine. *) Asterisk denotes no significant difference between chilled, frozen 1 week and frozen 1 month fillets within each treatment group (p>0.05). Statistical difference is listed in details in Appendix A.

4.2.5 Total yield

Evaluation of total yield after cooking is shown in Figure 4.10 and Figure 4.11. Evaluation of total yield of the fillets was determined by multiplying the mean value of yield after each processing step (injection, storage) and the mean value of cooking yield. The highest value of total yield of fresh cod fillets after chilled storage was obtained for fillets injected with HFP and the lowest for the control fillets. After frozen storage, the fresh cod fillets with added fish protein solutions showed also better total yield compared with control fillets and fillets injected with 1.5% salt. The light salted cod fillets (after frozen storage) gave on the other hand different results. Only fillets injected with HFP showed higher total yield than the control fillets. The fish protein solutions showed very different effects on the fresh and light salted cod fillets after frozen storage. HFP had greater impact on the light salted cod fillets while FPI showed better impact on the fresh cod fillets. FPH, on the other hand, gave similar total yield for both fresh and light salted cod fillets.

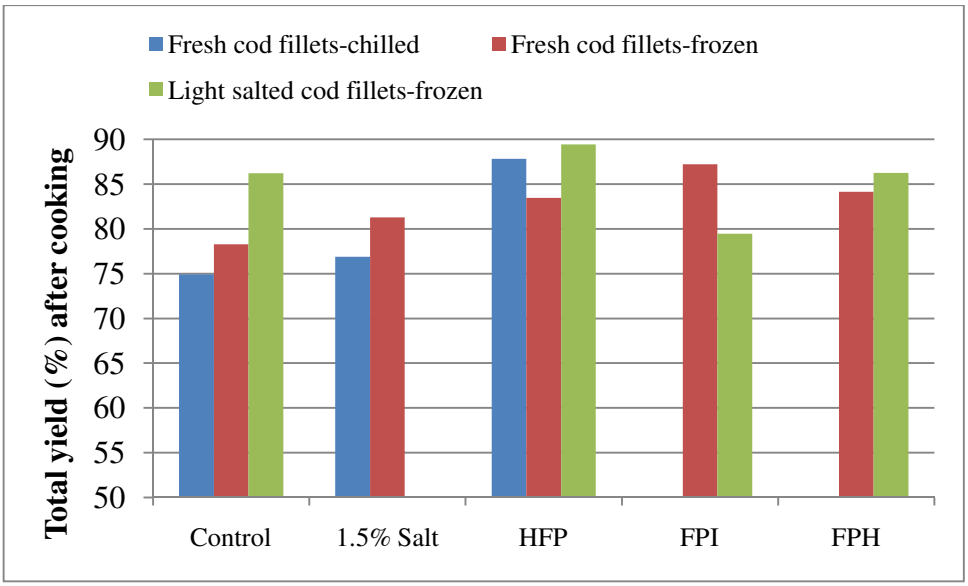

Figure 4.10. Total yield (%) after cooking of fresh cod fillets after 5 days of chilled storage and fresh and light salted cod fillets after 1 month of frozen storage

The total yield for the fresh saithe fillets were in contrast to the total yield of the fresh and light salted cod fillets. The highest value after chilling was obtained for fillets injected only with 4% salt, but the total yield decreased considerably after frozen storage and showed the lowest value after 1 month of frozen storage. After one month of frozen storage, the fillet injected with protein solutions showed higher values of total yield compared with the control group and the fillets injected only with 4% salt brine.

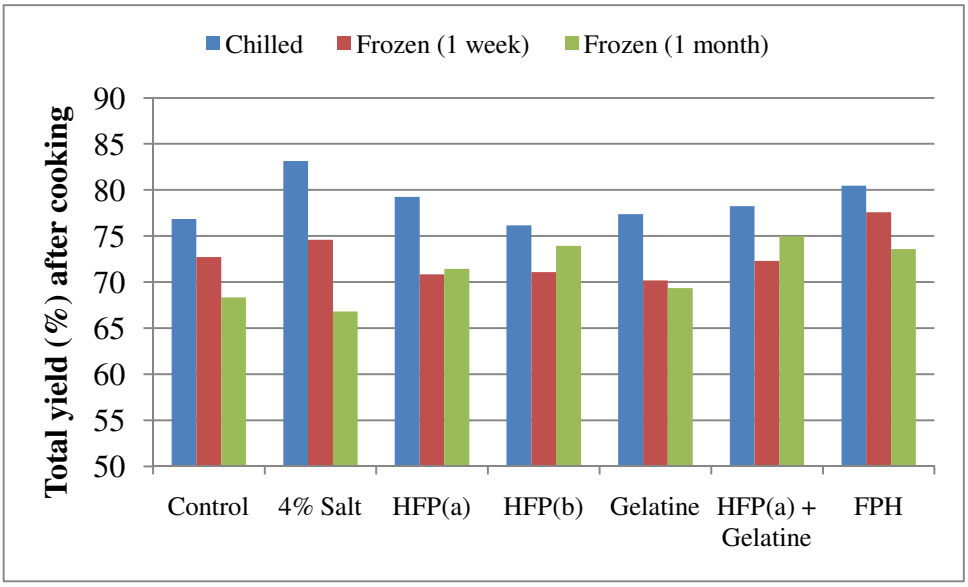

Figure 4.11. Total yield (%) after cooking of fresh skinless saithe fillets after chilled (4 days) and frozen (1 week and 1 month) storage. Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a) + Gelatine were first injected with 4% salt brine.

4.2.6 Water holding capacity

The water holding capacity (WHC) of the fresh and light salted cod fillets is shown in Figure 4.12 (and Table B.1). The difference in WHC of the fresh cod fillets after chilled storage was not significant (p>0.05). Addition of fish protein solutions to fresh and light salted cod fillets showed also no improvement after frozen storage compared with control fillets. The lowest value of water holding capacity of the frozen cod fillets was obtained for the fresh fillets injected with FPI (62.9% ± 3.5%). The highest value within the chilled fresh cod fillets was obtained in the fillets injected with FPH (76.9% ± 2.2%). The water holding capacity of the light salted fillets was always higher than for the fresh fillets but the difference between the groups of light salted fillets was not significant (p>0.05).

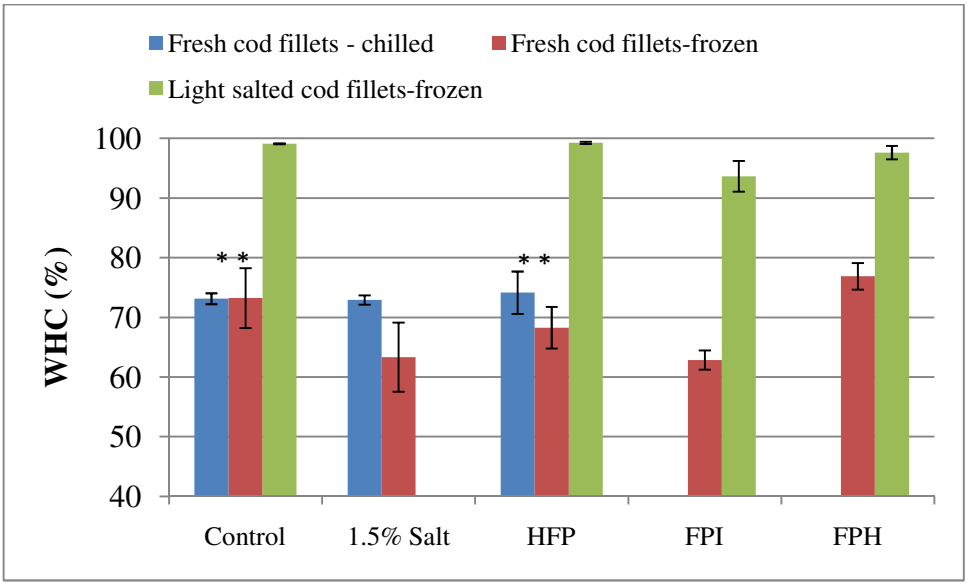

Figure 4.12. Water holding capacity (%) of fresh cod fillets after 5 days of chilled storage and fresh and light salted cod fillets after 1 month of frozen storage (n=4). (The chilled fresh cod fillets were not injected with FPI and FPH). *) Asterisk denotes no significant difference between values of fresh cod fillets (chilled and/or frozen) and light salted cod fillets within each treatment group (p>0.05). Statistical difference is listed in details in Appendix B.

The water holding capacity of the fresh saithe fillets is shown in Figure 4.13 (and Table B.2). The fish protein solutions showed no improvement of WHC after chilled or frozen storage compared with control fillets and fillets injected only with 4% salt brine. The WHC decreased considerably during frozen storage for all the fillets compared to chilled fillets (p<0.05). Addition of FPH before frozen storage scored

highest in WHC compared with other protein solutions and was even higher after 1 month of frozen storage compared with 4% salt brine.

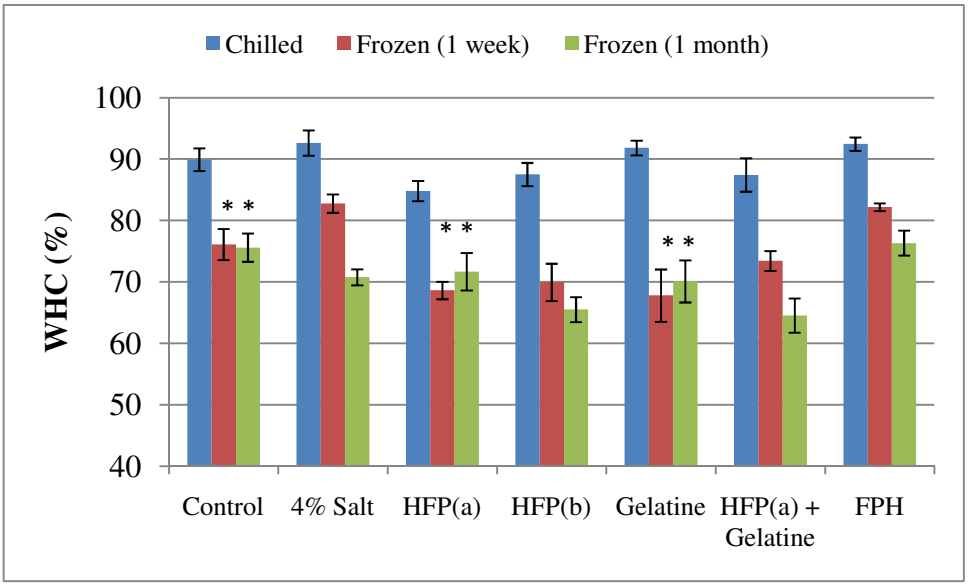

Figure 4.13. Water holding capacity (%) of fresh skinless saithe fillets (n=4) after chilled and frozen storage (1 week and 1 month). Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a) + Gelatine were first injected with 4% salt brine. *) Asterisk denotes no significant difference between chilled, frozen 1 week and frozen 1 month fillets within each treatment group (p>0.05). Statistical difference is listed in details in Appendix B.

4.2.7 T_2 transversal relaxation times

The transversal relaxation times were measured at room temperature. The T_{21} express the behaviour of tightly bound water between muscle cells, but T_{22} express on the other hand the behaviour of the less bound water or free water. The water molecules are therefore more bound as the T_2 is shorter.

The T_{21} relaxation times of fresh cod fillets after 1 month of frozen storage (Table 4.3) were significantly longer (p<0.05) for fillets treated with FPH (approximately 54 ms) compared with the control fillets (approximately 47 ms), indicating that the water mobility was lower in the control muscle structure than in the FPH. No significant difference was found between the control fillets and the fillets treated with FPI. The T_{22} relaxation times of fresh cod fillets after 1 month of frozen storage were not significant (p>0.05) between the groups.

Table 4.3. Transversal relaxation time T_2 for the fresh cod fillets (n=4) after 1 month of frozen storage (average ± stdv.). [a]) Values with the same superscript letter within a column are not significantly different (p>0.05).

Treatment	T_{21}(ms)	T_{22}(ms)
Control	46.9 ± 1.9[a]	264.6 ± 24.5[a]
FPI	47.3 ± 1.0[a]	238.1 ± 14.6[a]
FPH	53.7 ± 0.5	285.1 ± 8.3[a]

The T_{21} and T_{22} relaxation times of fresh saithe fillets after chilled and frozen (1 week and 1 month) storage are shown in Table 4.4. The chilled fillets with added fish protein solutions and/or 4% salt gave significantly (p<0.05) longer T_{21} relaxation times compared with the control fillet, except fillets injected only with FPH. The highest values were obtained for fillets with added HFP(a) and/or gelatine. The values of T_{21} relaxation times decreased significant during frozen storage (1 week and 1 month) compared with fillets after chilled storage. The differences between fillets with added fish protein solutions and control fillet or fillets injected with 4% salt were not significant.

The chilled fillets with added gelatine gave significantly (p<0.05) longer T_{22} relaxation time. The difference between other chilled fillets with added fish protein solutions and control fillets or fillets injected only with 4% salt was not significant. The values of T_{22} relaxation times decreased significant after 1 week of frozen storage compared with fillets after chilled storage, except for fillets injected with HFP(b) and FPH. The highest value (p<0.05) after 1 week of frozen storage were obtained for fillets with added HFP(b). The control fillets and fillets injected only with 4% salt were significantly lower compared with fillet with added fish protein solutions. The values of T_{22} relaxation times increased again after 1 month of frozen storage for most of the groups except for fillets injected with HFP(b) and FPH. The highest values (p<0.05) were obtained for fillets injected with HFP(a) and/or gelatine. Other treated fillets showed similar results (p>0.05) compared with control fillets. No significant differences were found between the values after chilled storage and values after 1 month of frozen storage for most of the groups.

Table 4.4. Transverse relaxation time T_2 for the fresh skinless saithe fillets (n=4) after 4 days of chilled storage, 1 week and 1 month of frozen storage (average ± stdv.). Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a) + Gelatine were first injected with 4% salt brine. [a-c]) Values with the same superscript letter within a column are not significantly different (p>0.05). *) Asterisk denotes significant difference within row. Each sample was measured in quadruple.

	Chilled		***Frozen (1 week)***		***Frozen (1 month)***	
Group	**T_{21}(ms)**	**T_{22}(ms)**	**T_{21}(ms)**	**T_{22}(ms)**	**T_{21}(ms)**	**T_{22}(ms)**
Control	50.8 ± 0.9^{a}	$248.4 \pm 19.6^{a*}$	$46.8 \pm 1.0^{a*}$	154.5 ± 10.8^{a}	$45.5 \pm 1.3^{a*}$	$215.2 \pm 18.7^{ab*}$
4% Salt	57.3 ± 1.0	$225.2 \pm 15.1^{abc*}$	$49.3 \pm 1.1^{bc*}$	164.4 ± 15.4^{a}	$50.9 \pm 1.7^{b*}$	$197.1 \pm 27.1^{a*}$
HFP(a)	60.5 ± 1.4^{b}	$242.2 \pm 21.3^{ac*}$	$46.1 \pm 0.9^{a*}$	190.0 ± 8.9^{b}	$47.8 \pm 1.0^{b*}$	$223.1 \pm 4.4^{b*}$
HFP(b)	54.4 ± 1.3	$205.0 \pm 8.6^{b*}$	$48.6 \pm 1.1^{c*}$	302.4 ± 18.9	$47.9 \pm 0.5^{ab*}$	$222.7 \pm 14.4^{b*}$
Gelatine	59.3 ± 0.7^{b}	274.8 ± 26.7*	50.5 ± 1.1^{b}	198.6 ± 7.6^{b}	47.6 ± 1.0^{b}	$258.1 \pm 12.3^{c*}$
HFP(a) +Gelatine	60.7 ± 1.5^{b}	$218.4 \pm 10.3^{bc*}$	$46.5 \pm 1.5^{a*}$	$191.9 \pm 23.4^{b*}$	$46.3 \pm 1.8^{a*}$	272.8 ± 14.4^{c}
FHP	51.3 ± 1.2^{a}	$215.9 \pm 12.6^{bc*}$	$45.6 \pm 0.3^{a*}$	$195.8 \pm 9.3^{b*}$	$46.7 \pm 0.4^{ab*}$	$201.7 \pm 11.0^{ab*}$

Figure 4.14 shows normalised distribution of water in fresh cod fillets after 1 month of frozen storage. 83-85% of the proton relaxation amplitude was reflected as T_{21} population (A_1), and the lowest T_{21} population values were obtained for the control fillets, where A_1 express the relatively amount of water which is strongly bound to the sample. The difference between the fillets were on the other hand not significant (p>0.05).

In Figure 4.15, normalised distribution of water in fresh saithe fillets after chilled and frozen storage are shown. 78-87%, 71-82% and 72-82% of the proton relaxation amplitude was reflected as T_{21} population (A_1) for chilled fillets, fillets frozen for 1 week and frozen for 1 month, respectively. There was significant difference (p<0.05) between the groups within each storage condition. For the chilled fillets, the highest value (p<0.05) of A_1 was obtained for fillets injected with HFP(b). When frozen, the highest A_1 values were obtained for control fillets and fillets injected only with FPH. The apparent population (A_1 and A_2) were compared within each group to investigate the effects from different storage conditions. The A_1 were always higher for chilled fillets compared to the frozen fillets in each group. When comparing the frozen fillets, the A_1 population decreased with time for most of the groups. There was no difference between the frozen fillets within control fillets and fillets injected with gelatine, and the A_1 population increased in fillets injected with HFP(b).

