The influence of salting procedures on the characteristics of heavy salted cod

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THE INFLUENCE OF SALTING PROCEDURES ON THE CHARACTERISTICS OF HEAVY SALTED COD

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Abstract

The production of heavy salted cod (bacalao) has changed from being a single-step process salting to a multi-step procedure. The aim of this study was to gain a deeper understanding of water retention and yields of heavy salted cod as influenced by salting procedures. The effects of different pre-salting methods (injection and brining, brining only, and pickling) were compared to a single kench salting step. The products were evaluated at different stages of the process: after pre-salting, dry salting, storage and rehydration, with regard to changes in yields, chemical content and salt-induced changes in muscle proteins and microstructure.

Injection was significantly different from other methods in increasing weight yields through brining, dry salting and rehydration. Salt concentrations of the brine did not influence the weight yields of dry-salted and rehydrated products. However, salt concentrations >20% had negative effects on the colour and commercial quality of the salted products. Addition of phosphates increased weight yield of salted but not of rehydrated products. Effects on quality varied between trials; both negative and positive effects were observed.

Strong salting-out effects on proteins were seen due to high salt contents in the salted products regardless of the procedures applied. The effects of salting procedures on yields and quality of heavy salted cod products were related to the pathway of solubilisation, denaturation and aggregation of proteins in the muscle. Myosin was less aggregated when injection was applied during pre-salting. A greater degree of protein aggregation correlated with higher initial salt concentrations and dehydration during the first days of salting, which was obtained with brining, pickling and kench salting. In addition, these methods resulted in stronger enzymatic degradation of proteins, shown as an increase in lower molecular weight subunits.

The connective tissue in the muscle was believed to play an important role in water retention of the muscle. Microstructural analysis of the dry-salted fillets showed that the intercellular spaces in injected and brined fillets were increased compared to only brining. However, the cross-sectional area of the fish fibres was similar in both groups.

Based on this, the main reason for higher weight yields of injected products after salting and rehydration were believed to result from irreversible effects on the connective tissue in the muscle and better retention of myosin structure, thereby better water-holding. The drawback of the wet-salting methods (brining and injection) was that the yield of nitrogenous compounds was lower for injected and brine-salted fillets. However, the main changes were due to higher losses of non-protein nitrogen, which is more likely to influence the organoleptic properties of the products than the weight yields.

Key words: Salting, cod, yield, water, protein, water holding capacity, protein denaturation, microstructure

Ágrip

Verkun saltfisks hefur þróast mikið undanfarna áratugi, frá því að vera einföld stæðusöltun yfir í nokkra þrepa verkunarferil. Fjöldi þrepa og val aðferða er mismunandi eftir því hver framleiðandinn er. Almennt hefst verkunin með forsöltun sem framkvæmd er með sprautun og pæklun eða pæklun/pækilsöltun sem fylgt er eftir með þurrsöltun (stæðusöltun). Eftir þurrsöltun er afurðum pakkað í viðeigandi umbúðir eftir afurðaflokkum og mörkuðum. Fyrir matreiðslu, eru afurðir útvatnaðar til að lækka saltinnihald þeirra.

Markmið þessarar rannsóknar var að dýpka þekkingu á áhrifum mismunandi verkunarferla með tilliti til vatnsheldni og nýtingar saltaðra þorskflaka. Fylgst var með breytingum á nýtingu, efnainnihaldi, afmyndun próteina og vöðvabyggingu þorsks í gegnum ferillinn; frá hráefni í gegnum forsöltun, þurrsöltun, geymslu og útvötnun.

Nýting sprautaðra afurða hélst hærri í gegnum allan ferilinn samanborið við aðrar aðferðir. Nýting afurða sem eingöngu voru pæklaðar í upphafi verkunar var óháð pækilstyrk að því undanskildu að áhrif voru merkjanleg við sjálfa pæklunina. Hins vegar voru áhrif á gæði neikvæð ef pækilstyrkur fór yfir 20%. Notkun fosfats jók nýtingu eftir söltun en ekki eftir útvötnun. Áhrif af viðbættu fosfati á gæði voru metin í tveimur tilraunum en niðurstöðum bar ekki saman á milli þeirra. Almennt er fosfat þó talið hafa jákvæð áhrif á blæ afurða og bæta þannig gæði. Áhrif fosfats á nýtingu samanborið við sprautun voru óveruleg.

Saltinnihald í vöðva var almennt >20% eftir söltun óháð verkunarferlum. Breytingar (afmyndun) á próteinum voru því miklar en mismunandi eftir söltunaraðferðum. Bygging myósíns virtist raskast minna við söltun í sprautuðum afurðum. Það var talið tengjast vægari hækkun á saltstyrk við upphaf söltunar sem leiddi til sterkari „salting-in“ áhrifa en með öðrum aðferðum.

Breytingar á bandvef við söltun voru einnig mismunandi eftir söltunaraðferðum. Millifrumubil eftir söltun var meira í afurðum sem voru sprautaðar og pæklaðar samanborið við afurðir sem eingöngu voru pæklaðar í upphafi verkunar. Aftur á móti var flatarmál fruma sambærilegt. Mismunur á vatnsheldni vöðvans og nýtingu eftir verkunarferlum var því tengdur breytileika í afmyndun bæði kollagens og myósíns en hingað til hafa niðurstöður fyrri rannsókna fyrst og fremst verið túlkaðar úr frá breytingum á vöðvatrefjum.

Tap þurrefnis við verkun var meira í sprautuðum og pækluðum afurðum. Fyrst og fremst var um „non protein nitrogen“ að ræða Hlutfall próteina sem tapaðist var lágt og því voru áhrif þurrefnistaps á vatnsheldni vöðvans talin óveruleg. Áhrifin voru fremur talin felast í breytileika í bragði og lykt afurða, vegna eðlis og eiginleika „non protein nitrogen“ efna en ekki var gerður samanburður á þessum eiginleikum í ritgerðinni.