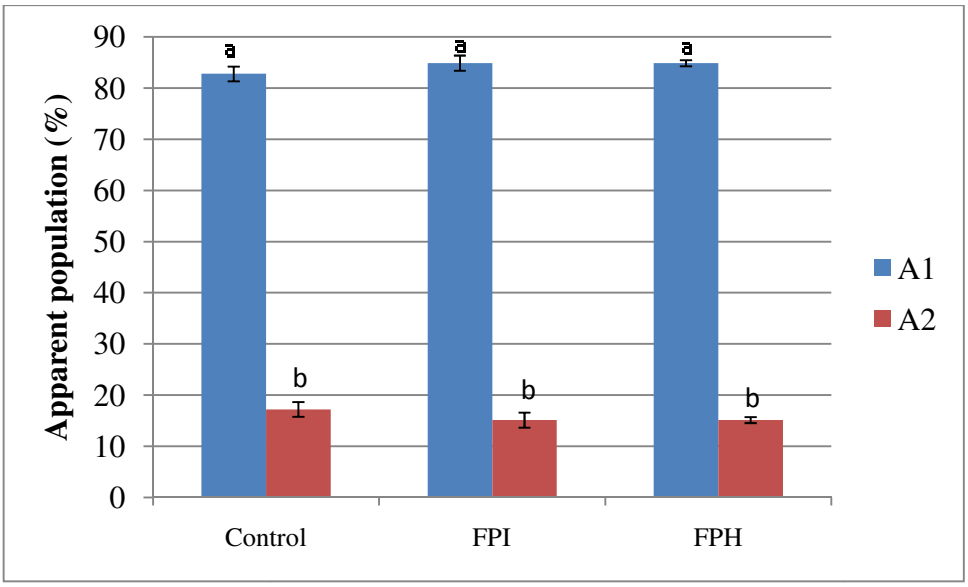

Figure 4.14. Normalised distribution of water in fresh cod fillets (n=4) after 1 month of frozen storage. The A_1 express the relatively amount of water which is strongly bound to the samples and the A_2 express the relatively amount of water which is loosely bound to the samples. [a-b]) Not significant difference between groups are market with the same letter (p>0.05).

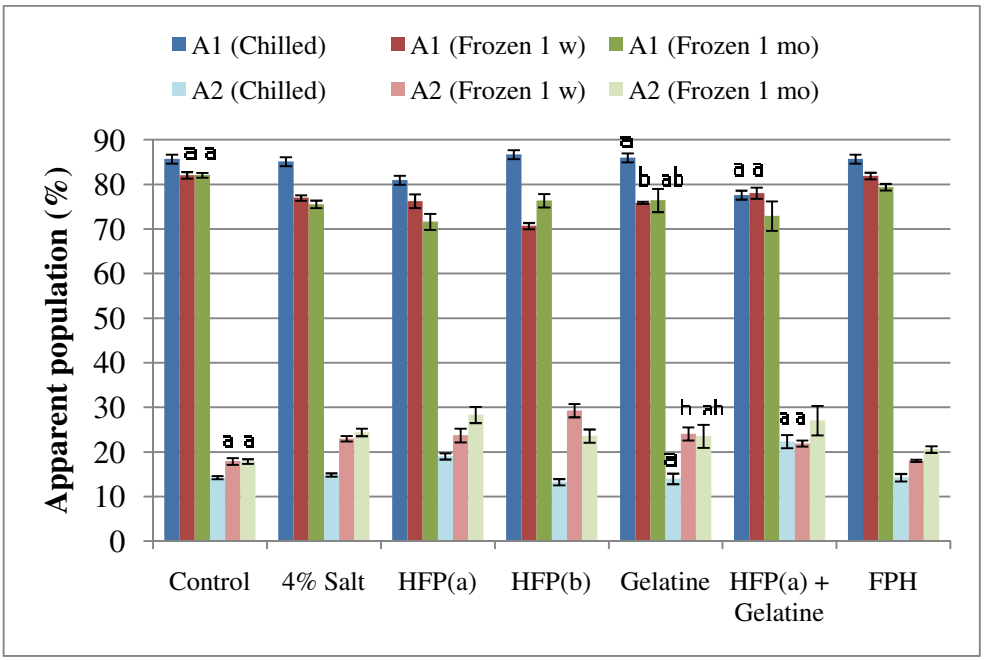

Figure 4.15. Normalised distribution of the water, in fresh skinless saithe fillets (n=4) after 4 days of chilled storage, 1 week and 1 month of frozen storage. A_1 express the relatively amount of water which is strongly bound to the samples and A_2 express the relatively amount of water which is loosely bound to the samples. Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a) + Gelatine were first injected with 4% salt brine. [a-b]) Not significant difference between values within each group are market with the same letter.

4.2.8 Chemical composition

The water content of fresh cod fillets before brine treatment and freezing was 81.7% ± 0.4%; the protein content was 17.4% ± 0.4% and the salt content was 0.1% ± 0.1%. For light salted cod fillets, the water content was 85.3% ± 0.4%; the protein content 12.1% ± 0.4% and the salt content 2.6% ± 0.1%. After 5 day at +2°C, the addition of HFP resulted in significant higher (p<0.05) water content (84.4% ± 0.4%) of the fresh cod fillets than in the control fillets (81.7% ± 0.4%) and fillets injected with 1.5% salt (82.9% ± 0.4%). After 1 month of frozen storage, the water contents of the fresh cod fillets were all similar, but fillets injected with FPI had slightly higher water content compared with other groups. The same trend was obtained for the light salted cod fillets after 1 month of frozen storage. The protein content of the chilled fresh cod fillets with HFP was lower than in the control fillets and fillets injected with 1.5% salt. The protein content of the fresh cod fillets after 1 month of frozen storage was very similar for all of the groups and the same trend was obtained for the light salted cod fillets. The dissimilarity between the fresh and light salted cod fillets after 1 month of frozen storage was that light salted fillets had higher water content than the fresh fillet and had therefore lower protein content, but it is known that changes in water and protein content are negatively correlated as could been seen in this trials (Figure 4.16 and Figure 4.17).

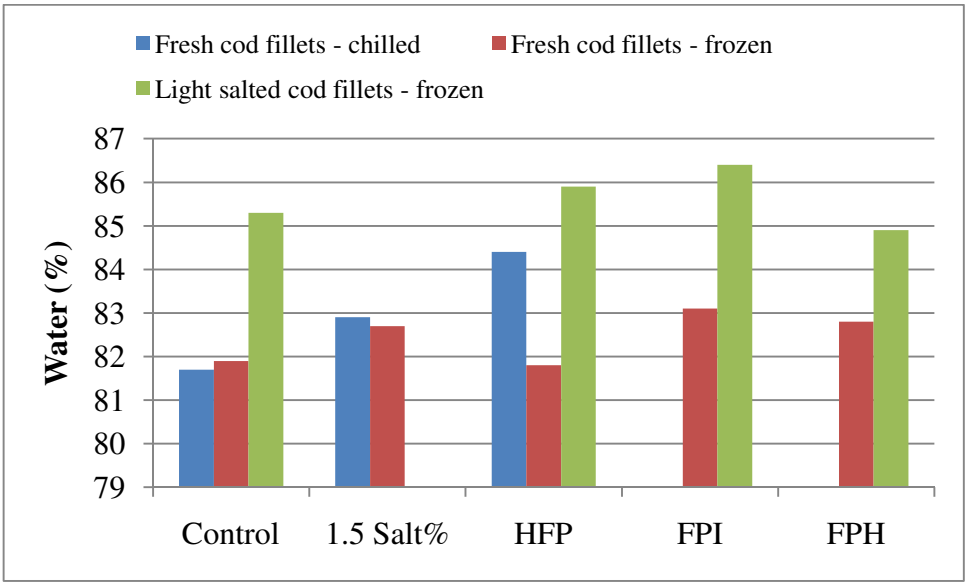

Figure 4.16. Water content of fresh cod fillets after 5 days of chilled storage and fresh and light salted cod fillets after 1 month of frozen storage (n = 3). Statistical difference is listed in details in Appendix D.

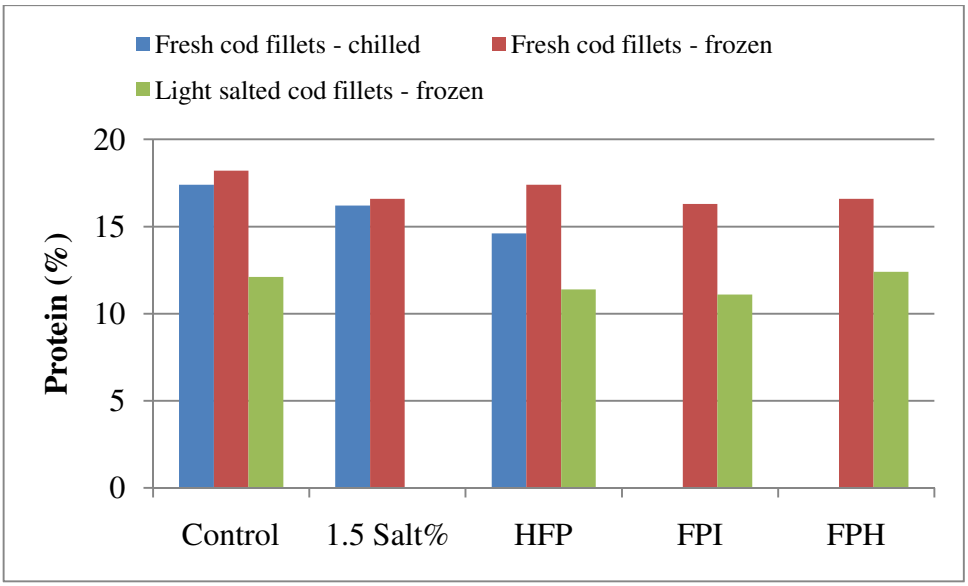

Figure 4.17. Protein content of fresh cod fillets after 5 days of chilled storage and fresh and light salted cod fillets after 1 month of frozen storage (n = 3). Statistical difference is listed in details in Appendix D.

Water and protein content of fresh saithe fillets after chilled (4 days) and frozen (1 week and 1 month) storage is shown in Figure 4.18 and Figure 4.19. The water content of the saithe fillets before brine treatment and chilling/freezing was 81.5% ± 1.2%; the salt content was 0.2% ± 0.1% and the protein content was 18.5% ± 0.4%. The addition of protein solutions and/or salt resulted in higher water content (83.3% to 85.4%) than in the control fillets. During frozen storage the water content of the fillets decreased. The protein content of the fillets treated with protein solutions and/or salt was generally lower than in the control fillets. This could be explained by dilution effects on the dry material in the fillets by added water. The protein content of the fillets generally increased during frozen storage compared to the chilled fillets. The saithe fillets with added FPH showed on the other hand very small changes in water and protein content during chilled and frozen storage.

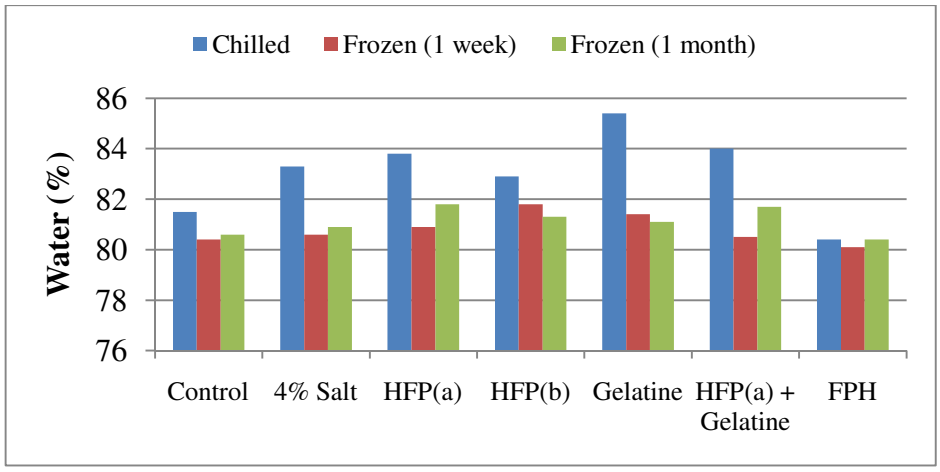

Figure 4.18. The water content of fresh skinless saithe fillets after 4 days of chilled storage, 1 week and 1 month of frozen storage (n=3). Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a) + Gelatine were first injected with 4% salt brine. Statistical difference is listed in details in Appendix D.

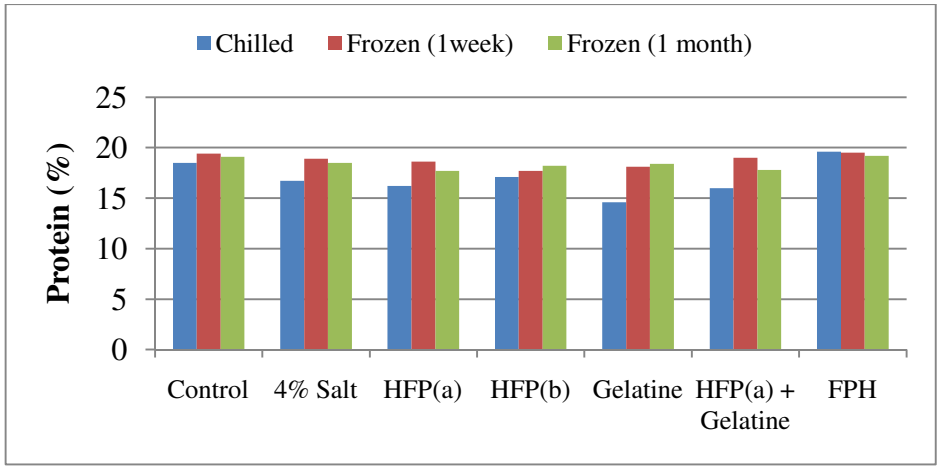

Figure 4.19. The protein content of fresh skinless saithe fillets after 4 days of chilled storage, 1 week and 1 month of frozen storage (n=3). Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a) + Gelatine were first injected with 4% salt brine. Statistical difference is listed in details in Appendix D.

Salt contents of the controls fillet and injected fillets are listed in Table 4.5 and Table 4.6. Injection with pure salt brine and/or brine containing fish protein solutions resulted in higher salt content of the fillets. Fresh cod fillets were injected with 1.5% salt brine while fresh saithe fillets were injected with 4% salt brine. The final salt concentrations in the cod and in the saithe fillets were similar, 0.4% and 0.5%, respectively. Addition of FPI and FPH into light salted cod fillets resulted in lower salt content of the fillets compared with the control fillets.

Table 4.5. Salt content (%) of fresh cod fillets and light salted cod fillets (n=3). Fresh fillets were kept for 5 days at chilled storage and 1 month at frozen storage. Light salted fillets were kept for 1 month at frozen storage. [a]) Values with the same superscript letter within a column are not significantly different (p>0.05).

	Fresh cod fillets		Light salted cod fillets
Treatment	***Chilled***	***Frozen***	***Frozen***
Control	0.1 ± 0.3^{a}	0.2 ± 0.3^{a}	2.6 ± 0.3^{a}
1.5% Salt	$0.4 \pm 0.^{3a}$	0.4 ± 0.3^{a}	--
HFP	0.4 ± 0.3^{a}	0.4 ± 0.3^{a}	2.7 ± 0.3^{a}
FPI	--	0.4 ± 0.3^{a}	2.0 ± 0.3^{a}
FPH	--	--	2.3 ± 0.3^{a}

Table 4.6. Salt content (%) of fresh skinless saithe fillets (n=3) after 4 days of chilled storage, 1 week and 1 month of frozen storage. Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine. [a]) Values with the same superscript letter within a column are not significantly different (p>0.05).

Treatment	Chilled	Frozen (1 week)	Frozen (1 month)
Control	0.2 ± 0.3^{a}	0.2 ± 0.3^{a}	0.3 ± 0.3^{a}
4% Salt	0.5 ± 0.3^{a}	0.5 ± 0.3^{a}	0.6 ± 0.3^{a}
HFP(a)	0.5 ± 0.3^{a}	0.5 ± 0.3^{a}	0.5 ± 0.3^{a}
HFP(b)	0.5 ± 0.3^{a}	0.5 ± 0.3^{a}	0.5 ± 0.3^{a}
Gelatine	0.5 ± 0.3^{a}	0.5 ± 0.3^{a}	0.5 ± 0.3^{a}
HFP(a)+Gelatine	0.5 ± 0.3^{a}	0.5 ± 0.3^{a}	0.5 ± 0.3^{a}
FPH	0.4 ± 0.3^{a}	0.4 ± 0.3^{a}	0.4 ± 0.3^{a}

4.2.8.1 Protein and water yield

The yield with respect to protein and water content was calculated to observe changes in these factors during processing. The water and protein yields were calculated as the quantity of water or protein after chilling/thawing divided by the original quantity in the raw material. Addition of HFP into chilled fresh cod fillets resulted in higher water yield than in control fillets and fillets injected with 1.5% salt, but the water yield decreased again during frozen storage (Figure 4.20). Addition of FPI and FPH increased the water yield in both fresh and light salted cod fillets after frozen storage compared with the control fillets. Water yield was highest in fresh cod fillets treated with FPH, but this group was also relatively low in thaw drip. The control groups of fresh cod fillets had the lowest water yield.