Lykilorð: Söltun, þorskur, nýting, vatn, prótein, vatnsheldni, afmyndun, vöðvabygging, smásæ myndgreining

List of papers

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

I. Effects of Phosphate on Yield, Quality and Water Holding Capacity in the Processing of Salted Cod (*Gadus morhua*).
Kristin Anna Thorarinsdottir, Sigurjon Arason, Sigurður G. Bogason and Kristberg Kristbergsson.
Journal of Food Science, 66(6) 821-826 (2001).

II. Changes in myofibrillar proteins during processing of salted cod (*Gadus morhua*) as determined by electrophoresis and differential scanning calorimetry.
Kristin Anna Thorarinsdottir, Sigurjon Arason, Margret Geirsdottir, Sigurður G. Bogason and Kristberg Kristbergsson.
Food chemistry, 77, 377-385 (2002).

III. The effects of various salt concentrations during brine curing of cod (*Gadus morhua*).
Kristin Anna Thorarinsdottir, Sigurjon Arason, Sigurður G. Bogason and Kristberg Kristbergsson.
International Journal of Food Science & Technology, 39, 79-89 (2004).

IV. The effects of pre-salting methods from injection to pickling on the yields of heavily salted cod (bacalao).
Kristin Anna Thorarinsdottir, Sigurjon Arason, Gudjon Thorkelsson, Sjofn Sigurgisladottir, Eva Tornberg.
Submitted to Journal of Food Engineering, January, 2010.

V. Effects of different pre-salting methods on protein aggregation during heavy salting of cod fillets.
Kristin Anna Thorarinsdottir, Sigurjon Arason, Sjofn Sigurgisladottir, Thora Valsdottir, Eva Tornberg.
Submitted to Food Chemistry, January, 2010.

VI. Effects of pre-salting methods on the microstructural and rheological properties of heavy salted and rehydrated cod.
Kristin Anna Thorarinsdottir, Sigurjon Arason, Sjofn Sigurgisladottir, Valur N. Gunnlaugsson, Jonina Johannsdottir, Eva Tornberg.
Submitted to Food Research International, January, 2010.

The author's contributions to the papers

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

II. The author planned the experiments and wrote the paper in collaboration with co-authors. The author carried out the experiment and analysis, except for the DSC analysis, which was carried out by Margret Geirsdottir.

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

IV. The author planned the experiment and wrote the paper in collaboration with co-authors. The experimental work was carried out by technicians and scientists at Matís ohf.

V. The author planned the experiment and wrote the paper in collaboration with co-authors. The experimental work was carried out by technicians and scientists at Matís ohf, the DSC analysis and SDS-PAGE were carried out by Thora Valsdottir and the author.

VI. The author planned the experiment and wrote the paper in collaboration with co-authors. The experimental work was carried out by technicians and scientists of Matís ohf, and the microstructural analysis was carried out by Valur N. Gunnlaugsson, Jonina Johannsdottir and the author.

Abbreviations and symbols

MHC = heavy chain

HMM = heavy meromyosin

LMM = light meromyosin

TVB-N (TVN) = total volatile nitrogen bases

TMA = trimethyl amine

DSC = differential scanning calorimetry

NMR = nuclear magnetic resonance

WHC = water-holding capacity

LP = liquid phase

SP = solid phase

TN = total nitrogen

PN = protein nitrogen

NPN = non-protein nitrogen

TCA = trichloroacetic acid

TMAO = trimethylamine oxide

TMA = trimethylamine

DMA = dimethylamine

a_w = water activity

RH = relative humidity

SDS = sodium dodecyl sulfate

PAGE = Poly acrylamide gel electrophoresis

Eq. = equation

Y_t^o = total weight yield at time t (%)

M_0^o = initial weight of the fillets before salting (g)

M_t^o = weight of the fillets at time t (g)

ΔM_B^o = proportional weight changes during a processing step (from A to B) (%)

Yx_t^C = yield or recovery of chemical components (x) at time t (%)

x = weight fraction of a component chemical component (C = protein/water)

t = time

δ = phase shift

G′ = storage or elastic modulus

G″ = loss or viscous modulus

Contents

1 Introduction

Heavy salted cod products have been exported from Iceland since the 16th century. Since then, storage techniques like refrigeration and freezing have developed and have increasingly been applied to retard postmortem spoilage of fish. Therefore, the role of salting has changed from primarily being a storage method to being a curing method in order to obtain the unique organoleptic characteristics of heavily salted cod (bacalao) products.

1.1 Processing of salted cod

Salting and curing of cod is a long process that can be divided into several steps (Figure 1). Salting of the fish starts after filleting or splitting (butterfly filleting) when salt and possibly other ingredients are added to the products. The processes used for bacalao production have changed rapidly over the past decades. Originally, the fish was only pile salted by stacking splitted fish with alternating layers of coarse salt, often termed kench salting. It was restacked several times to obtain an even pressure and curing of the fish. Finally, it was sun dried. In the late 20th century in Iceland, producers started to pre-salt fish before the pile salting step, using pickling (from ~1980) and brining (from ~1990). The main difference between pickling and pile salting is that pickling is carried out in closed tubs, in some cases with addition of water/brine. On the other hand, the brine formed during pile salting is allowed to drain away. Exporters started to control the ambient temperature in storage, which made it possible to shorten the salting process and produce lightly cured products. The fish was only pile salted for 10 to 12 days after pre-salting and packed as lightly/wet cured. This curing stage of the products is known in Iceland as “tandurfiskur”. Today, different combinations of injection, brining, pickling and dry salting/pile salting/stacking are used, varying with producers and production countries.