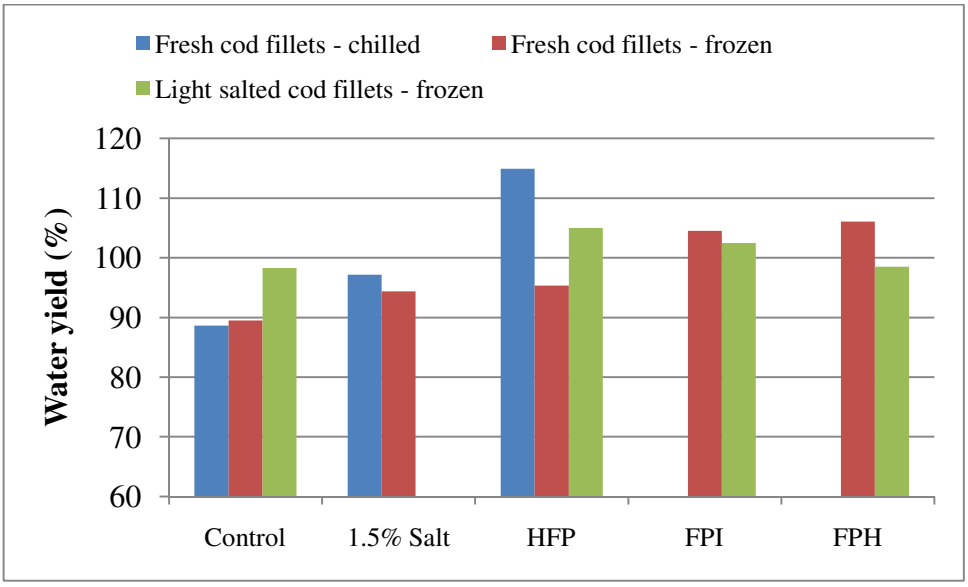

Figure 4.20. Average water yield (%) in fresh cod fillets after chilled storage and in fresh and light salted cod fillets after 1 month of frozen storage.

Addition of HFP to chilled fresh cod fillets resulted also in higher protein yield than in control fillets and fillets injected with 1.5% salt, and increased during frozen storage (Figure 4.21). Addition of fish protein solutions into fresh cod fillets increased the protein yield after 1 month of frozen storage compared to the control fillets. The same trend was obtained for the light salted cod fillets after 1 month of frozen storage, with the exception of FPI where the protein yield was lower compared with the control fillets. Addition of FPH into both fresh and light salted cod fillets had the most impact on protein yield, increased the protein yield from 94.5% to 100.4% and from 96.0% to 104.9%, respectively.

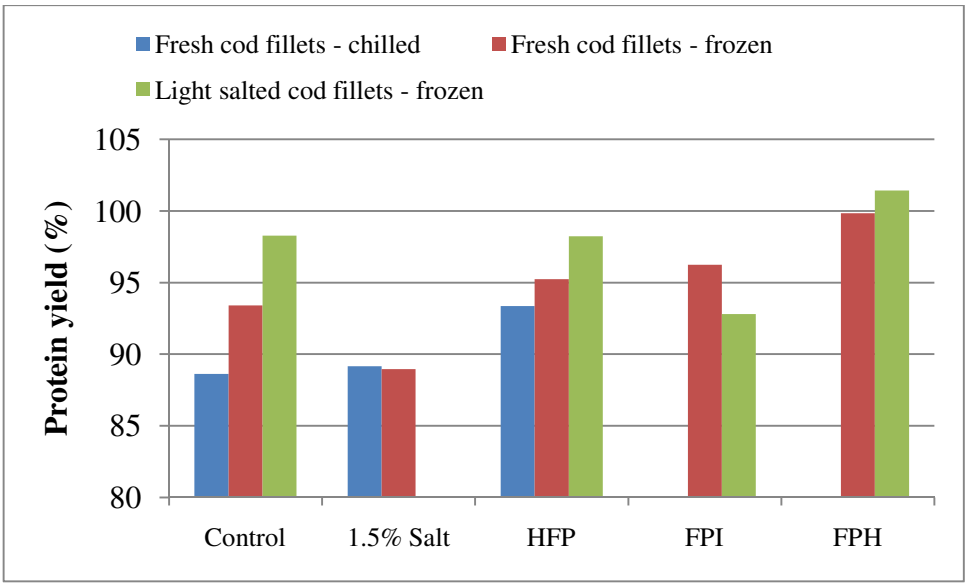

Figure 4.21. Average protein yield (%) in fresh cod fillets after chilled storage and in fresh and light salted cod fillets after 1 month of frozen storage.

Evaluation of yield with respect to water and protein of fresh saithe fillets after chilled and frozen storage is shown in Table 4.7 and Table 4.8. Addition of protein solutions and/or salt, into the chilled fresh saithe fillets, increased the water yield (97.1% to 108.9%) compared with the control fillets (96.6%). Water yield was highest in fillets injected with HFP(a) and HFP(a)+Gelatine. Control fillets and fillets injected only with FPH had the lowest water yield after 4 days at chilled storage. After one week of frozen storage the water yield decreased for all the fillets but saithe fillets injected only with FPH showed the lowest (3%) deterioration compared to the chilled fillets. After one month of frozen storage the water yield continued to decrease. Only fillets with HFP(a) and HFP(a)+Gelatine gave higher water yield compared to the fillets after one week of frozen storage. The yield with respect to protein was highest for the saithe fillets injected only with FPH after 4 days at chilled storage but decreased during frozen storage (1 week and 1 month). Nevertheless, the FPH fillets were at all times higher in protein yield compared with other fillets. The protein yield increased for most of the fillets after one week of frozen storage compared with the chilled fillets and highest for fillets injected with gelatine. On the other hand, after one month of frozen storage the protein yield decreased compared to the fillets after one week of frozen storage, except for fillets treated with HFP(b).

Table 4.7. Average water yield of fresh skinless saithe fillets after chilling and frozen (1 week and 1 month) storage. Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine.

Treatment	Chilled	Frozen (1 week)	Frozen (1 month)
Control	96.6	92.6	91.2
4% Salt	102.1	90.8	86.3
HFP(a)	108.9	90.7	92.9
HFP(b)	106.9	96.7	96.7
Gelatine	103.0	90.7	90.7
HFP(a)+Gelatine	108.7	91.4	91.4
FPH	97.1	94.0	94.0

Table 4.8. Average protein yield of fresh skinless saithe fillets after chilling and frozen (1 week and 1 month) storage. Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine.

Treatment	Chilled	Frozen (1 week)	Frozen (1 month)
Control	96.6	99.5	96.7
4% Salt	88.5	94.8	88.4
HFP(a)	90.8	92.8	89.5
HFP(b)	95.3	93.2	95.6
Gelatine	75.7	89.8	89.3
HFP(a)+Gelatine	89.3	96.0	91.4
FPH	103.3	101.9	97.4

4.2.9 Sensory analysis

Average of sensory attributes for each group and p-values for difference between groups are shown in Appendix E, but only chilled (5 days) cod fillets with added HFP and 1.5% salt were evaluated. The greatest differences between the groups were in flavour and texture. Fillets with added HFP showed a larger difference than fillets injected with 1.5% salt compared with the control fillets. The HFP fillets had more mushy texture and stronger frozen and bitter taste compared to the other two groups and less metallic taste than the control fillets. Fillets injected with HFP and 1.5% salt showed more white precipitation and stronger salt taste compared with the

control fillets. Overall, the salt injected fillets had stronger odour and taste compared with the other groups. The protein injected fillets had higher scores for quality deterioration attributes.

4.2.10 Principal component analysis

Principal component analysis (PCA) was made on comparison of the mean values from most of the groups, after chilled and frozen storage. Principal component analysis does not allow missing values. Therefore two PCA were made, one only with group where T_2 transversal relaxation time was measured and one with all the groups (excluding T_2 transversal relaxation time measurements).

4.2.10.1 PCA with T_2 transversal relaxation time data

The measured parameters were water-, salt- and protein content, drip loss, water holding capacity, T_2 transversal relaxation times (T_{21} and T_{22}) and apparent populations, cooking yield and storage yield.

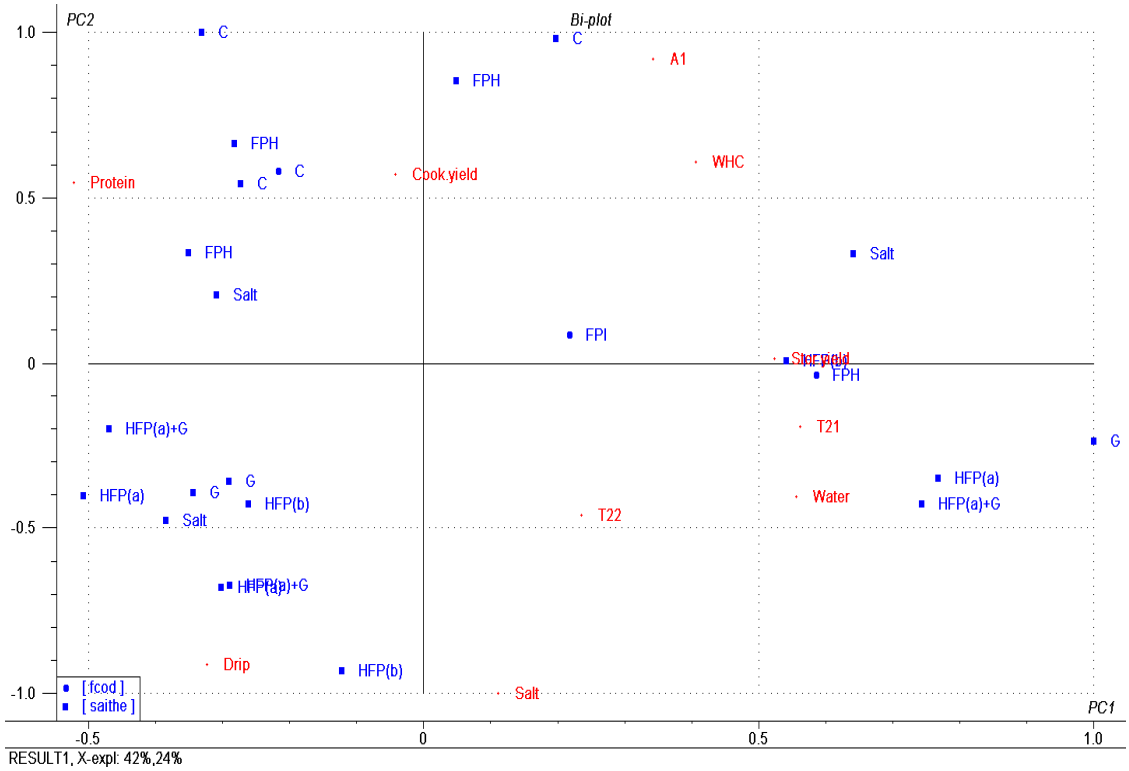

Figure 4.22. Principal component analysis. Bi-plot showing the 1st and 2nd principal components of measured parameters of different treated fillets after chilled and frozen storage.

The first 3 principal components explained 80% of the variation in the model. The 1st component (42%) explained how storage yield, T_{21} relaxation times, water holding capacity and chemical composition (protein and water) varied with storage condition (chilled or frozen) and fish species (cod or saithe). The frozen saithe fillets are located to left of the PC1 axes while the chilled fillets are located to the right of the PC1 axes.

The 2nd component (24%) showed the difference in drip and cooking yield between treatments before chilling or freezing. The results indicated that the drip was lower in the control fillets and fillets treated only with salt (1.5% and 4%) or FPH. It may also be concluded that the water holding capacity of these fillets were higher. Additional, these fillets were also only injected once which may indicate that double injection of saithe fillets is not favourable.

A correlation between water holding capacity, storage yield, T_{21} relaxation time and population (A_1), and water content was observed, but the groups scoring high in these factors were the chilled groups and groups with cod fillets. These factors were negatively correlated to drip loss and protein content where the groups with the highest protein content were the frozen groups. A negative correlation between A_1 populations (assigned to water located within organized protein structure) and drip was observed, as expected, where the drip loss is mainly loosely bounded water and are easily lost.

A plot of scores and loadings from the 1st and 3rd principal components (Figure 4.23) highlighted how the variation could be attributed to storage condition and species.

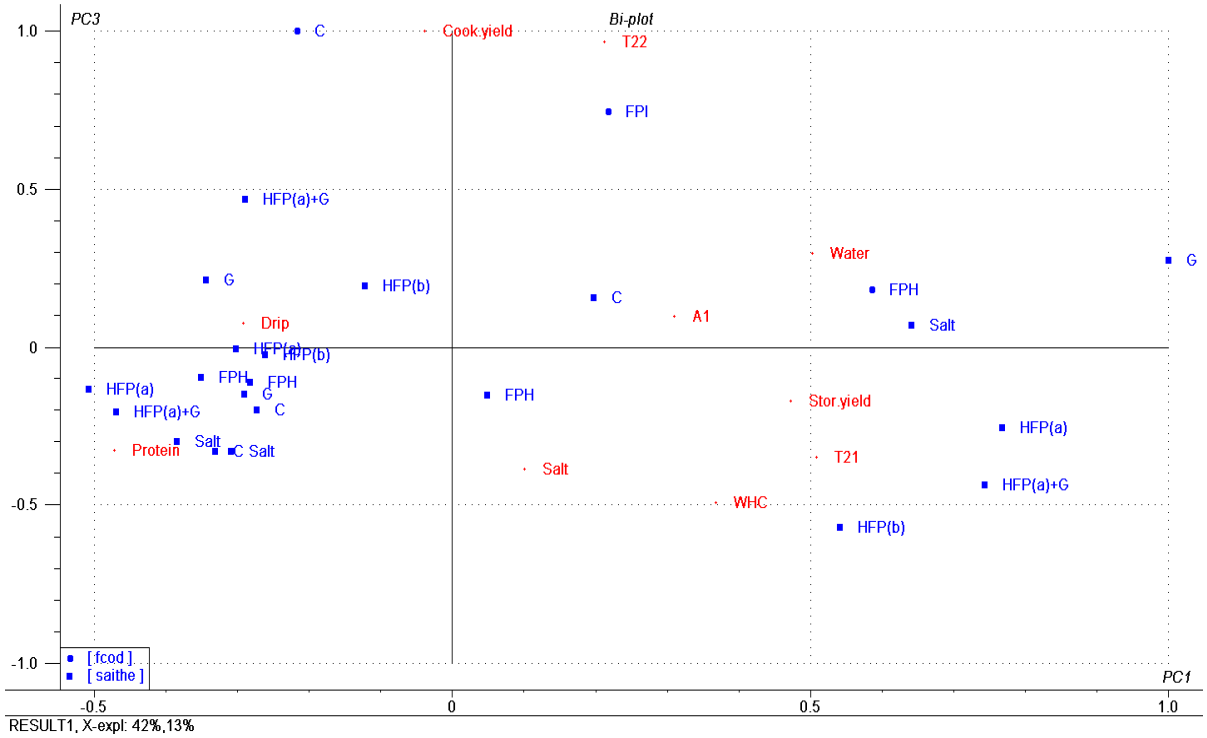

Figure 4.23. Principal component analysis. Bi-plot showing the 1st and 3rd principal components of measured parameters of different treated fillets after chilled and frozen storage

4.2.10.2 PCA for all groups – excluding T_2 transversal relaxation time data

The measured parameters were water-, salt- and protein content, drip loss, water holding capacity, cooking yield and storage yield.

The first 3 principal components explained 86% of the variation in the model. The 1st component (56%) explained how storage yield, water holding capacity and chemical composition (protein, water and salt) varied with storage condition (chilled or frozen) and fish species (cod or saithe). The frozen saithe fillets are located to left of the PC1 axes while the chilled fillets are located to the right of the PC1 axes. These results were similar to the PCA results described above (4.2.10.1). The light salted cod fillets showed notably different behaviour than the other fillets. The model can therefore explain the variation between fresh and light salted fillets.

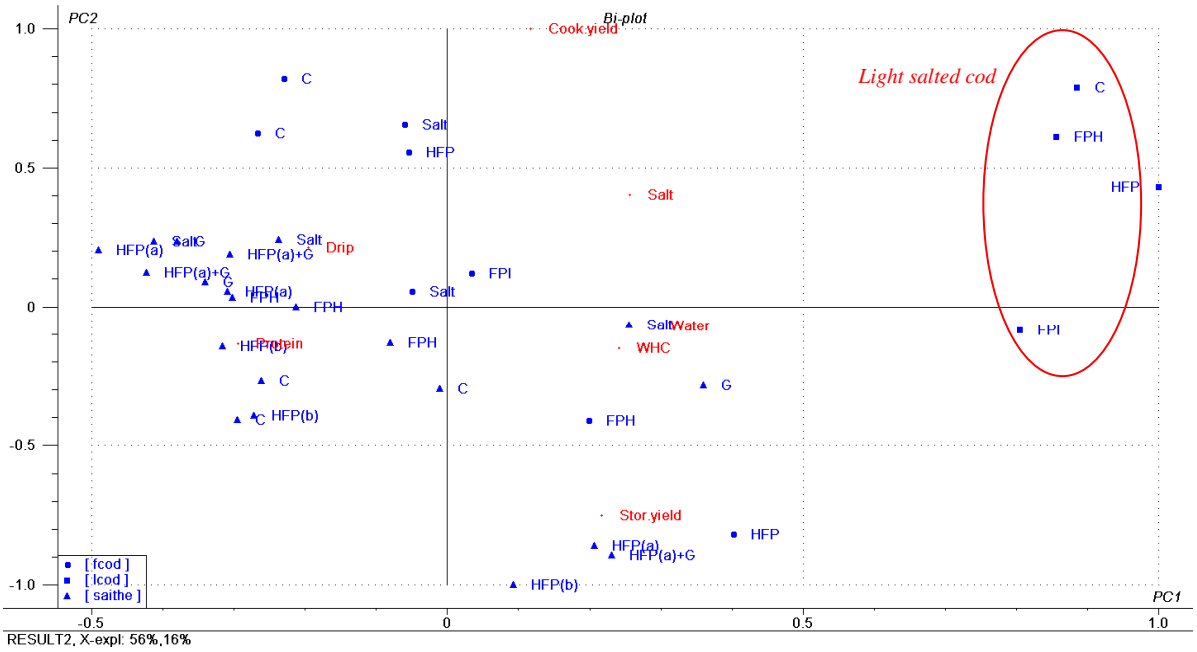

Figure 4.24. Principal component analysis. Bi-plot showing the 1st and 2nd principal components of measured parameters of different treated fillets after chilled and frozen storage. (fcod=fresh cod fillets; lcod=light salted cod fillets).