The changes in salting procedures and curing conditions have altered the characteristics of the products, increased weight yields and improved commercial quality (Lindkvist, Gallart-Jornet & Stabell, 2008). The shorter curing time and lower temperature during curing and storage conditions have resulted in milder curing flavours and a whiter appearance (Barat et al., 2003; Lindkvist et al., 2008). Another important factor is that the raw material is of better quality than before as a result of improvements in catching, handling and storage techniques.

Weight yields and commercial quality are highly important with regard to process return, market share and prices. Salted products are rated according to size and commercial quality (SPIG/PORT) at packaging. PORT refers to export of the products to Portugal and SPIG to Spain, Italy and Greece.

The appearance and colour of the fish is of great importance. Higher standards are used for SPIG fish; it is supposed to be whiter and thicker than the PORT fish.

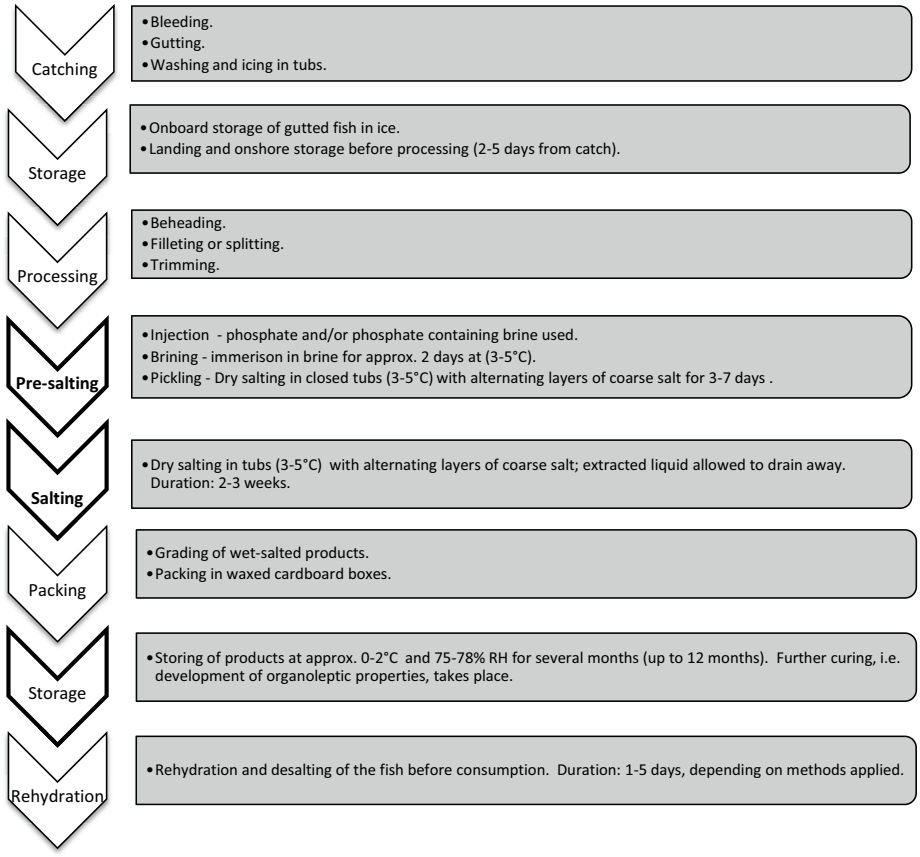


Figure 1. The process for the salted cod in the study, from catch to a product ready for cooking. The steps of interest in the study were the pre-salting and salting steps. Different pre-salting methods were used: injection and brining, brining only and pickling. (RH = relative humidity)

1.2 Products and chemical content

The water content of lightly cured fish is about 55-57% and the salt content about 18-21%. The water content of the lightly cured products has increased due to changes in salting procedures, mainly by the addition of a pre-salting step prior to dry salting, where the fish is wet salted through methods such as injection and brining. Medium-cured products are obtained by restacking the fillets and further salting for 1 to 3 weeks. Fully cured fish of this type has about 50-52% water and 19-21% salt content. Drying of the salted products can be carried out to obtain semi-dried or extra-dried products, with a water content of 40-44% and 30-32%, respectively (Table 1). In Table 1, the water and salt content of

salted cod at different curing stages are presented. However, no official standards are available for chemical content of salted cod in Iceland. The condition of the product is usually agreed upon between exporters and buyers and in accordance to consumer preference.

Table 1. Water and salt content of heavy salted cod at different curing stages. Values for fresh and rehydrated fish are shown for comparison. These values are presented to give a basic reference, although no official standards are available in Iceland.

Product	Water (%)	Salt (%)
Fresh fish	80-82	0.2-0.3
Lightly cured/wet salted (Tandurfiskur)	55-58	18-21
Medium cured	53-54	19-21
Fully cured	50-52	20-22
Ordinary cured	44-50	20-23
Dried (Brasilia*)	43-46	21-23
Semi-dried (Cura Corrente 7/8*)	40-42(44)	22-24
Extra dried*	30(-35)	23-25
Rehydrated	82-84	0.9-1.5

*(Eiríksson & Guðmundsson, 1982; Jónas Bjarnason, 1986)

Changes in salting procedures and shortening of the curing time have resulted in higher water content and increased weight yields of the salted products. These changes are accompanied by a higher uptake of salt and maintaining salt saturation of the liquid phase in the products, which is the prerequisite for the long shelf life of the products. Precise control of temperature and humidity is essential with regard to the growth of halophilic bacteria and the stability of quality parameters. It must be kept in mind that some microorganisms and enzymes are still active despite the high salt content and chilled storage conditions (Pedro S., 2004; Rodrigues, Ho, Lopez-Caballero, Vaz-Pires & Nunes, 2003). To minimize weight changes during storage, it is also important to maintain equilibrium between the water activity (a_w) in the salted fish and the relative humidity (RH) in air where the fish is stored. When the RH is higher than a_w in fully salted muscle (0.75), the salted fillets absorb water and gain weight, whereas the opposite is observed when RH < a_w (Doe, Hashmi, Poulter & Olley, 1982).