5 DISCUSSIONS

5.1 The fish protein solutions

The particular process used for protein isolation affects their functional properties and must be controlled with respect to the desired characteristics of the isolated proteins. The functionality of added proteins depends on their origin, molecular structure, method of isolation, various modifications of the isolated proteins, and their interactions with other ingredients in the food system.

The chemical compositions of the fish protein solutions were quite similar except for the FPH. The FPH had significantly higher protein content, and therefore, lower water content compared with the other fish protein solutions. The FPH were also considerably higher in salt content (3.6±0.1%). The different effects of the fish protein solutions on the fish muscle may therefore be particularly explained by this variation in chemical composition.

Results obtained with SDS-PAGE showed how the fish protein solutions differ in molecular distribution. The FPH and the gelatine solutions contained much smaller protein units while the other fish protein solutions contained much larger protein units and even myofibrils. The FPH showed even distribution i.e. contains many difference sizes of molecules from 2 – 212 kDa. The gelatine, on the other hand contained no molecules bigger than ~66 kDa.

Weight loss of the fish protein solutions can show the ability of the proteins (in the solution) to retain water. The FPI showed the lowest amount of weight loss, while there was no significant difference between other solutions. Studies have shown that increased pH, above pI, can increase water holding capacity (Wagenknecht & Tuelsner 1975; Thorkelsson 2007). The FPI was significantly more alkaline (9.28±0.1) compared with the other fish protein solutions, which can explain the difference in weight loss. In contrast to these results, addition of FPI into fish muscle had on the other hand no affect to improve the water holding capacity of the fish muscle. According to Fennema (1990), the mean isoelectric point (pI) of the myofibrillar proteins are about pH 5-6. Minimum water holding capacity, swelling

and protein solubility of meat has been observed around the pI, but it increases again with either decreasing or increasing pH value. The most promising fish protein solutions on water holding capacity of the fish muscle was the FPH solution. The pH of the FPH was 7.67, but it has been reported that increasing pH from 6.4 to 7.4 increased the water holding capacity in fish protein isolate (Kristinsson & Hultin 2003).

In this study the viscosity of the fish protein solutions were measured with two methods, Bohlin and Brabender, but no correlation was seen between the results. Gunnarsson *et al.* (2005) reported that Brabender viscosity of fish hydrolysed samples did not correlate with results from Bohlin viscosity, although some of the results correlated, indicating that factors like shear rate, and shape induced force can affect viscosity especially when viscosity of the same sample is measured by different methods (Gunnarsson *et al.* 2005). The lowest viscosity was obtained for the FPH which may be correlated to considerable small protein units. Also the FPH contained higher amount of salt which can lead to more soluble solution and therefore lower viscosity. The viscosity of the fish protein solutions may affect how well they are retained within the fish muscle.

5.2 Weight gain

Weight gain of fillets injected with salt brines (1.5% and 4% salt concentration) was relatively low (4.9% to 5.1%) compared with fillets injected with fish protein solutions (up to 16.1%). This difference in weight gain may be due to that most of protein solutions contains similar protein component, particularly myofibrillar proteins. It has been shown that myofibrillar proteins are effective in cross-linking ability between proteins and water holding capacity, which may explain why the protein solutions are more easily kept within the fillets after injection. Thorarinsdottir *et al.* (2004) observed 4% to 7% weight gain of cod fillets injected with salt, protein, phosphate or combination of all. The weight gain after salt injection in this experiment was similar and for Thorarinsdottir *et al.* (2004), but the weight gain after protein injection was considerable higher in this experiment. The protein concentration of the protein solutions may affect the weight gain ability of the fillets. The protein concentration in this trial was, for most solutions, ~3% but

10% in Thorarinsdottir *et al.* (2004) trials. This can be supported with this experiment. Fillets injected with FPH gained the lowest weight (5.1% to 6.9%) compared with other fish protein solutions, but the protein concentration of the FPH solution was 14.2%. Higher protein concentration has therefore negative effect with respect to weight gain of injected fillets. High protein concentration may prohibit protein-water connections in the muscle and therefore result in lower weight gain

The viscosity of the fish protein solutions may also affect the weight gain in the fillets. It has been shown that very high viscosity can result in low weight gain (Qiancheng 2008) due to difficulty during injection. In this experiment, most of the protein solutions had similar viscosity except FPH which obtained the lowest viscosity. It may therefore be concluded that certain viscosity interval are more suitable with respect to weight gain of fillets after injection.

In addition, the FPH solution contained much smaller protein units while the other protein solutions contain whole proteins and even myofibrils. This difference may explain partially the difference in weight gain and the retain ability of the protein solutions in the fillets. Also, the FPH solution contained considerable higher salt content compared with the other fish protein solutions.

Weight gain of injected fillets can also be influenced by many factors, such as injection pressure, needle density, needle speed and injection direction (when the brine is deposited) (Freixenet 1993; Birkeland *et al.* 2003; Birkeland *et al.* 2007). In present study, the same injection settings were used in for all the fish protein solutions.

5.3 Drip loss

In this experiment, FPH injection showed relatively low thaw drip after frozen storage compared with control and salt injected cod fillets and the other fish protein solutions. The FPI fillets showed the highest value of drip loss compared with other injected fillets, but were still significant lower than the control fillets. Thorarinsdottir *et al.* (2004) showed that salt injection had higher drip loss than fish protein hydrolysate injection, but the control fillets showed the lowest drip loss after

frozen storage. Previous studies have claimed that drip loss can be reduced by using different protein materials (Kristinsson & Rasco 2000a). Thaw drip has been linked to partial denaturation of proteins during freezing, which leads to decreased water holding capacity (Shenouda 1980; Mackie 1993). Addition of salt into fish prior freezing has shown to increase water holding capacity and decrease drip loss (Ragnarsson 1988). Phosphates have also been used to reduce drip loss as was shown in Thorarinsdottir *et al.* (2004) study. Their study showed that fish protein hydrolysates gave lower drip loss than phosphate, but it was more effective to use the fish protein combined with salt and phosphate. In this experiment, addition of fish protein solutions (HFP) into fresh cod fillets decreased the drip loss significantly during chilled storage compared with control fillets and salt injected fillets. The difference between the fish protein solutions (HFP, FPI and HFP) may be due to the particular process used for the protein isolation.

Treated saithe fillets showed considerably higher values of drip loss when compared with cod fillets. Saithe fillets are said to have lower quality due to more gaping problems than cod fillets, which may partially explain the difference. It has been claimed that thaw drip can be reduced by using high-quality raw materials and good control of storage conditions (Cormier & Leger 1987). The treatment of the fillets in this experiment may also explain the relatively high drip loss of the saithe fillets. The rough treatment, double injection and slow freezing, may have considerably negative effect on the quality of the saithe fillets. These treatments seem not to affects the cod fillets as much. Gaping problems are less known in cod fillets. During *rigor mortis* the glycolysis leads to formation of lactic acid and the pH is lowered. A low pH value can cause rupture of connective tissue that underlies gaping (Love 1997). The decrease in pH occur a lot faster for the saithe fillets which can explain the difference between cod and saithe fillets.

5.4 Yield after storage

Injection of fish protein into fresh cod fillets before chilled storage showed considerable higher storage yield compared with control fillets and salt injected fillets. Addition of FPI and FPH increased the storage yield of fresh cod fillets after frozen storage compared with the control fillets, but had no additional improvement

to the salt effects of the light salted cod fillets. HFP showed on the other hand more positive effects on the light salted cod fillets than the fresh fillets after frozen storage. Results from Thorarinsdottir et.al. (2004) showed that cod fillets treated with protein (soy and fish protein) resulted in lower yield after frozen storage compared with control fillets, salt (5%) and phosphate (3%) treated fillets. The combined use of salt, phosphate and protein, on the other hand, resulted in increased yield. It has been suggested that the combined use of salt and phosphates might be effective in opening the myofibrillar structure resulting in increased water retention and yield (Peterson *et al.* 1988).

The treated saithe fillets showed in general a substantial decrease in storage yield after frozen storage compared to after chilled storage. The fillets were frozen in freeze cabin which is slow freezing method; but IQF, which is rather fast freezing method, would be more favourable. Freezing affects the physical properties of the fish muscle (Arason & Ásgeirsson 1984) and changes the muscle structure. The muscle cells shrink causing liquid leak out of the cells to the inter-cellular space (Bello *et al.* 1981; Hurling & Mcarthur 1996). The freezing rate is a very important factor, where slow freezing results in more detrimental changes to the fish muscle (Arason & Stefánsson 1999; Belitz *et al.* 2004).

Higher salt concentration can result in higher water holding capacity (Peterson *et al.* 1988), which causes more retention of injection solutions. Lower protein concentration, and therefore higher water content, can on the other hand result in less water holding in the fillet. Higher yield may also be obtained by immersing the fillets in the same brine solution immediately after injection (Thorarinsdottir *et al.*, 2004).

5.5 Cooking yield

Addition of protein solutions and/or salt before chilled or frozen storage showed no improvement on yield after cooking compared with the control fillets. After 5 days at chilled storage, the cod fillets injected with fish proteins showed lower cooking yield compared with the control fillets. The control fillets had considerable higher drip loss (Figure 4.2) and the difference of cooking yield between the groups may

therefore be due to the fact that the control fillets had lost most of their loosely bound and free moving water during storage, while the strongly bound water remains. In other words, the chilled control fillets had less water to lose than the protein injected fillets. The light salted cod fillets showed similar values of cooking yield compared with the fresh fillets after frozen storage, with or without added protein solutions. These results are quite surprising where it have been claimed that increased salt concentration increase cooking yield (Jittinandana *et al.* 2002).

Other studies (Thorarinsdottir *et al*., 2004) have shown that injection with salt and or phosphate resulted in higher cooking yield compared with fillets injected protein solutions brine before frozen storage. It has also been suggested that the protein concentration of the injection brine can affect the cooking yield (Shahidi *et al.* 1995) and water-binding varies with the type of proteins and their functional properties (Karmas & Turk 1976). The combination of capelin hydrolysate and salt has been shown to increase the cooking yield of pork, but increasing the capelin hydrolysate concentration led to increased cooking yield (Shahidi *et al.*, 1995). Similar results were obtained with addition of shark protein hydrolysate (Onodenlore & Shahidi 1996). In this experiment, higher protein concentration in the fish protein solution (FPH) (Table 4.1) showed slight improvement of cooking yield (compared with control fillets) when injected into saithe fillets, but no improvement for the cod fillets.

The cooking yield is important for the consumers. It is important for the consumers to get what they buy, e.g. that the product does not shrink during cooking and becomes less juicy, and no disadvantage texture changes.

5.6 Total yield

Evaluation of total yield after cooking is a good way to observe the total yield through all the process steps. The total yield considers each step, i.e. the weight gain, drip loss, yield after storage and the cooking yield. The total yield can be divided into two main parts, i.e. the production capacity and sales and consumers capacity. The yield from the processing lines and the weight gain can be labelled as

the production capacity while the yield after storage and cooking yield can be labelled as the sales and consumers' capacity.

Addition of fish proteins into chilled and frozen cod fillets resulted in higher total yield compared with control fillets and salt (1.5%) injected fillets. Addition of HFP showed the lowest decrease in weight through the process (closest to the original weight of the fillets). Control fillets of light salted cod showed, as expected, higher total yield after frozen storage compared with control fillets of fresh cod. Addition of FPH decreased this difference and resulted in similar total yield of the fresh and light salted cod fillets. The FPI fillets gave also higher total yield compared with the control fillets but were more effective on fresh cod fillets than light salted cod fillets after frozen storage.

The saithe fillets showed at all time lower total yields compared with the cod fillets. For the saithe fillets, FPH showed at all time better total yield compared with the other fish proteins and the control fillets. The FPH fillets showed also less deterioration during frozen storage compared with the other treated fillets which is a good advantage. The salt injected fillets showed much more and unexpected quality decline during frozen storage compared with the fillets treated with fish protein solutions, but it has been claimed that increased salt concentration can increase yield and water holding capacity of the muscle (Jittinandana *et al.*, 2002; Peterson *et al.*, 1988). Perhaps a higher salt concentration (>4%) is needed for injection into saithe fillets to obtain desirable results. The results from this experiment can also indicate that injection of protein solutions into saithe fillets is more favourable when fillets are frozen rather than chilled.

There may also be other factors that affect the total yield, e.g. raw material conditions, time of the year and other things.

5.7 Water holding capacity

Generally in this experiment, injection of protein and/or salt showed no improvement on the water holding capacity of the fish muscles. Addition of fish protein hydrolysate (FPH) resulted in slightly higher value of water holding capacity after

frozen storage. Thorarinsdottir *et al.* (2004) showed that addition of fish protein hydrolysate combined with phosphate and/or salt increased the water holding capacity considerably. The pH value is known to affect the water holding capacity of fish muscle. The water holding capacity is at its minimum at the myofibrillar proteins isoelectric point (pH 5-6) (Belitz *et al.*, 2004). The pH value of the injection solution may therefore influence the water holding capacity of the muscle, where the pH of the FPH (Table 4.1) was 7.67 (>pI). In contrast to this, the FPI was very alkaline (9.28) but had no affect to improve the water holding capacity of the fish muscle.

Increased salt concentration (1.5% to 4%) of the injection brine resulted in higher water holding capacity. Salt is known to increase swelling of the muscle and the muscle ability to retain water. It has been established that water holding capacity increases with increased salt concentration up to 6% (Offer & Knight 1988; Fennema 1990), but studies have shown that fish muscle loses water at higher salt concentration (>10%) which results in decreased water holding capacity (Thorarinsdottir *et al.*, 2002). The salt content in the salt injected fillets was 0.4% to 0.6% (Table 4.5 and Table 4.6), but the maximum water holding capacity is reached at approximately 6% salt content in the muscle.

In the present study, the water holding capacity generally decreased during frozen storage compared with chilled storage. Similar results were obtained from Erikson *et al.* (2004). This effect could be explained by that water-protein associations in fresh raw material were partly replaced by protein-protein interactions during frozen storage.

The considerably slow freezing method used may also cause decrease in water holding capacity during frozen storage. The structure of the fresh fish muscle changes during freezing and frozen storage. The muscle cells shrink, causing liquid leak out of the cells to the inter-cellular space (Bello *et al.*, 1981; Hurling & McArthur, 1996). Is has been shown that ice crystals are formed within the muscle cells and between them during freezing. The location and size of the ice crystals are dependent of the freezing conditions (Howgate, 1979). During slow freezing, big ice crystals are formed outside the muscle cells and extract water from the cells causing

ice crystal formation between the cells and disrupting them. This decreases the fish muscle water binding ability which can lead to lower quality and yield when thawed.

5.8 T_2 transversal relaxation times

Betram et.al. (2001) concluded that a multiexponential transverse relaxation cannot be simply explained by a simple intracellular and extracellular compartmentalization model. T_{21} population is tentatively assigned to water located within organized protein structure, whereas T_{22} population may reflect water being expelled to the space between fiber bundles (Betram *et al.* 2001).

In this experiment, the bi-exponential fit suggested two water populations, one with relaxation times of 46-61 ms (T_{21}) and another at 155 to 302 ms (T_{22}) with values depending on treatment. These T_{21} values approximately correspond to previously reported values in fresh cod at 45 ms (Erikson *et al.* 2004) and 50 ms (Andersen & Rinnan 2002). In the former study, however, the second water population at 124 ms (assigned extracellular water) was considerably shorter than observed in this experiment at 264 ms. Furthermore, Anderson & Rinnan (2002) showed that the magnitude of the relaxation time depends on the position along the fish, being shorter near the head and longer near the tail where the total water content was approximately 2% higher than near the head section. The effects were explained by smaller muscle cell/fiber size in the tail section affecting the water distribution and thus the water mobility.

The untreated raw material, in this experiment, exhibited generally lower T_{21} value compared with injected fillets. This suggested lower water mobility in the fresh tissue having retained more of its original structure. The T_{22} relaxation times were also generally longer compared with control fillets (increased mobility of the free water). The effect of fish protein solutions and/or salt addition compared with untreated fillets may be induced swelling and thus improved water mobility as reflected by higher T_{21} values. Addition of salt (5.5% to 7.5%) into fresh cod fillets have shown significantly increase in water mobility (T_{21} relaxation time) (Erikson *et al.*, 2004). Erikson *et al.* (2004) hypothesized that increase in the 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).

In this experiment, freezing and thawing had significantly impact on the T_{21} value, resulted in decreased water mobility. The effects of freezing and thawing on the T_{22} value were quite interesting, where the value decreased significantly after one week of frozen storage but increased again after 1 month at frozen storage. Erikson *et al.* (2004) observed no significant changes for the T_{22} values after freezing and thawing, but this water population may be assigned as drip loss (Erikson *et al.*, 2004).

Comparison of the apparent population showed that addition of protein solutions and/or salt did not improve the T_{21} population (less mobile water) compared with the control fillets after chilled or frozen storage. Treated fillets showed therefore also generally higher value of the T_{22} population (more mobile water) than the control fillets.

The frozen fillets had lower T_{21} population (A_1norm) values compared with chilled fillets, corresponding to higher amount of water reflected in the T_{22} population (A_2norm). Similar results were obtained from Aursand *et al.* (2009), but their result indicated that frozen fillets clearly possessed a more open. By comparing T_{21} populations with corresponding water holding capacity values indicate correlation between the T_{21} population and WHC. The same results were observed with the total yield.

LF NMR T_2 relaxation is considered to be effective tool for obtaining further understanding of the relationship between the microstructure of fish muscle and its water mobility (Aursand *et al.* 2009). The rapid LF NMR method may have the potential to replace traditional salt and water-related analytical methods and may also be implemented for at-line quality control (Erikson *et al.*, 2004).

5.9 Sensory analysis

Results from sensory analysis showed a difference among the groups with regard flavour and texture, where fillets with added HFP showed more negative effects.

One of the main disadvantages of the injection method is the risk of microbial contamination and damages of the muscle structure. Freezing after injection has therefore been considered to be more suitable storage condition for the fillets. The evaluated cod fillets were kept at chilled storage for 5 days, which may therefore partly explain the difference between the control fillets and the protein injected fillets. Vann & DeWitt (2007) showed that protein solutions had positive effects on sensory attributes. They claimed that beef with added acid solubilised protein solution into beef was comparable to phosphate-enhanced steaks for percent discoloration and overall acceptability (Vann & Dewitt 2007). Hagen & Sandnes also claim that injection of fish protein hydrolysate into salmon fillets show no changes in odour and taste (Hagen & Sandnes 2005).

5.10Chemical composition

The addition of protein solutions and/or salt (4%) resulted generally in slightly higher water content of the fillets than in the control fillets, except for fillets with added FPH. This resulted also in higher yield with respect to the water.

The protein content of the fillets injected with protein solutions and/or salt was generally lower than in the control fillets. This could be explained by dilution effects on the dry material in the fillet by added water. The additives also increased the yield with respect to protein of the cod fillets compared with the control fillets, and decreased the dilution effects from the added water. Thorarinsdottir *et al.* (2004) showed similar results, and also that fish protein may be added to fillets parallel to salt and phosphate to reduce changes in the relative protein content and thereby in the amount of dry material.

The final salt concentration of the fillets, in these experiments, shows how the muscles in cod and saithe differ. The fresh cod fillets were injected with 1.5% salt brine while fresh saithe fillets were injected with 4% salt brine, but the final concentration of the fillets were similar. By using salt concentration of 4%, it was expected to reach ~0.7% final salt concentration in the fillets. This may indicate that the cod muscle has better binding ability than the saithe muscle, but gaping is well known problem for saithe fillets.

It is known that condition and chemical composition of the fish muscle varies with the season of the year (Dambergs 1964; Botta *et al.* 1987a). Addition of fish proteins might be used to compensate for a reduction in protein content, such as after spawning period.

In present study, the saithe fillets were processed in May while the cod fillets mainly were processed in October. During the summertime the fish regenerate its fat supplies and in the fall (October-November) the proportion of protein is maximised and the proportion of water-soluble material minimised (Dambergs, 1964). Therefore, the fish condition is good in the fall and the muscles are firm (Raversu & Krzynowek 1991), which may partially explain the difference between the raw materials used.

6 CONCLUSION

Additions of fish proteins solutions increased the weight gain considerably after injection of the fillets, compared with salt treated fillets, but varied with types of fish protein solutions. The protein concentration of the injection brine can be limiting factor for the weight gain of fillets after injection. It can be concluded that lower protein concentration (~3%) in the solutions are more suitable than higher concentration (~14%) with regard to weight gain of injected fillets. But it must be kept in mind that the fish protein solutions used are generally very different. They differ mainly in the protein composition. The FPH solution contained much smaller protein units while the other protein solutions contain whole proteins and even myofibrils.

By adding fish protein solutions into cod fillets before chilled or frozen storage, storage yield can be increased and drip loss reduced, compared with control and salt treated fillets. Addition of FPH and HFP were particularly effective. These protein solutions showed also considerable improvement with regard to the total yield of the cod fillets, i.e. through the whole process from the producer to the consumer. The FPI solution was also effective with regard to fresh cod fillets after frozen storage. Addition of gelatine showed the weakest influence on the fillets. The concentration (2%) and/or the gelatine type (collagen peptide) are therefore not suitable as additive in fish fillets to improve the fillets stability and quality. Perhaps higher concentration is needed or different type of gelatine (different isolation method).

Addition of fish proteins and/or salt into fillets showed less effect on the water holding capacity of the muscle, but the influence were expected to be greater. The most promising fish protein solutions to increase water holding capacity was the FPH. The pH of the protein solutions may affect how well the proteins retain the water inside the muscle. Perhaps higher water holding capacity can be received if the injected protein solutions are more alkaline (>pI) up to certain point. The FPI was very alkaline (9.28) but had no affect to improve the water holding capacity of the fish muscle.

The saithe fillets seem to be more insensitive injection and freezing than cod fillets, but gaping is well known problem for saithe fillets. Frozen storage seems therefore to have dramatic effects on the yield and quality of the saithe fillets. It has to be taken into consideration that freezing methods and freezing speed matter, therefore for example IQF or other fast freezing methods would be more suitable to maintain the quality of the saithe fillets. In addition, injection is a rather rough treatment and the numbers of injection may therefore have great influence on the saithe muscle. There is a need to optimize the treatment for saithe fillets, perhaps it is more suitable to immerse the fillets in fish protein solutions rather than incorporate the fish proteins through injections.

The present study indicates that cod muscle has greater binding ability than saithe muscle. Addition of salt into fillets is a very common method to increase the binding properties and quality of fillets. The final salt concentration in the fillets can, according to European standards, be approximately 0.7% to be considered fresh product (not light salted). The saithe fillets seem to need higher salt concentration in the brine to reach this limits compared with the cod fillets.

Overall, injection of protein solutions into cod and saithe fillets is an effective means to improve or stabilize the weight and quality of the fillets, but more optimisation is needed with regard to raw material.

REFERENCES

Aitken, A. (2001). "Polyphospates in fish processing." Ministry of agriculture, fisheries and food - Torry research station Retrieved August 16, 2009, from http://www.fao.org/wairdocs/tan/x5909e/x5909e00.htm#Contents.

Alvarez, J. A., Pozo, R. and Pastoriza, L. (1996). "Effect of a cyroprotectant agent (sodium tripolyphosphate) on hake slices preserved in modified atmosphere packing." *Food Science Technology International* **2**: 177-181.

An, H., Peters, M. Y. and Seymour, T. A. (1996). "Roles of endogenous enzymes in surimi gelation." *Trends in Food Science & Technology* **7**(10): 321-327.

Andersen, C. M. and Rinnan, Å. (2002). "Distribution of Water in Fresh Cod." *Lebensm.-Wiss. u.-Technol.* **35**: 687-696.

Arason, S. and Ásgeirsson, L. (1984). Um frystingu sjávarafurða. Reykjavík, IFL.

Arason, S. and Stefánsson, G. (1999). Rf pistill nr. 13 - Frysing og geymsla frystra sjávarafurða. Reykjavík, IFL.

Aursand, I. G., Veliyulin, E., Böcker, U., Ofstad, R., Rustad, T. and Erikson, U. (2009). "Water and salt distribution in Atlantic Salmon (Salmo salar) studied by low-field 1H NMR, 1H and 23Na MRI and light microscopy: Effects of raw material quality and brine salting." *Journal of Agricultural and Food Chemistry* **57**: 46-54.

Bárzana, E. and García-Garibay, M. (1994). Production of fish protein concentrates. In *Fisheries processing - Biotechnological applications*. London, Chapman & Hall**:** pp.494.

Belitz, H.-D., Grosch, W. and Schieberle, P. (2004). Food Chemistry, 3rd revised Edition. Germany, Springer-Verlag Berlin Heidelberg.

Bello, R. A., Luft, J. H. and Pigott, G. M. (1981). "Improved histological procedures for microscopic demonstration of related changes in fish muscle tissue structure during holding and freezing." *Journal of Food Science* **46**: 733-740.

Betram, H. C., Engelsen, S. B., Busk, H., Karlsson, A. H. and Andersen, H. J. (2004a). "Water properties during cooking of pork studied by low-field NMR relaxation: effects of curing and the RN gene." *Meat Science* **66**: 437-446.

Betram, H. C., Karlsson, A. H., Rasmussen, M., Petersen, O. D., Dönstrup, S. and Andersen, H. J. (2001). "Orgin of multiexponential T2 relaxation in muscle myowater." *J Agric Food Chem* **49**: 3092-3100.

Betram, H. C., Schäfer, A., Rosenvold, K. and Andersen, H. J. (2004b). "Physical changes of significance for early post mortem water distribution in porcine M. longissimus." *Meat Science* **66**: 915-924.

Bigelow, W. and Lee, C. M. (2007). "Evaluation of various infused cryoprotective ingredients for their freeze-thaw stabilising and texture improving properties in frozen red hake muscle." *Journal of Food Science* **72**(1): C56-C64.

Birkeland, S., Akse, L., Joensen, S., Tobiassen, T. and Skåråå, T. (2007). "Injection-salting of pre-rigor fillets of Atlantic Salmon (Salmo salar)." *Journal of Food Science* **72**(1): E29-E35.

Birkeland, S., Skåra, T., Bjerkeng, B. and Rörå, A. M. B. (2003). "Product yield and gaping in cold-smoked atlantic salmon (Salmo salar) fillets as influenced by different injection-salting techniques." *Journal of Food Science* **68**(5): 1743-1748.

Bobkova, E. A., Bobkow, A. A., Levitsky, D. I. and Reisler, E. (1999). "Effects of SH1 and SH2 Modifications on Myosin Similarities and Differences." *Biophysical Journal* **76**: 1001-1007.

Botta, J., Bonnell, G. and Squires, B. (1987a). "Effect of method of catching and time of season on sensory quality of fresh raw Atlantic cod (Gadus morhua)." *Journal of Food Science* **52**(4): 928-931.

Botta, J., Kennedy, K. and Squires, B. (1987b). "Effect of method of catching and time of season on the composition of Atlantic cod (Gadus morhua)." *Journal of food Science* **52**(4): 922-924.

Brown, W. (1986). Fish muscle as food. In *Muscle as Food*. P. Bechtel. London, Academic Press Inc.**:** pp.406-453.

Bröndum, J., Munck, L., Henckel, P., Karlsson, A., Tornberg, E. and Engelsen, S. B. (2000). "Prediction of water-holdin capacity and composition of porcine meat by comparative spectroscopy." *Meat Science* **55**(2): 177-185.

Colmenero, F. J. (1996). "Technologies for developing low-fat meat products." *Trends in Food Science & Technology* **7**: 41-48.

Cormier, A. and Leger, L. W. (1987). "Effect of sodium polyphosphates on frozen cod fillets (Gadus morhua." *Canadian Institute of Food Science and Technology Journal* **20**: 222-228.

Dambergs, N. (1964). "Extractives of fsih muscle. 4. Seasonal variations of fat, water solubles, protein and water in cod (Gadus morhua L.) fillets." *J. Fish. Res. Bd. Can.* **21**: 703-709.

Damodaran, S. (1996). Amino Acids, Peptides, and Proteins. In *Food Chemistry*. New York, Marcel Dekker, Inc.**:** pp.321-429.

Dauksas, E., Falch, E., Slizyte, R. and Rustad, T. (2005). "Composition of fatty acid and lipid classes in bulk products generated during enzymic hydrolysis of cod (Gadus morhua) by-products." *Process Chemistry* **40**(8): 2659-2670.

Dziezak, J. D. (1990). "Phosphates improve many foods." *Food Technology* **44**(4): 80-91.

Eide, O., Borresen, T. and Strom, T. (1982). "Minced fish production from capelin (Mallotus villosus) A new method for gutting, skinning and removal of fat from small fatty fish species." *Journal of Food Science* **47**: 347-349.

Erikson, U., Veliyulin, E., Singstad, T. E. and Aursand, M. (2004). "Saling and Desalting of Fresh and Frozen-thawed Cod (Gadus morhua) Fillets: A Comparative Study Using 23Na NMR, 23Na MRI, Low-field 1H NMR, and Physicochemical Analytical Methods." *Journal of Food Science* **69**(3): 107-114.

Fairbanks, G., Steck, T. L. and Wallach, D. F. H. (1971). "Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane." *Biochemistry* **10**: 2606-2617.

FAO (2008). The State of World Fisheries and Aquaculture. Rome, Italy, FAO Fisheries Department, Food and Agricultural Organisaton of the United Nations.

Fennema, O. R. (1990). "Comparative water holding properties of various muscle foods. A critical review relating to definitions, methods of measurement, governing factors, comparative data and mechanistic matters." *Journal of Muscle Foods* **1**: 363-381.

Foster, R. J. (2004, January 12). "Meat-ing consumer expectations." Food Product Design Retrieved September 1, 2009, from http://www.foodproductdesign.com/articles/2004/12/meat-ing-consumer-expectations.aspx.

Freixenet, L. (1993). "Spray injection of meta: Influence of the brine pressure in the quality of injected products." *Fleischwirtshaft International* **3**: 16-20.

Geirsdóttir, M. (2006). "Nýfæði og markfæði." *Matur er mannsins megin. Rit Matvæla- og næringafélag Íslands* **18**(1): 13.

Giese, J. (1994). "Proteins as ingredients: types, function, application." *Food Technol.* **48**: 49-54,56,58,60.

Gopakumar, K., Muraleedharan, V. and Bhattacharyya, S. K. (1992). "Preperation and properties of surimi from tropical fish." *Food Control* **3**(2): 109-112.

Griffin, N. (2005a). "BUSINESS: High-tech processing - at Nort Atlantic Inc., the war against bacteria ends." *The working Waterfront*: http://www.workingwaterfront.com/article.asp?storyID=20050303.

Griffin, N. (2005b). "FISHING: Changing the Chemistry of a Fish." *The Working Waterfront*: http://www.workingwaterfront.com/article.asp?storyID=20050303.

Gudjonsdottir, M., Gunnlaugsson, V. N., Finnbogadottir, G. A., Sveinsdóttir, K., Magnússon, H. and Arason, S. (2009). Low field NMR study on wild and farmed Atlantic cod (Gadus morhua). In *Magnetic resonance in food science*. Cambridge, Royal Society of Chemistry**:** pp.231-240.

Guðmundsdóttir, Á. (2005). "Kennsluhefti í námskeiðinu Matvælaefnafræði I (09.81.44)." *www.hi.is/~ag/matvefn*.

Guðmundsson, M. and Hafsteinsson, H. (1997). "Gelatin from cod skins as affected by chemical treatments." *Journal of Food Science* **62**(1): 37-39,47.

Gunnarsson, J. R., Guðmundsdóttir, G., Thorarinsdottir, K. A., Arason, S. and Gíslason, J. (2005). Functional properties of protein hydrolysates from fish by-products. Project report to the EU, IFL.

Hagen, H. and Sandnes, K. (2005). "Process for improvement of meat quality in fish, protein hydrolysate and method of producing a protein hydrolysate", Europe. Patent: EP1605767

Hansen, C. L. (2005). "High pressure injection technologies." *Encyclopedia of Agricultural, Food, and Biological Engineering*: 1-3.

Hao, S., Li, L., Yang, X., Cen, J., Shi, H., Bo, Q. and He, J. (2009). "The characteristics of gelatin extracted from sturgeon (Acipenser baer) skin using various pretreatments." *Food Chemistry* **115**: 124-128.

Hastings, R. J., Rodger, W., Park, P., Matthews, A. D. and Anderson, E. M. (1985). "Differential scanning calorimetry of fish muscle: the effect of processing and species variation." *Journal of Food Science* **50**: 503-510.

Howgate, P. (1979). Fish. In *Food Microscopy*. London, Academic Press, Inc.**:** pp.343-389.

Hultin, H. O. (1976). Characteristics of muscle tissue. In *Food Chemistry*. New York, Marcel Dekker, Inc. **4:** pp.577-614.

Hurling, R. and McArthur, H. (1996). "Thawing, refreezing and frozen storage effects on muscle functionality and sensory attributes of frozen cod (Gadus morhua)." *Journal of Food Science* **61**(6): 1289-1296.

ICES. (2009a). "ICES-FishMap." International Council for the Exloration of the Sea Retrieved August 9, 2009, from http://www.ices.dk/marineworld/fishmap/ices/pdf/cod.pdf.

ICES. (2009b). "ICES-FishMap." International Council for the Exloration of the Sea Retrieved August 9, 2009, from http://www.ices.dk/marineworld/fishmap/ices/pdf/saithe.pdf.

Insel, P., Turner, R. E. and Ross, D. (2004). Nutrition Second Edition. Sudbury, Massachusetts, Jones and Bartlett Publishers, Inc.