Curing of the fish continues after packing, during storage and transport of products. The curing time, and whether or not further processing such as drying is applied, depends on the markets. Before consumption the products are rehydrated. Rehydration involves soaking of the fish in water, which results in water uptake and desalting of the muscle. The shelf life of rehydrated products is short, meaning that rehydrated fillets can only be stored for a couple of days at refrigerated conditions. The rehydrated products can also be frozen and stored in that form.

1.3 Quantity and value of salted fish exported

Cod (*Gadus morhua*) is the most common fish species used for the production of heavy salted fish, but other species used are saithe (*Gadus virens*), ling (*Molva vulgaris*), tusk (*Brosme bromse*), and to a minor extent haddock (*Melanogrammus aeglefinus*) and blue ling (Table 2).

Table 2. Quantity of catch (tonnes) of gutted fish used for salt fish production in Iceland, in the years 2001 to 2007 (Statistics Iceland).

Species	2001	2002	2003	2004	2005	2006	2007
Cod	102.032	82.095	73.628	81.237	74.708	67.298	59.821
Haddock	-	-	1	498	57	43	119
Saithe	11.096	9.501	8.474	8.410	7.613	10.768	8.270
Ling	2.321	2.253	2.687	2.375	2.779	4.889	5.130
Blue ling	78	279	145	19	49	199	190
Tusk	2.550	3.119	2.656	1.441	1.344	2.764	3.880
Total	**118.077**	**97.247**	**87.591**	**93.980**	**86.550**	**85.961**	**77.410**

The total quantity of salted ground fish products exported was 34 to 43 thousand tons per annum in the years 2001 to 2007. The export value was in the range of 15 to 20.000 milliards Icelandic krona per annum from 1999 to 2006. Products were mostly wet-salted cod products. The export value was higher than that of both chilled and frozen cod products in 2008.

1.4 Market and consumer profiles

Iceland's salted cod products are mainly exported to Portugal and Spain (Figure 2). Nearly 20% is exported to the Netherlands, according to export reports (Statistics Iceland). However, this proportion of the products is mainly transported further to the Mediterranean countries.

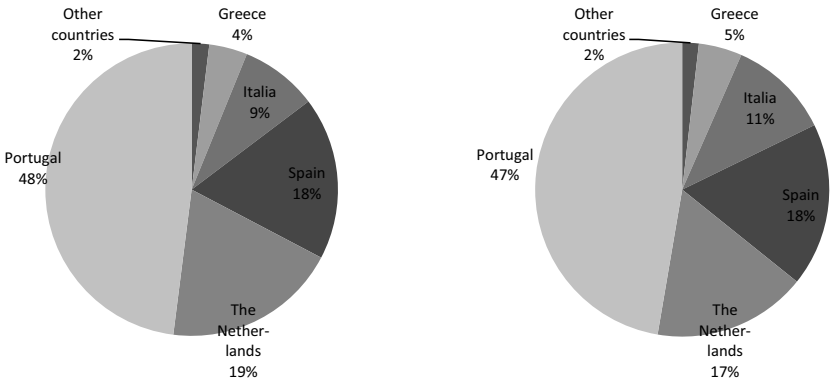


Figure 2. Export countries for wet-salted cod products from Iceland, ratios of quantities on the left, ratios of values on the right (Statistics Iceland).

The reason for the popularity of heavy salted cod is a result of the unique organoleptic properties of the salted products, which differ from fresh products. These are preferred by consumers in Southern Europe and Latin America. On the other hand, rehydration of the fish is time-consuming, and younger consumers aim for faster food preparation and in some cases also simpler and cheaper food. To fill this market gap, a new product, light salted cod fillets with only ~2% salt content, has been introduced to the market. The distribution and consumption of these products has increased rapidly over the last decade. The process and characteristics of the light salted products vary considerably from the traditional bacalao. It is only light salted by injection and/or brining and then stored as frozen. Therefore the characteristic flavour and texture, which are formed as proteins and fats are degraded during the curing of heavy salted cod, are missing.

Despite the increased consumption of light salted cod fillets, the market volume for heavy salted cod has not decreased but stagnated. This shows that there is still a group of consumers that prefers the traditional characteristics of the products and continues to buy them, even though the prices are higher (Lindkvist et al., 2008).

2 Objectives

In Iceland, the wet salting methods, injection and brining, are the most widely used for pre-salting cod. These methods can be used to control the rate of salt and water fluxes in the muscle and between the muscle and the surrounding brine. The salting mechanisms and process-related denaturation of proteins are essential with regard to different characteristics of the products: the weight yield, the commercial quality, and the organoleptic properties. However, a deeper knowledge about the exact influences and mechanisms of salting is needed for optimisation of the processes.

The objectives of this study were therefore:

- To find the relationship between yields and different salting procedures (Paper IV).
- To determine how quality and chemical content of the fish fillets are influenced by the different salting procedures (Paper IV).
- To compare the effects of traditional brine salting methods with a process with better control of the salt flux, either by gradually increasing the salt concentration in the brine or by keeping the salt concentration in the brine constant by compensating for the reduction in salt during processing (Paper III).
- To determine the effects of polyphosphates on yield, quality and sensory characteristics of the salt-cured cod during salting and after rehydration (Papers I and IV).
- To study the conformational stability, aggregation and degradation of proteins in the processing of high salt-content fish, using DSC and SDS-PAGE (Papers II and V).
- To compare the influence of different pre-salting procedures (injection and brine salting versus brining only) on microstructural and rheological characteristics of salted and rehydrated cod (Paper VI).

The results provided insight into the underlying reasons, i.e. salting-in and salting-out effects on proteins. These parameters induce structural changes like swelling and shrinkage of the muscle; in other words, they are determinant for the volumetric space available for the liquid phase to be held within the fish fillet. The results can also be the basis for an advisory tool used by salt fish producers to gain better control of changes in weight, chemical content and quality during the production. This would ensure less variation in the products, increase consumer appeal and strengthen market shares.

3 Theoretical background

Heavy salting involves extreme changes in water and salt content of the fish muscle, which are the prerequisite for the long storage life of the products. During salting and following storage, specific organoleptic characteristics of bacalao develop due to biochemical activities in the muscle (Lauritzen, 2004), known as curing of the fish. The characteristics of the products are influenced by postmortem changes of the fish, processing, salting methods and storage conditions (Figure 3). The main issues of this thesis are the mass transfer and yields of salted products (gray shaded box in Figure 3), which are of high economic importance for salt fish producers. These parameters are believed to be strongly affected by salting procedures and salt-related changes in proteins.

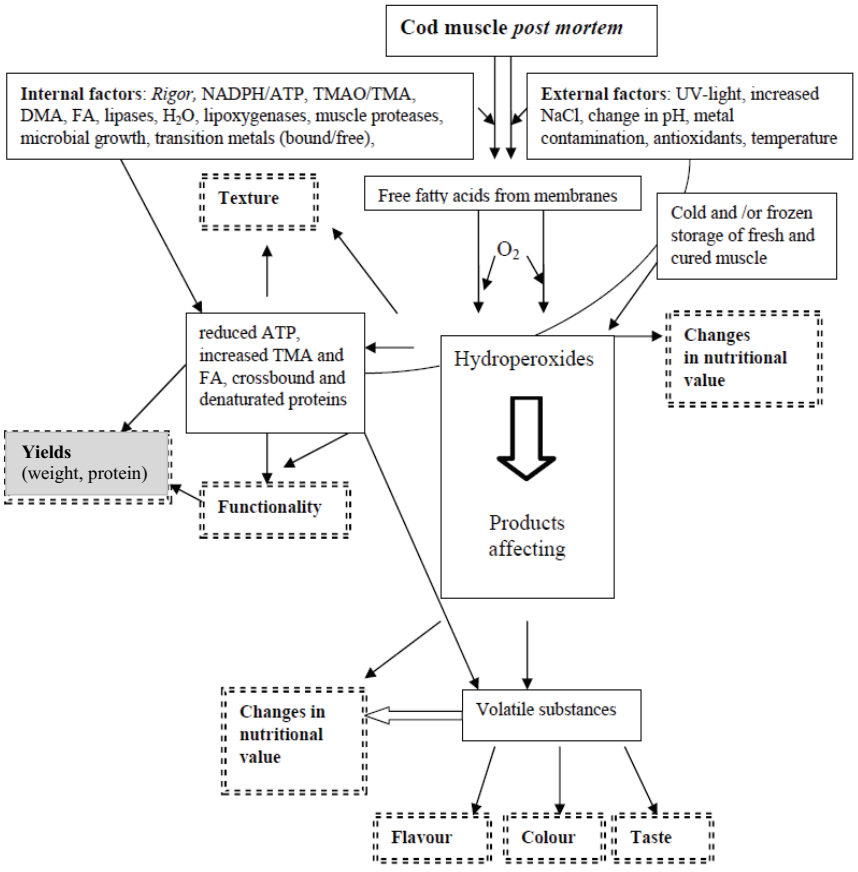


Figure 3. Biochemical processes in cod *post mortem* muscle that affect the quality of salt-cured products. Yields, changes in nutritional value, flavour, colour, taste, texture, and functionality are used as quality indicators (modified by (Lauritzen, 2004) from (Shenouda, 1980), (Martinsen, 1995)).
NADPH = Nicotinamide adenine dinucleotide phosphate hydrated, ATP = Adenosine triphosphate, TMAO = Trimethylamine oxide, TMA = Trimethylamine, DMA = Dimethylamine, FA = Formaldehyde, UV-light = Ultra violet light.

3.1 Salting methods

The methods applied for salting differ with regard to mass transfer mechanism and weight yields. The heavy salting of cod has developed from being a single step to multi-step process, involving a pre-salting step. Pre-salting is followed by a modified and shortened version of the old kench salting method, which has also been termed pile or dry salting. The main difference lies within a pre-salting step, which may involve brine injection, brining or pickling. In brine injection and brining, a pre-prepared brine with a controlled salt concentration is used, whereas dry salt is added afterwards to the fish when pickling and kench salting are employed.

3.1.1 Injection

Injection results in a relatively homogenous salt concentration in the muscle in a short time, compared to other salting methods. Additionally, it makes it possible to add other ingredients to the products, such as proteins, which would not be possible by brining. However, injection alone can only be used to add small quantities of salt to the fish because only limited amounts of brine can be pumped into the muscle. Injection volumes, distribution of brine and retention of the brine in the muscle depend on the characteristics of the raw material, composition of the brine, and the equipment and settings used, such as type of needle, needle density, needle-strokes per minute, release of the brine (in/out), dwelling time and pressure applied. The following treatments of the injected product and storage conditions also influence the retention of the injected brine. Disadvantages of injection are risk of microbial contamination and possible damage of the muscle structure due to the pressure applied and needle holes in the muscle (Bakowski, Riewe, Borys & Straszewski, 1970; Birkeland, Akse, Joensen, Tobiassen & Skara, 2007; Birkeland, Skara, Bjerkeng & Rora, 2003; Boles & Swan, 1997a; Boles & Swan, 1997b; Brunk, 1985).