Jacquier, J. L., Vouille, D. and Fouillet, C. (2000). "Method of injection of suspension of fish meat into fish pieces, particulary tuna", Europe. Patent: EP 0974273

Jespen, S. M., Pedersen, H. T. and Engelsen, S. B. (1999). "Application of Chemometrics to Low-Field H1 NMR Relaxation Data of Intact Fish Flesh." *Journal of the Science of Food and Agriculture* **79**(13): 1793-1802.

Jittinandana, S., Kenney, P. B., Slider, S. F. and Kiser, R. A. (2002). "Effect of brine concentration and brining time on quality of smoked rainbow Trout fillets." *Journal of Food Science* **67**(6): 2095-2099.

Jóhannsson, R., Sigurgísladóttir, S., Thorkelsson, G. and Bergsson, A. B. (2009). Hrein vöðvaprótein úr fiski. Reykjavík, Skýrsla Matís 19-09**:** 10.

Jónsson, G. (1992). Íslenskir fiskar. Reykjavík, Fjölvi ehf.

Jul, M. (1984). Thawing. In *The quality of frozen food*. London, Academic Press Inc.**:** pp.261-270.

Karim, A. A. and Bhat, R. (2009). "Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins." *Food Hydrocolloids* **23**: 563-576.

Karmas, E. and Turk, K. (1976). "Water binding of cooked fish in combination with various proteins." *Journal of Food Science* **41**: 977-979.

Kelleher, S. D. (2005). "Process for retaining moisture in cooked animal muscle", U.S. Patent: 6,855,364

Kelleher, S. D. and Hultin, H. O. (1999). "Protein composition isolated from a muscle source", U.S. Patent: 6,005,073

Kim, C. R., Hearnsberger, J. O., Vickery, A. R., White, C. H. and Marshall, D. L. (1995). "Extending shelf life of refrigerated catfish fillets using sodium acetate and monopotassium phosphate." *Journal of Food Protection* **58**: 644-647.

Kim, J. S. and Park, J. W. (2006). Mince from seafood processing by-products and surimi as food ingredients. In *Maximising the value of marine by-products*. London, Woodhead Publishing Cambridge**:** pp.198-227.

Kristbergsson, K. and Arason, S. (2006). Utilizaton of By-Products in the Fish Industry. In *Utilization of By-Products and Treatment of Waste in the Food Industry*, Springer US**:** pp.233-258.

Kristinsson, H. G. (2006). Aquatic food protein hydrolysates. In *Maximising the value of marine by-products*. Cambridge, Woodhead**:** pp.229-247.

Kristinsson, H. G. and Hultin, H. O. (2003). "Effect of Low and High pH Treatment on the Functional Properties of Cod Muscle Proteins." *Journal of Agricultural and Food Chemistry* **51**(17): 5103-5110.

Kristinsson, H. G. and Rasco, B. A. (2000a). "Biochemical and functional properties of Atlantic salmon (Salmo salar) muscle protein hydrolyzed with various alkaline proteases." *Journal of Agriculture and Food Chemistry* **48**: 657-666.

Kristinsson, H. G. and Rasco, B. A. (2000b). "Fish protein hydrolysates: production, biochemical and functional properties. Critical reviews." *Food Science and Nutrition* **40**(1): 43-81.

Kristinsson, H. G., Theodore, A. E. and Ingadóttir, B. (2006). Chemical processing methods for protein recovery from marine by-products and underutilized fish species. In *Maximising the value of marine by-products*. London, Woodhead Publishing Cambridge**:** pp.144-168.

Laemmli, U. K. (1970). "Cleavage of structural proteins during the assembly of the head of bacteriophage T4." *Nature* **227**: 680-685.

Lambelet, P., Renevey, F., Kaabi, C. and Raemy, A. (1995). "Low-field nuclear magnetic resonance relaxation study of stored or processed cod." *Journal of Agricultural and Food Chemistry* **29**: 688-691.

Lanier, T. C. (1986). "Functional properties of surimi." *Food Technology* **40**: 107-114.

Lastein, N. (2002). Analysis of Water Pools in Cod using Slicing/PARAFAC - master thesis. Copenhagen, The Royal Veterinary and Agricultural University - KVL**:** 116.

Lindsey, R. C. (1996). Food additives. In *Food chemistry*. New York, Marcel Dekker**:** pp.767-823.

Love, R. M. (1997). Biochemical dynamics and the quality of fresh and frozen fish. In *Fish processing technology*. G. M. Hall. London, Blackie Academic and Professional**:** pp.1-26.

Lynch, B. (2006, January 1). "Fishy Foodservice Trends." Prepared Foods Network Retrieved September 1, 2009, from http://www.preparedfoods.com/Articles/Feature_Article/ff4766cf40ad8010VgnVCM100000f932a8c0____.

Mackie, I. M. (1993). "The effects of freezing on flesh proteins." *Food Reviews International* **9**(4): 575-610.

Masniyom, P., Benjakul, S. and Visessanguan, W. (2005). "Combination effect of phosphate and modified atmosphere on quality and shelf-life extension of refrigerated seabass slices." *LWT* **38**: 745-756.

Matís. (2009). "Vinnsluleiðir." Fræðsluvefur Matís Retrieved August 10, 2009, from http://fraedsluvefur.rf.is/Undirflokkur/Vinnsluleidir/frysting/.

Matlock, R., Terrell, R. N., Savell, J. W., Rhee, K. S. and Dutson, T. R. (1984). "Factors affecting properties of raw-frozen pork sausage patties made with various NaCl/phosphate combination." *Journal of Food Science* **49**: 1363-1366.

Murphy, S. C., Gilroy, D., Kerry, J. F. and Kerry, J. P. (2005). "Assessment of added protein/starch on the functional properties of surimi gels manufactured from Atlantic whiting." *Food Chemistry and Toxicology* **70**(1): C21-C24.

Murray, J. and Burt, J. R. (2001). "The Composition of Fish." Ministry of technology, Torry research station Retrieved August 10, 2009, from http://www.fao.org/wairdocs/tan/x5916E/x5916e00.HTM.

Nettlon, J. A. (1985). Seafood Nutrition. Facts, issues and marketing of nutrition in fish and shellfish. NY, Osprey Books.

Offer, G. and Knight, P. (1988). The structural basis of water-holding in meat. In *Developments in meat science*. London, Elsevier**:** pp.63-243.

Onodenlore, A. C. and Shahidi, F. (1996). "Protein dispersions and hydrolysates from sharks (Isurus oxyrinchus)." *J. Aquat. Food Prod. Technol.* **5**: 43-59.

Park, J. W. (1994). "Functional protein additives in surimi gels." *Jorunal of Food Science* **59**(3): 525-527.

Park, J. W. and Lanier, T. C. (1989). "Scanning calorimetric behavior of tilapia myosin and actin due to processing of muscle and protein purification." *Journal of Food Science* **54**: 49-51.

Pellegrini, M. (2009). "Marinades Go High-Tech." The National Provisioner Retrieved September 1, 2009, from http://www.provisioneronline.com/Archives_Davinci?article=2154.

Peterson, B. C., Parrish, F. C., Stromer, J. and Sromer, M. H. (1988). "Effects of salt and pyrophosphate on the physical and chemical properties of beef muscle." *Journal of Food Science* **53**: 1258-1265.

Pétursson, S. (2006). "Nýjar matvælarannsóknir á Íslandi - Fiskiprótein lofa góðu sem yfirborðsvirk efni fyrir ýrulausnir." *Tímarit um raunvísindi og stærðfræði* **4**(1): www.raust.is/2007/1/14.

Protein. (2007, July 28). "Nutrition Fact Sheet: Protein." Retrieved August 10, 2009, from http://www.feinberg.northwestern.edu/nutrition/factsheets/protein.html.

Qiancheng, Z. (2008). Effects of salt and protein injection on yield and quality changes during storage of chilled and frozen saithe fillets. Reykavík, UNU-Fisheries Training Programme**:** 55.

Ragnarsson, K. (1988). The effect of various salts on the chemical and textural changes in frozen gadoid and non-gadoid fish mince. Cornell, Cornell University**:** 2510-25.

Raversu, E. M. and Krzynowek, J. (1991). "Variability of salt absorption by brine dipped fillets of cod (Gadus morhua), blackback flounder (Pseudopleuronectes americanus), and ocen perch (Sebastes marinus)." *Journal of Food Science* **56**: 648-652.

Rörå, A. M. B., Furuhaug, R., Fjæra, S. O. and Skjervold, P. O. (2004). "Salt diffusion in pre-rigor filleted Atlantic salmon." *Aquaculture* **232**: 255-263.

Sánchez-Alonso, L., Solas, M. T. and Borderías, A. J. (2007). "Physical study of minced fish muscle with a White-grape by-product added as an ingredient." *Journal of Food Science* **72**(2): 94-101.

SeaWorld. (2007). "SeaWorld/Busch Gardens ANIMALS." Retrieved August 10, 2009, from http://www.seaworld.org/animal-info/info-books/bony-fish/anatomy.htm.

Shahidi, F., Han, X. and Synowiecki, J. (1995). "Production and characteristics of protein hydrolysates from capelin (Mallotus villous)." *Food Chemistry* **53**(3): 285-293.

Shenouda, S. Y. K. (1980). "Theories of protein denaturation during frozen storage of fish flesh." *Advances in Food Research* **26**: 275-311.

Sikorski, E. Z. (1995). Seafood: Resources, Nutritional Composition and Preservation, CRC Press.

Simon, F. J., Reinke, W. C., Soo, H. M., Lanning, C. L. and Richert, S. H. (1981). "Process for producing a fish product", U.S. Patent: 4,301,180

Slizyte, R., Dauksas, E., Falch, E., Storrö, I. and Rustad, T. (2005). "Characteristic of protein fractions generated from hydrolysed cod (Gadus morhua) by-products." *Process Biochemistry* **40**: 2021-2033.

STATICE (2008). Catch, value and processing of catch 2007. Reykjavík Statistics Iceland (www.statice.is/series).

STATICE (2009). Catch, value and processing of catch 2008. Reykjavík, Statistics Iceland (www.statice.is/series)**:** 52.

Steen, C. and Lambelet, P. (1997). "Texture changes in frozen cod mince measured by low-field nuclear magnetic resonance spectroscpopy." *Journal of the Science of Food and Agriculture* **75**: 268-272.

Stone, H. and Sidel, J. L. (1985). Sensory evaluation practices. Orlando, Fla, Academic press Inc.

Strategro (2005). U.S. Market for protein ingredients. Developed for IcePro. Strategro International LLC, 28. February.

Strategro (2006). U.S. Protein injection marinade market. Developed for IcePro. Strategro International LLC, 25. February.

Swartz, W. E. (1983). "Whey protein fortified fish and process for preparation", U.S. Patent: 4,407,831

Thorarinsdottir, K. A., Arason, S., Bogason, S. G. and Kristbergsson, K. (2001). "Effects of phosphate on yield, quality, and water holding capacity in the processing of salted cod (Gadus morhua)." *Journal of Food Science* **66**(6): 821-826.

Thorarinsdottir, K. A., Arason, S., Geirsdóttir, M., Bogason, S. G. and Kristbergsson, K. (2002). "Changes in myofibrillar proteins during processing of salted cod (Gadus morhua) as determined by electrophoresis and differential scanning calorimetrhy." *Food Chemistry* **77**(3): 377-385.

Thorarinsdottir, K. A., Gudmundsdottir, G., Arason, S., Thorkelsson, G. and Kristbergsson, K. (2004). "Effefts of added salt, phosphates, and proteins on the chemical and pysicochemical characteristics of frozen cod (Gadus morhua) fillets." *Food Engineering and Physical Properties* **69**(4): 144-152.

Thorarinsdottir, K. A., Karlsdottir, M. G., Klonowski, I., Bergsson, A. B., Sigurdsson, S. and Arason, S. (2009). Jöfnun - aukin gæði og bættir eiginleikar marnings. Reykjavík, Matís Report 15-09**:** 28.

Thorkelsdottir, Á., Sveinsdóttir, K., Thorarinsdottir, K. A., Tryggvadottir, S. V., Martinsdottir, E. and Pálsson, P. G. (2005). Shelf life of radio-frequency and conventional heated frozen cod and salmon fillets. Reykjavík, IFL Report**:** 41.

Thorkelsson, G. (2007). "Kennsluglærur í námskeiðinu Matvælavinnsla 1 (09.81.45)."

Thorkelsson, G. and Gunnlaugsdóttir, H. (2005). Lífvirk efni í íslensku sjávarfangi - Yfirlitsskýrsla. Reykjavík, IFL Report.

Thorkelsson, G., Sigurgísladóttir, S., Geirsdóttir, M., Jóhannsson, R., Guérard, F., Chabeaud, A., Bourseau, P., Vandanjon, L., Jaouen, P., Chaplain-Derouiniot, M., Fouchereau-Peron, M., Martinez-Alvarez, O., Le Gal, Y., Ravallec, R., Picot, L., Berge, J. P., Delannoy, C., Jakobsen, G., Johannsson, I., Batista, I. and Pires, C. (2008). Mild processing techniques and development of functional marine proteins and peptide ingredients. In *Improving seafood products for the consumer*. T. Borresen. Cambridge, Woodhead publishing limited**:** pp.363-398.

Toldrá, F. (2003). "Muscle Foods: Water, Structure and Functionality." *Food Science and Technology International* **9**(3): 173-177.

Valsdóttir, T., Finnbogadóttir, G. A., Thorarinsdóttir, K. A. and Arason, S. (2006). Notkun fiskpróteina í flakavinnslu. Sprautun með smækkuðum vöðva. Reykjavík, IFL Report.

Vann, D. G. and DeWitt, C. A. M. (2007). "Evaluation of solubilized proteins as an alternative to phosphates for meat enhancement." *Journal of Food Science* **72**(1): C72-C77.

Voet, D. and Voet, J. G. (2004). Bioschemistry, 3rd edition. USA, John Wiley & Sons.

Wagenknecht, W. and Tuelsner, M. (1975). "Studies on technologically determined changes in the water binding capacity of fish muscle.] Untersuchungen ueber technologisch bedingte Veraenderungen der Wasserbindung des Fischmuskels." *Fischerei Forschung* **13**: 57-65.

Wikipedia. (2009a, August 10). "Actin." Retrieved August 10, 2009, from http://en.wikipedia.org/w/index.php?title=Actin&oldid=307179606

Wikipedia. (2009b, July 17). "Atlantic cod." Retrieved August 9, 2009, from http://en.wikipedia.org/wiki/Atlantic_cod.

Wikipedia. (2009c, July 30). "Myosin." Retrieved August 10, 2009, from http://en.wikipedia.org/w/index.php?title=Myosin&oldid=305027558.

Wikipedia. (2009d, June 23). "Sacromere." Retrieved August 10, 2009, from http://en.wikipedia.org/w/index.php?title=Sarcomere&oldid=298052141.

Xiong, Y. L. L. (2005). "Role of myofibrillar proteins in water binding in brine-enchanced meats." *Food Research International* **38**: 281-287.

Zhou, P., Mulvaney, S. J. and Regenstein, J. M. (2006). "Properties of Alaska pollock skin gelatin: A comparison with tilapia and pork skin gelatins." *Journal of Food Science* **71**(6): C313-C321.

APPENDIX A

This appendix contains tables with values with regard to yield after injection, storage yield, drip loss and cooking yield of the fillets.

Table A.1 Yield (%) after injection of fresh and light salted cod fillets (n=20) (average ± stdv.). [a-e]) Values with the same superscript letter within a column are not significantly different (p>0.05).

Material	Treatment	After injection (%)
Fresh cod fillets	*1.5% Salt*	105.1 ± 2.1^{ab}
	HFP	116.1 ± 2.4^{c}
	FPI	112.2 ± 1.0^{cd}
	FPH	106.9 ± 1.3^{ae}
Light salted cod fillets	*HFP*	108.9 ± 1.0^{de}
	FPI	108.1 ± 1.0^{ade}
	FPH	103.2 ± 0.9^{b}

Table A.2. Yield (%) after injection of fresh skinless saithe fillets (n=26) (average ± stdv.). [a-b]) Values with the same superscript letter within a column are not significantly different (p>0.05). (Saithe fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine).

	Treatment	After injection (%)
Fresh saithe fillets	*4% Salt*	104.9 ± 1.3^{a}
	HFP(a)	115.7 ± 2.8^{b}
	HFP(b)	115.2 ± 2.0^{b}
	Gelatine	105.6 ± 1.8^{a}
	HFP(a)+Gelatine	113.3 ± 1.7^{b}
	FPH	105.1 ± 0.9^{a}

Table A.3. Yield (%) after chilled storage of fresh cod fillets (average ± stdv.). [a-b]) Values with the same superscript letter within a column are not significantly different (p>0.05). [1-2]) Values with the same superscript number within a row are not significantly different (p>0.05).

Treatment	Day 1	Day 3	Day 5	Day 10
Control	^{1}90.7 ± 1.7	^{1}91.6 ± 1.0^{a}	^{1}88.6 ± 0.7	^{1}91.5 ± 2.5
1.5% Salt	^{1}95.6 ± 1.9	^{1}97.2 ± 3.8^{ab}	^{1}95.7 ± 2.3	^{1}97.1 ± 2.7
HFP	^{1}113.9 ± 1.3	^{2}110.3 ± 1.5^{b}	^{12}111.3 ± 1.3	^{2}109.1 ± 2.3

Table A.4. Yield (%) after 1 month of frozen storage of fresh and light salted cod fillets (average ± stdv.). [a-d]) Values with the same superscript letter within a column are not significantly different (p>0.05).