The brine is believed to be located in brine pockets around the injection site initially after brining. The salt concentration is quickly diluted by the liquid in the muscle as the salt migrates from the injection site to other parts of the muscle (Offer, 1988a). The effects of injection can partly be described as brining, where the salt migrates from the injection site due to osmotic forces. What makes the process different from brining is mainly the smaller diffusion distance of the brine, which depends on the equipment used. In addition, the pressure exerted on the muscle during injection may affect structural components of the muscle depending on the condition of the raw material.

Needles with large diameters, high pressures, and continuous jets during injection are factors that can lead to breakage of the muscle structure, formation of channels or brine pockets in the muscle. Brine that is located in holes made by the needle is not fixed in the structure and can easily drip off. High needle density can be more effective than increased pressure to improve weight yields of fish muscle

without increasing risk of structural defects (Birkeland et al., 2003). Pressure that is too high can easily cause damage to the muscle structure, such as increased gaping of the muscle segments and breakage of muscle fibres. It must be kept in mind that the structure of fish muscle is more sensitive than that of meat (Freixenet, 1993). Therefore milder treatments must be used. Methods like tumbling cannot be applied to shorten the time needed for the migration of salt into the muscle, as used in meat products.

3.1.2 Brining

In the late 20th century, brine salting became the main pre-salting method in Iceland. The use of brining as a pre-salting step before pile salting is believed to improve quality and weight yields of salt products. Brining is carried out by immersing fillets into a brine prepared from coarse salt and tap water. The diffusion of salt into the muscle depends on several factors, such as concentration and composition of the brine, the shape and thickness of the product, ratio of brine to product and duration of brining. The temperature is kept low (2-4°C) to minimise bacterial growth. Several studies have been carried out in which the muscle is brined in a high brine-to-fish ratio to minimise dilution effects from liquid diffusing from the muscle to the brine (Andres et al., 2002; Andres, Rodriguez-Barona, Barat & Fito, 2005b; Barat et al., 2002; Barat et al., 2003). In praxis, common initial ratios are 1.6:1 or 2:1, meaning that significant changes are observed in the salt concentration of the brine during brining.

The relationship between salt concentration of brine and chemical changes in the fish muscle have long been known. Already in the early 20th century, the influence of salt concentration in the brine on weight uptake and extraction of muscle proteins during brining were described (Callow 1931 (as cited by Lawrie 1998); Callow, 1947). It was found that gradual increase in salt concentration of the brine (from 2% salt) resulted in higher weight gains than by immediate immersion of pork muscle in strong brine (>6%) (Figure 4). The differences in weight yields obtained can be explained by salting-in effects on proteins at the lower salt concentrations, whereas this does not occur if the initial salt concentration is in the salting-out region (above 4-5%), and these changes appeared to be irreversible. These findings were confirmed in later studies (Duerr & Dyer, 1952; Knight & Parsons, 1988; Offer, 1988a). Duerr & Dyer (1952) described the relationship between the solubility of cod muscle proteins, salt concentration and weight uptake. The solubility of cod muscle proteins—mainly myosin—and weight uptake decreased when the salt concentration in the muscle exceeded 5-6% salt, which occurred after about 48 hours when the cod fillets were immersed in saturated sodium chloride solution at 0°C (Figure 5).

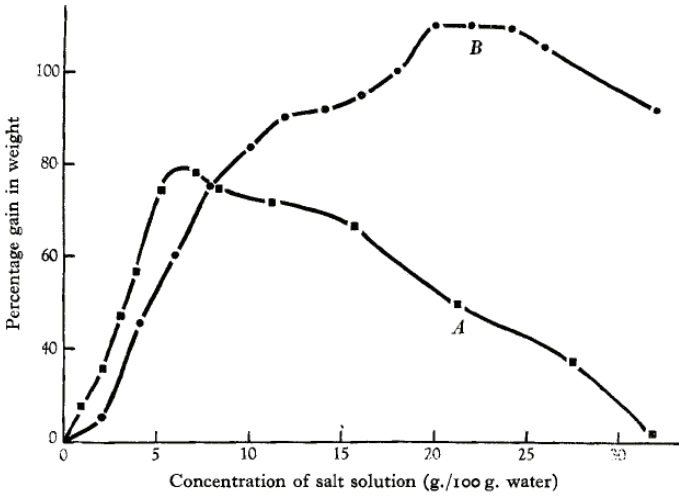


Figure 4. Swelling of pork muscle at 0°C by A: immersion in solutions containing various amounts of salt, and B: immersion in a 2% solution of salt which was progressively increased in strength during brining (Callow, 1947).

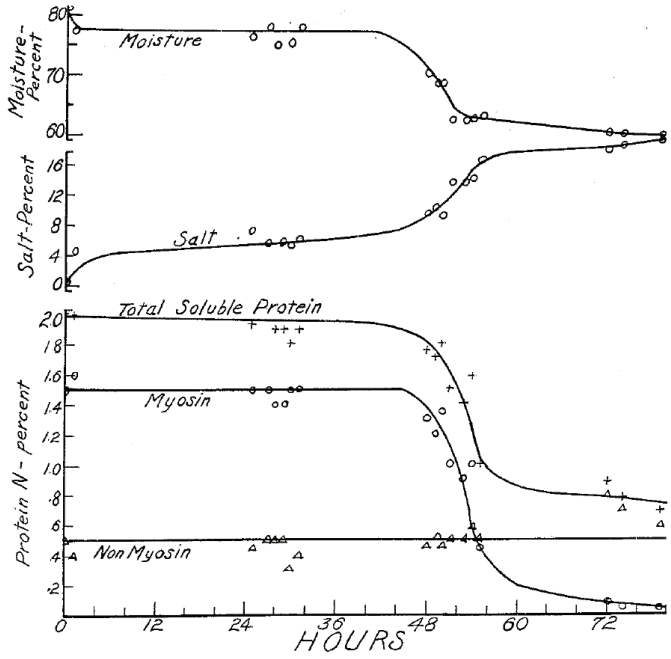


Figure 5. Loss of water, uptake of sodium chloride and changes in total soluble protein, myosin protein, and non-myosin protein in cod fillets immersed in saturated sodium chloride at 0°C (Duerr et al., 1952).

3.1.3 Pickling

In pickle salting, the fish is stacked with alternating layers of dry salt. The salt uptake is initiated by extraction of liquid from the fish muscle and solubilisation of the salt. Saturated brine is formed in a surface layer on the fillets which further extracts moisture from the muscle. Salt moves from the surface to the interior of the muscle, whereas water migrates in the opposite direction, to the surrounding solution (van Klaveren & Legrende, 1965). The ratio of brine to fish is much lower than in brining. Usually, the brine drains to the bottom of tub, where it forms a thin layer. Therefore, this method is more similar to dry salting than brining. The salting rate and the salt concentration cannot be controlled in the same way as in brining. Lower weight yields are obtained than with brining (Andres et al., 2005b). The coarseness of the salt influences the salting rate of the fish, as smaller crystals dissolve more rapidly than large ones (van Klaveren et al., 1965).

3.1.4 Dry salting

Dry or kench salting was the original method used for production of bacalao. It is similar to pickling, except that the brine formed is allowed to drain off. Butterfly filleted (splitted) or filleted fish are stacked with alternating layers of dry, coarse salt. In the old version of kench curing, the fish was restacked several times and kept in stacks for weeks. Today, dry or pile salting is mainly used as the step following brining or pickling. The fish is stacked into plastic tubs in which the stacks are only about 30-40 cm high. It remains stacked for 10 to 12 days for pile salting and finally packed (van Klaveren et al., 1965; Vas-Velche, Capell & Gibbs, 1998).

3.2 Rehydration

Rehydration is carried out by soaking the fish in fresh water. The rehydration rate is influenced by ratio of water to fish, replacement of water with fresh water during the rehydration process, stirring level, thickness and size of the fish and whether it is skinned or not. The curing stage and water content of the salted products also influence the desalting process (Andres, Rodriguez-Barona & Barat, 2005a; Andrés, Rodriguez-Barona, Barat & Fito, 2001; Barat et al., 2004a; Barat, Rodriguez-Barona, Castello, Andres & Fito, 2004c; Martinez-Alvarez, 2002; Martinez-Alvarez, Borderias & del Carmen Gomez-Guillen, 2005).

Rehydration involves leaching of sodium and chloride ions from the muscle to the surrounding water and hydration of the muscle. There is an inverse relationship between water uptake and quantity of salt lost during rehydration. The adverse diffusion proceeds until equilibrium with the surrounding water (brine) is reached (Crean, 1961b). The weight of the fish increases simultaneously. Usually,

rehydration yields show an adverse relationship to curing yields, which is influenced by the condition of the raw material and salting methods (Akse & S., 1996). The final salt content after rehydration can vary from 1% to 3%, depending on storage after rehydration and cooking methods.

3.3 Mass transfer mechanisms

The mechanism of salting of fish is influenced by the method applied, the condition of the muscle, denaturation of proteins, and the degradation of the muscle structure. The main components transferred during salting are water, salt, salt-soluble proteins, smaller peptides and other nitrogenous compounds. The mass transfer is complicated due to the fact that fish is a multi-component system, which changes with aging after death, processing and addition of other ingredients or additives. The structure or "solid phase" (SP) can be considered as capillary, porous, and colloidal in size. In the liquid phase (LP) of the muscle, water acts as a solvent for salt and muscle components, mainly proteins that are solubilised by the salt. In addition, salt-induced denaturation and aggregation of proteins influence the structural changes in the intra- and extracellular matrix and thereby also chemical fluxes in the muscle. This makes the transfer of different components in the muscle interdependent of each other. The mass transfer can be categorised as microscopic (diffusion) and macroscopic (convection) (Figure 6), depending on the driving forces for the chemical fluxes.

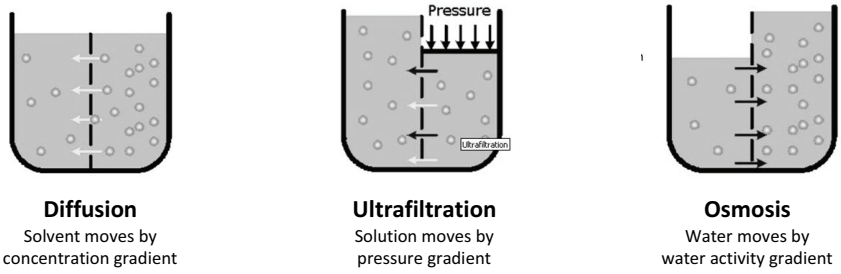


Figure 6. Simplified graphical explanation of different terms used for mass transfer in the muscle, convection of water due to pressure gradient can be compared to the ultra filtration in this figure (Toltec International, 2006).

Diffusion is the most important mechanism during salting and is caused by concentration gradients. It involves movement of solutes like NaCl from a region of high concentration into a region of low concentration. The transfer is a non-steady state diffusion described by Fick's second law (Eq. 3.1). It predicts how diffusion causes the concentration field to change with time. Fick's first law is applied to steady state diffusion, where the concentration within the diffusion volume does not change with respect to time (Kovácsné Oroszvári, 2004). At salt concentrations above 4%, salting-out effects will occur (Hamm, 1972), i.e. the meat proteins will start to aggregate, and that can cause a lateral contraction of the myofibrillar compartment (Tornberg, personal communication, 2009).

$$J = D * \frac{\Delta C}{\Delta x} \quad (3.1)$$

Where J is the rate of diffusion of a solute in a given time
D is the diffusion coefficient
$\frac{\Delta C}{\Delta x}$ is the concentration gradient over a diffusion distance.