Material	Treatment	After frozen storage (%)
Fresh cod fillets	*Control*	89.3 ± 3.0
	1.5% Salt	93.3 ± 2.5
	HFP	95.2 ± 2.3
	FPI	102.7 ± 1.9^{ab}
	FPH	104.6 ± 1.7^{a}
Light salted cod fillets	*Control*	98.3 ± 0.9^{c}
	HFP	104.3 ± 1.6^{a}
	FPI	101.2 ± 2.7^{bd}
	FPH	99.0 ± 2.0^{cd}

Table A.5. Yield (%) of the fresh skinless saithe fillets after chilling (n=6), 1 week (n=10) and 1 month (n=10) in frozen storage (thawing) (average ± stdv.). [a-c]) Values with the same superscript letter within a column are not significantly different (p>0.05). (Saithe fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine).

Treatment	4 days chilled (%)	1 w frozen (%)	1 mo frozen (%)
Control	96.6 ± 0.9^{a}	93.6 ± 1.6^{abc}	92.2 ± 1.8^{abc}
4% Salt	99.9 ± 1.9^{b}	91.8 ± 2.8^{a}	86.9 ± 9.4^{b}
HFP(a)	105.9 ± 1.7^{c}	91.3 ± 2.8^{a}	92.5 ± 6.4^{acb}
HFP(b)	105.1 ± 1.0^{c}	96.4 ± 3.6^{b}	96.2 ± 2.2^{a}
Gelatine	98.3 ± 2.8^{b}	90.8 ± 4.5^{a}	88.8 ± 2.7^{bc}
HFP(a)+Gelatine	105.4 ± 2.3^{c}	92.5 ± 4.3^{ac}	94.0 ± 3.3^{ab}
FPH	98.4 ± 1.1^{ab}	95.6 ± 3.3^{bc}	92.9 ± 2.9^{ab}

Table A.6. Drip loss (%) of fresh and light salted cod fillets after 1 month of frozen storage (thawed) (average ± stdv.). [a-b]) Values with the same superscript letter within a column are not significantly different (p>0.05).

Material	Treatment	Drip loss (%)
Fresh cod fillets	*Control*	10.7 ± 3.0
	1.5% Salt	6.7 ± 2.5[a]
	HFP	5.2 ± 1.6[a]
	FPI	8.7 ± 2.1
	FPH	3.4 ± 1.1[b]
Light salted cod fillets	*Control*	5.4 ± 0.7[a]
	HFP	6.4 ± 1.1[a]
	FPI	6.7 ± 1.8[a]
	FPH	2.3 ± 0.8[b]

Table A.7. Drip loss (%) of fresh skinless saithe fillets after chilling, 1 week and 1 month of frozen storage (average ± stdv.). [a-c]) Values with the same superscript letter within a column are not significantly different (p>0.05). (Saithe fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine).

Treatment	4 days chilled (%)	1 w frozen (%)	1 mo frozen (%)
Control	3.4 ± 0.9	3.6 ± 1.8	4.7 ± 1.1
4% Salt	5.5 ± 1.7[a]	10.0 ± 2.8[a]	12.3 ± 2.7[a]
HFP(a)	8.0 ± 1.6[bc]	18.9 ± 1.9	15.0 ± 3.3
HFP(b)	9.1 ± 1.9[c]	13.0 ± 2.8[b]	12.3 ± 2.1[a]
Gelatine	7.4 ± 1.8[abc]	11.6 ± 3.2[ab]	12.3 ± 1.6[a]
HFP(a)+Gelatine	8.1 ± 1.9[c]	15.7 ± 3.2	13.5 ± 3.1[a]
FPH	6.5 ± 1.0[ab]	7.0 ± 3.1	8.7 ± 1.7

Table A.8. Yield (%) after cooking (n=6) of fresh cod fillets after chilled storage and fresh and light salted cod fillets after 1 month of frozen storage (average ± stdv.). [a-b]) Values with the same superscript letter within a column are not significantly different (p>0.05).

Material	Treatment	After chilled storage (%)	After frozen storage (%)
Fresh cod fillets	*Control*	84.5 ± 0.4	87.7 ± 1.4[a]
	1.5% Salt	80.3 ± 1.2[a]	87.2 ± 0.6[a]
	HFP	78.9 ± 0.5[a]	87.6 ± 1.4[a]
	FPI	--	84.9 ± 2.2[a]
	FPH	--	80 4 ± 3.1[b]
Light salted cod fillets	*Control*	--	87.7 ± 2.3[a]
	HFP	--	85.8 ± 3.1[a]
	FPI	--	78.6 ± 5.3[b]
	FPH	--	87.2 ± 3.0[a]

Table A.9. Yield (%) after cooking (n=6) of fresh skinless saithe fillets after chilling, 1 week and 1 month of frozen storage (average ± stdv.). [a-d]) Values with the same superscript letter within a column are not significantly different (p>0.05). (Saithe fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine).

Treatment	4 days chilled (%)	1 w frozen (%)	1 mo frozen (%)
Control	79.5 ± 1.0[ab]	77.5 ± 1.6[b]	74.1 ± 4.5[a]
4% Salt	83.2 ± 1.7[c]	81.2 ± 2.9[a]	76.9 ± 3.4[ab]
HFP(a)	74.9 ± 2.0 [d]	77.5 ± 1.7[b]	77.2 ± 2.0[ab]
HFP(b)	72.5 ± 2.7[d]	73.8 ± 2.9	76.9 ± 1.8[ab]
Gelatine	78.7 ± 1.6[a]	77.3 ± 3.5[b]	78.1 ± 2.0[b]
HFP(a)+Gelatine	74.2 ± 3.1[d]	78.2 ± 2.0[ab]	79.7 ± 0.7[b]
FPH	81.8 ± 1.9[bc]	81.1 ± 3.3[a]	79.3 ± 2.6[b]

APPENDIX B

This appendix contains measured values of water holding capacity of the fillets studied.

Table B.1. Water holding capacity (%) of fresh cod fillets after chilled storage and fresh and light salted cod fillets after 1 month of frozen storage (n=3) (average ± stdv.). [a-d]) Values with the same superscript letter within a column are not significantly different (p>0.05).

Material	Treatment	After chilled storage (%)	After frozen storage (%)
Fresh cod fillets	*Control*	73.1 ± 0.9[a]	73.2 ± 5.0[a]
	1.5% Salt	72.9 ± 0.8[a]	63.3 ± 5.8[b]
	HFP	74.1 ± 3.6[a]	68.3 ± 3,5
	FPI	--	62.9 ± 1.6[b]
	FPH	--	76.9 ± 2.2[a]
Light salted cod fillets	*Control*	--	99.1 ± 0.1[c]
	HFP	--	99.3 ± 0.2[c]
	FPI	--	93.7 ± 2.6[d]
	FPH	--	97.6 ± 1.1[cd]

Table B.2. Water holding capacity (%) of fresh skinless saithe fillets (n=3) after chilled and frozen (1 week and 1 month) storage (average ± stdv.). [a-d]) Values with the same superscript letter within a column are not significantly different (p>0.05). (Saithe fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine).

Treatment	4 days chilled (%)	1 w frozen (%)	1 mo frozen (%)
Control	89.9 ± 1.9[ab]	76.1 ± 2.5[a]	76.5 ± 2.3[a]
4% Salt	92.6 ± 2.1[a]	82.8 ± 1.5[b]	70.8 ± 1.3[b]
HFP(a)	84.8 ± 1.6[c]	68.6 ± 1.4[c]	71.7 ± 3.0[b]
HFP(b)	87.2 ± 1.9[c]	70.0 ± 3.0[cd]	65.5 ± 2.0[c]
Gelatine	91.8 ± 1.2[a]	67.8 ± 4.3[c]	70.1 ± 3.4[b]
HFP(a)+Gelatine	87.4 ± 2.7[b]	73.4 ± 1.6[ad]	64.5 ± 2.8[c]
FPH	92.5 ± 1.1[a]	82.2 ± 0.6[b]	76.3 ± 2.0[a]

APPENDIX C

This appendix contains graphical representations of data fromT_2 transverse relaxation time (T_{21} and T_{22}) measurements of the fillets. There are also tables containing measured values of T_{21} and T_{22} populations of the fillets, A_1 and A_2, respectively.

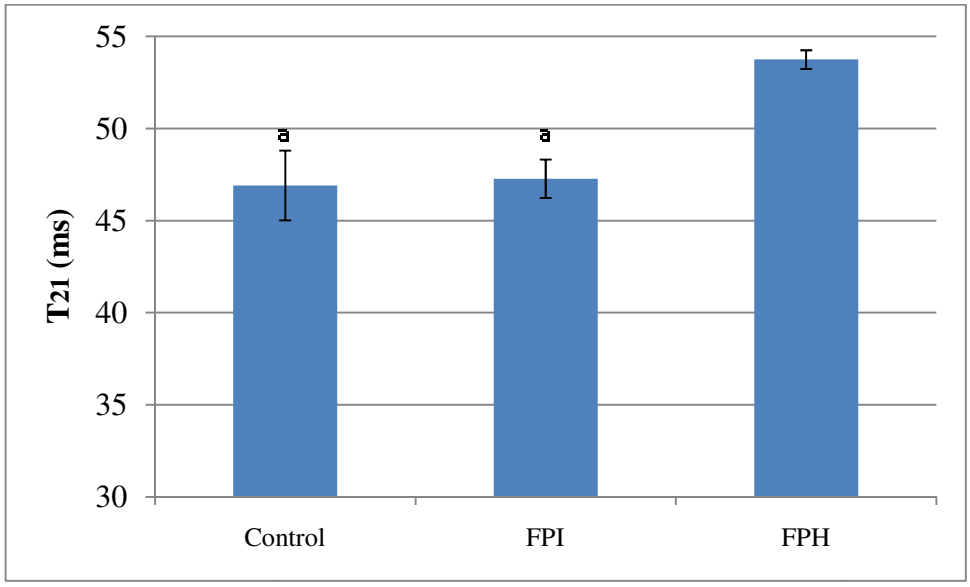

Figure C.1. The shorter (T_{21}) transversal relaxation time, T_{21}, of the fresh cod fillets after 1 month of frozen storage. [a-b]) Not significant difference between groups are market with the same letter.

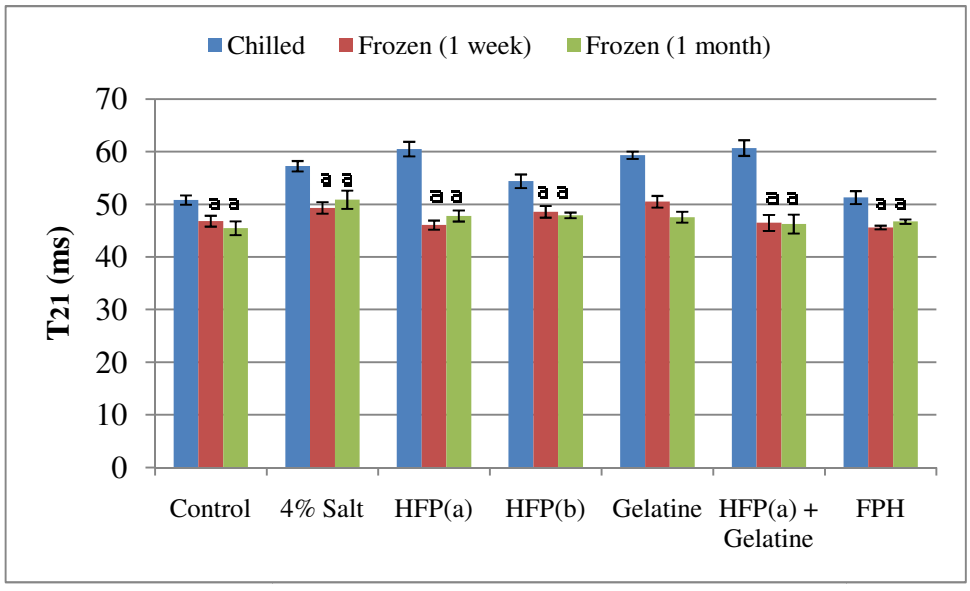

Figure C.2 The shorter transversal relaxation time, T_{21}, of fresh skinless saithe fillets after 4 days of chilled storage, 1 week and 1 month of frozen storage. Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a) + Gelatine were first injected with 4% salt brine. [a-b]) Not significant difference between values (chilled, frozen 1 week and frozen 1 month) within each treatment group are market with the same letter (p>0.05).

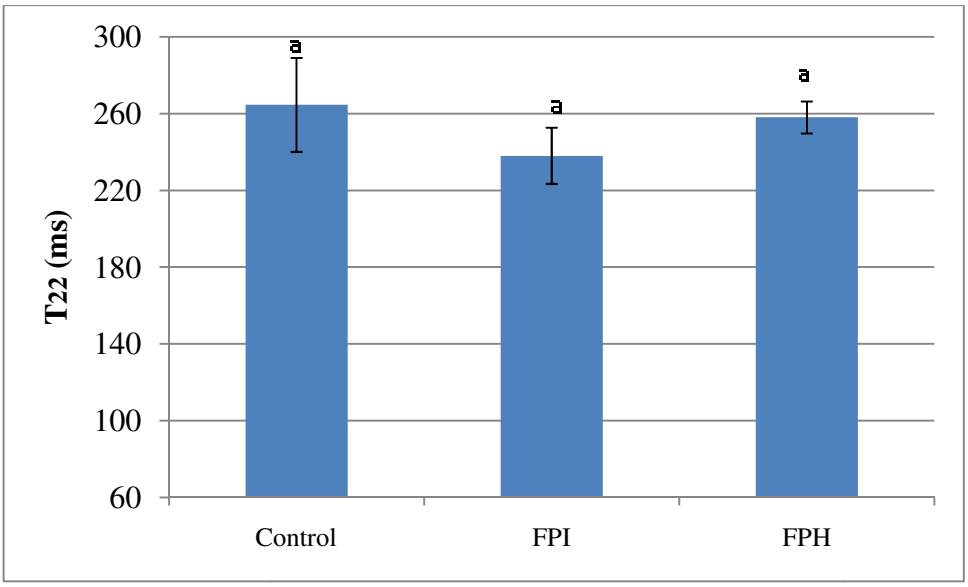

Figure C.3. The longer transversal relaxation time, T_{22}, of the fresh cod fillets after 1 month of frozen storage. [a]) Not significant difference (p>0.05) between groups are market with the same letter.

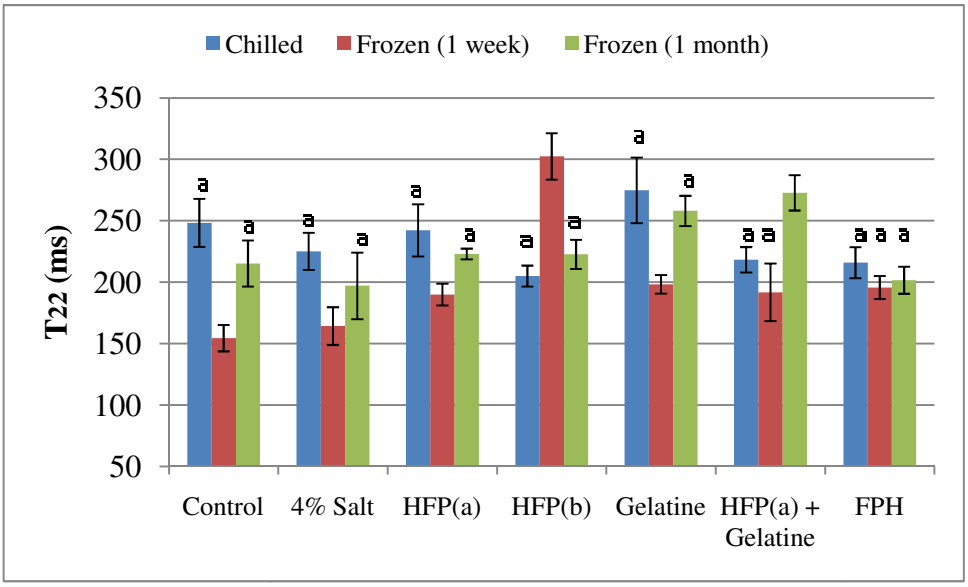

Figure C.4 The longer transversal relaxation time, T_{22}, of fresh skinless saithe fillets after 4 days of chilled storage, 1 week and 1 month of frozen storage. Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a) + Gelatine were first injected with 4% salt brine. [a-b]) Not significant difference (p>0.05) between values (chilled, frozen 1 week and frozen 1 month) within each treatment group are market with the same letter.

Table C.1. Normalised distribution of water in fresh cod fillets after 1 month of frozen storage. The A_1 express the relatively amount of water which is strongly bounded to the samples and the A_2 express the relatively amount of water which is loosely bounded to the samples (average ± stdv.). [a-b]) Values with the same superscript letter within a column are not significantly different (p>0.05).

Treatment	A_1 population (%)	A_2 population (%)
Control	82.8 ± 1.4^{a}	17.2 ± 1.4^{a}
FPI	84.9 ± 1.5^{a}	15.1 ± 1.5^{a}
FPH	84.9 ± 0.6^{a}	15.1 ± 0.6^{a}

Table C.2. A_1 population. Normalised distribution of water in fresh saithe fillets after chilled and frozen (1 week and 1 month) storage (average ± stdv.). The A_1 express the relatively amount of water which is strongly bounded to the samples. [a-c]) Values with the same superscript letter within a column are not significantly different (p>0.05). (Saithe fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine).

Treatment	4 days chilled	1 w frozen	1 mo frozen
Control	85.7 ± 0.4^{ab}	82.1 ± 0.7^{a}	82.1 ± 0.5^{a}
4% Salt	85.1 ± 0.4^{bc}	77.0 ± 0.6^{bc}	75.6 ± 0.9^{abc}
HFP(a)	81.0 ± 1.2^{c}	76.3 ± 1.5^{b}	71.6 ± 1.8^{b}
HFP(b)	86.7 ± 1.5	70.7 ± 0.7	76.4 ± 1.5^{abc}
Gelatine	86.0 ± 0.8^{b}	75.9 ± 0.2^{b}	76.4 ± 2.6^{abc}
HFP(a)+Gelatine	77.6 ± 3.0^{c}	78.1 ± 1.3^{c}	72.9 ± 3.3^{bc}
FPH	85.7 ± 0.7^{ab}	81.9 ± 0.7^{a}	79.4 ± 0.7^{ac}

Table C.3. A_2 population. Normalised distribution of water in fresh saithe fillets after chilled and frozen (1 week and 1 month) storage (average ± stdv.). The A_2 express the relatively amount of water which is loosely bounded to the samples. [a-c]) Values with the same superscript letter within a column are not significantly different (p>0.05). (Saithe fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine).

Treatment	4 days chilled	1 w frozen	1 mo frozen
Control	14.3 ± 0.4^{ab}	17.9 ± 0.7^{a}	17.9 ± 0.5^{a}
4% Salt	14.9 ± 0.4^{bc}	23.0 ± 0.6^{bc}	24.4 ± 0.9^{abc}
HFP(a)	19.0 ± 1.2^{c}	23.7 ± 1.5^{b}	28.4 ± 1.8^{b}
HFP(b)	13.2 ± 1.5	29.3 ± 0.7	23.6 ± 1.5^{abc}
Gelatine	14.0 ± 0.8^{b}	24.1 ± 0.2^{b}	23.6 ± 2.6^{abc}
HFP(a)+Gelatine	22.4 ± 3.0^{c}	21.9 ± 1.3^{c}	27.1 ± 3.3^{bc}
FPH	14.3 ± 0.7^{ab}	18.1 ± 0.7^{a}	20.6 ± 0.7^{ac}

APPENDIX D

This appendix contains values with water and protein contents of the fillets (Table D.1 and Table D.2). In table D.3 and D.4 the yield with respect to water and protein of the fresh and light salted cod fillets are listed. There is also graphical representation of yield with respect to water and protein of the saithe fillets (Figure D.1 and Figure D.2).

Table D.1. Water (±0.4%) and protein (±0.4%) content of fresh and light salted cod fillets after chilled (5 days) and frozen (1 month) storage (n=3). [a-b]) Values with the same superscript letter within a column are not significantly different (p>0.05).

		Chilled		*Frozen*	
Material	**Treatment**	**Water (%)**	**Protein (%)**	**Water (%)**	**Protein (%)**
Fresh cod fillets	*Control*	81.7[a]	17.4[a]	81.9[a]	18.2[a]
	1.5% Salt	82.9[a]	16.2[a]	82.7[a]	16.6[b]
	HFP	84.4	14.6	81.8[a]	17.4[ab]
	FPI	--	--	83.1[a]	16.3[b]
	FPH	--	--	82.8[a]	16.6[b]
Light salted cod fillets	*Control*	--	--	85.3[ab]	12.1[a]
	HFP	--	--	85.9[ab]	11.4[a]
	FPI	--	--	86.4[a]	11.1[a]
	FPH	--	--	84.9[b]	12.4[a]

Table D.2. Water (±0.4%) and protein (±0.4%) content of fresh saithe fillets after chilled (4 days) and frozen (1 week and 1 month) storage (n=3). Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine. [a-c]) Values with the same superscript letter within a column are not significantly different (p>0.05).

	Chilled		*Frozen (1 week)*		*Frozen (1 month)*	
Treatment	**Water (%)**	**Protein (%)**	**Water (%)**	**Protein (%)**	**Water (%)**	**Protein (%)**
Control	81.5[a]	18.5[ac]	80.4[abc]	19.4[a]	80.6[a]	19.1[abc]
4% Salt	83.3[b]	16.7[b]	80.6[abc]	18.9[ab]	80.9[a]	18.5[abc]
HFP(a)	83.3[b]	16.2[b]	80.9[abc]	18.6[ab]	81.8[a]	17.7[b]
HFP(b)	82.9[ab]	17.1[bc]	81.8[b]	17.7[b]	81.3[a]	18.2[abc]
Gelatine	85.4[c]	14.6	81.4[abc]	18.1[ab]	81.1[a]	18.4[abc]
HFP(a)+Gelatine	84.0[bc]	16.1[b]	80.5[abc]	19.0[ab]	81.7[a]	17.8[abc]
FPH	80.4[a]	19.6[a]	80.1[c]	19.5[a]	80.4[a]	19.2[c]

Table D.3. Average water yield (%) of fresh cod fillets after chilled storage and fresh and light salted cod fillets after 1 month of frozen storage.

Material	Treatment	Chilled	Frozen
Fresh cod fillets	*Control*	88.6	89.5
	1.5% Salt	97.2	94.4
	HFP	114.9	95.3
	FPI		104.5
	FPH		106.1
Light salted cod fillets	*Control*		98.3
	HFP		105.0
	FPI		102.5
	FPH		98.5

Table D.4 Average protein yield (%) of fresh cod fillets after chilled storage and fresh and light salted cod fillets after 1 month of frozen storage.

Material	Treatment	Chilled	Frozen
Fresh cod fillets	*Control*	88.6	93.4
	1.5% Salt	89.2	89.0
	HFP	93.4	95.2
	FPI		96.2
	FPH		99.8
Light salted cod fillets	*Control*		98.3
	HFP		98.2
	FPI		92.8
	FPH		101.4

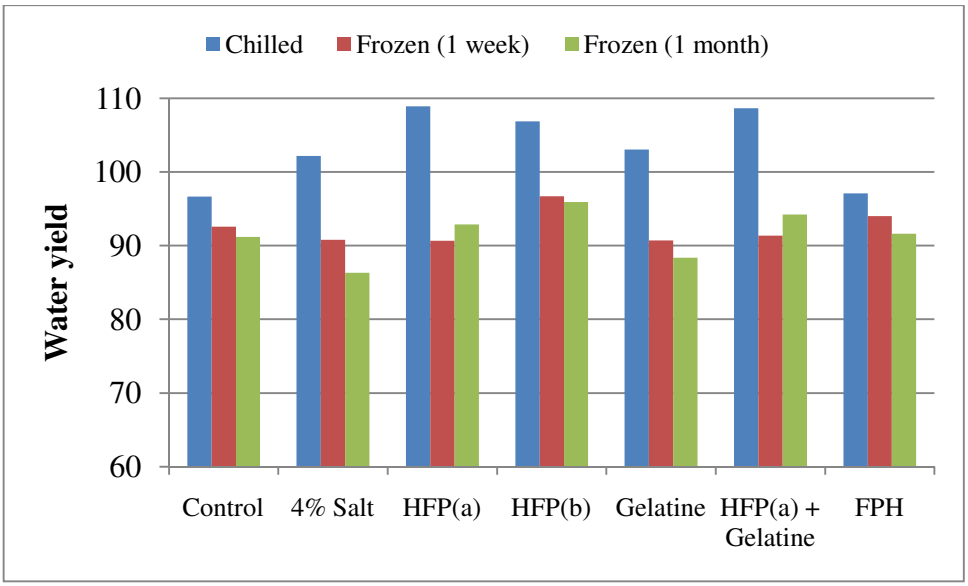

Figure D.1 Water yield (%) of fresh skinless saithe fillets after chilled and frozen (1 week and 1 month) storage. Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine.

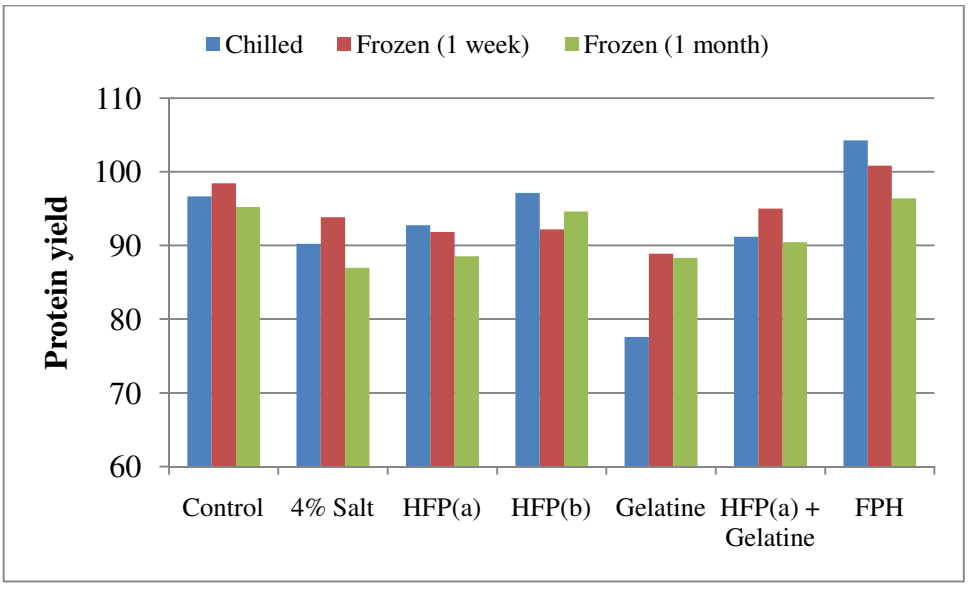

Figure D.2. Protein yield (%) of fresh skinless saithe fillets after chilled and frozen (1 week and 1 month) storage. Fillets injected with HFP(a), HFP(b), Gelatine and HFP(a)+Gelatine were first injected with 4% salt brine.

APPENDIX E

Results from the sensory analysis of fresh cod fillets after days at chilled storage, according to QDA, are listed in Table E.1.

Table E.1. The mean of QDA sensory attributes (scale 0-100), for fresh cod fillets after 5 days at chilled storage. (ms: marginal significance, p = 0.05-0.10); *(p<0,05); **(p<0.01); *(p<0.001).**

Sensory attributes		HFP	1.5% Salt	Control	p-value
Odour					
Sweet		38	44	40	0.292
Shellfish		34	40	35	0.143
Meat		28	31	29	0.379
Vanilla/boiled milk		31	34	32	0.796
Potatoes		35	37	39	0.514
Frozen		11	9	8	0.231
Table cloth		16	18	17	0.833
TMA		13	12	14	0.570
Sour		7	9	10	0.269
Sulphur		5	5	4	0.581
Appearance					
Colour		32	28	34	0.358
Appearance		38	33	33	0.254
Precipitation	**	50	46	37	0.009
Flakes		47	45	51	0.130
Taste					
Salt	***	25	24	12	0.000
Metallic	ms	25	30	30	0.055
Sweet		29	35	30	0.256
Meat		25	29	30	0.298
Frozen	*	17	12	13	0.035
Pungent	**	14	9	9	0.006
Sour	ms	8	7	5	0.073
TMA		14	11	14	0.163
Off		17	14	15	0.798
Texture					
Soft		60	54	54	0.385
Juicy		48	50	49	0.856
Tender		52	49	46	0.564
Mushy	*	52	44	40	0.014
Meat		33	38	38	0.334
Clammy		39	38	39	0.975
Rubbery		26	25	27	0.910

APPENDIX F

Results from measurement of the injected fish protein solutions (FPS) and their raw material are listed in this Appendix. Chemical compositions of the FPS raw material and colorimetric results are listed in Table F.1 and Table F.2, respectively. Additional, the molecular weight distribution of the fish protein solutions are shown in Figure F.1.

Table F.1. Chemical composition of the raw material used in the injected protein solutions.

Raw material	Water (%)	Protein (%)	Salt (%)
Mince for HFP	84.3 ± 0.4	15.3 ± 0.4	0.4 ± 0.1
Mince for HFP(a)	83.0 ± 0.4	16,5 ± 0.4	0.4 ± 0.4
Mince for HFP(b)	82.1 ± 0.4	17,4 ± 0.4	0.4 ± 0.4
FPH concentrate	50.8 ± 1.2	39.1 ± 0.4	10.29 ± 0.1
Dried gelatine (CP)	4.7 ± 0.4	92.5 ± 0.4	2.4 ± 0.1

Table F.2. Whiteness of the fish protein solutions (FPS) used for injection and the raw material used to prepare the HFP solutions.

	Sample	Whiteness
Material	*Mince for HFP*	51.5 ± 4.4
	Mince for HFP(a)	27.5 ± 1.4
	Mince for HFP(b)	28.8 ± 2.2
Injection FPS	*HFP*	84.2 ± 4.7
	HFP(a)	48.7 ± 1.5
	HFP(a)+Gelatine	39.8 ± 2.3
	HFP(b)	52.7 ± 1.7
	Gelatine	13.01 ± 3.8
	FPH	14.2 ± 0.6
	FPI	59.6 ± 0.4

Figure F.1 shows how the molecular distribution of the fish protein solutions differs. The hydrolysed fish proteins (FPH) shows even distribution i.e. contains many difference sizes of molecules from 2 – 212 kDa. The gelatine, on the other hand contains no molecules bigger than ~66 kDa.

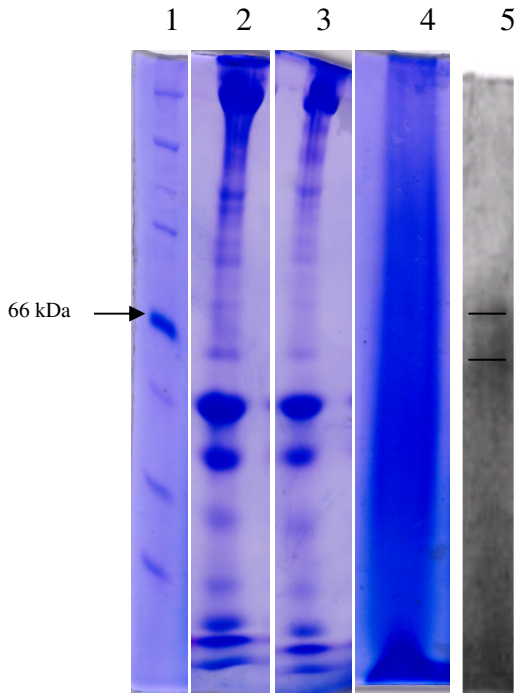

Figure F.1. Protein pattern of fish proteins used for injection into cod and saithe fillets. Line 1: Ladder; 2: HFP; 3: HFP(b); 4: FPH; and 5: Collagen peptide (low molecular weight gelatine).

APPENDIX G

Pearson´s correlation analysis was performed for the samples of chilled and frozen fillets. The correlation between two variables reflects the degree to which the variables are related. The correlation coefficient and corresponding p-value are listed in Table G.1 and Table G.2 for the chilled and frozen fillets, respectively. The pair of variables with positive correlation coefficients and p values below 0.050 tends to increase together. For the pairs with negative correlation and p value below 0.050, one variable tends to decrease while the other increases. For pairs with p value greater than 0.050, there is no significant relationship between the two variables.

The results show some distinguish between the variables after chilled and frozen storage.

Table G.1. Pearson´s correlation between measured variables of chilled fillets.

	Drip	Cooking yield	WHC	A_1 population	Water	Salt	Protein
Storage yield	**0.798**	**-0.813**	**-0.834**	-0.631	0.365	0.642	-0.365
	0.0314	**0.0261**	**0.0198**	0.129	0.420	0.120	0.420
Drip	--	-0.727	-0.517	-0.315	0.468	**0.822**	-0.468
	--	0.0639	0.235	0.491	0.289	**0.0234**	0.289
Cooking yield		--	**0.865**	0.424	-0.359	-0.327	0.359
		--	**0.0119**	0.343	0.430	0.474	0.430
WHC			--	0.591	-0.246	-0.204	0.246
			--	0.162	0.595	0.661	0.596
A_1 population				--	0.327	-0.303	0.327
				--	0.474	0.509	0.474
Water					--	0.645	**-1.000**
					--	0.118	**6.148E-31**
Salt						--	-0.645
						--	0.118

Table G.2 Pearson´s correlation between measured variables of frozen fillets.

	Drip	Cooking yield	WHC	A_1 population	Water	Salt	Protein
Storage yield	-0.025	0.109	-0.309	-0.0244	0.276	-0.365	-0.190
	0.957	0.815	0.500	0.959	0.550	0.421	0.683
Drip	--	0.536	-0.671	**-0.963**	0.826	**0.849**	**-0.888**
	--	0.215	0.0991	**0.0005**	0.0221	**0.0156**	**0.00759**
Cooking yield		--	-0.358	-0.495	0.273	0.402	-0.314
		--	0.431	0.259	0.553	0.372	0.493
WHC			--	0.636	-0.733	-0.601	**0.763**
			--	0.125	0.0609	0.153	**0.0460**
A_1 population				--	**-0.900**	-0.751	**0.939**
				--	**0.00572**	0.0515	**0.00168**
Water					--	0.508	**-0.990**
					--	0.245	**1.9E-5**
Salt						--	-0.624
						--	0.134