Convection takes place due to hydrostatic pressure differences in the muscle. It involves the movement of a solvent with its solutes from a compartment of high pressure to a low pressure area. In salting, the relocalisation of liquid in muscle compartments can often be pressure driven. The pressure gradients (ΔP) can result from external forces like gravity (*g*), or intramuscular forces: capillary forces and contraction pressure resulting from changes in the porosity of the muscle. For example, from changes in intercellular spaces (pores) due to denaturation and aggregation of muscle proteins or due to pressure (gravity) resulting from stacking of cod fillets during salting. The relevance of different pressure gradients (ΔP_1 and ΔP_2) on mass transfer in the muscle have been described by Kovácsné Oroszvári, Bayod, Sjöholm & Tornberg (2006). The flow in capillaries generated by pressure is described by Darcy's law. It is used to show the relationship between a volumetric flow through a porous medium to the viscosity of the fluid and the pressure drop over a given distance. The mass transfer generated by ΔP_1 (Eq. 3.2) is counteracted by ΔP_2 (Eq. 3.3), which is generated by capillary forces. The capillaries in the myofibrils are within the nano range, i.e. the capillary forces are so strong that only very high pressures can move water from that region of the muscle. In the extracellular space, the porosity of the muscle is larger (10-100 μm) and capillary forces are weaker, i.e. the liquid is more easily removed (Tornberg, personal communication, 2009).

$$\Delta P_1 = \frac{Q\eta L}{AK} \quad (3.2)$$

Where ΔP_1 is the pressure gradient either generated by gravity (ρgh) or by contraction pressure,
Q is the volumetric flow, η is the water viscosity,
K is the permeability of the porous media,
L is the length of the sample,
A is the cross-sectional area of the sample.

$$\Delta P_2 = \frac{2\gamma cos\Theta}{r} \quad (3.3)$$

Where ΔP_2 is the counteracting pressure gradient generated by capillary forces (Figure 7)
γ is the surface tension of water,
Θ is the contact angle of water with the meat capillary,
r is the radius of the meat capillary.

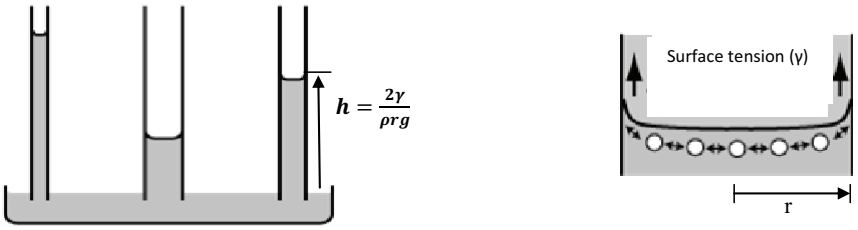


Figure 7. Capillary action. The height (h) to which capillary action will take water in a uniform circular tube is limited by surface tension (γ) and is inversely related to the radius (r) of the capillary and density of the liquid (ρ) (HyperPhysics, 2009)

Osmosis is the third type of mass transfer in the muscle, where water diffusion is driven by a difference in chemical potential, i.e. by a water activity gradient. The direction of the diffusion is in the opposite direction to the salt flux. The water activity decreases due to the interaction of salt with water via dipole ions bonds (Barat et al., 2003).

3.4 Mass transfer kinetics during salting

The mass transfer kinetics depend on salting methods, concentration gradients between the muscle and surrounding media, and pressure gradients in the muscle. In addition, the skin may retard salt uptake and, in the case of fatty species, the fat content influences the rate of salting (Gallart-Jornet, Barat, Rustad, Erikson, Escriche & Fito, 2007a). The salting rate and the time required to approach equilibrium between a surrounding brine solution and fillets is strongly related to the concentration gradients and the salt-related effects on the muscle proteins. Immersion in saturated brine leads to significant weight losses, similar to pickle and dry salting (Figure 8). On the other hand, soaking in 4-20% NaCl brine leads to an overall weight uptake (Barat et al., 2002; Gallart-Jornet et al., 2007b). Cod fillets lose weight during the first 24 hours of brining at 20% (Figure 9), but subsequently the weight starts to increase. In spite of the long brining time (300-400 hours), the muscle does not reach equilibrium with the surrounding salt solution after brining. Weight changes still occur, except in the saturated brine (Barat et al., 2002). Concentration gradients within the muscle during salting (Erikson, Veliyulin, Singstad & Aursand, 2004) can be the reason for the observed weight gains after 24 hours of brining. If the salt concentration of the inner part of the fillet was still below the salting-out level (4-5%), it may have started to swell and gain weight, whereas the outer part lost weight due to salting-out effects from the surrounding brine.

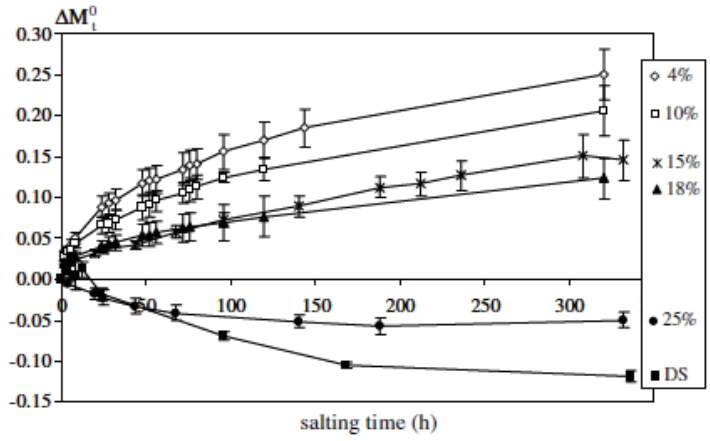


Figure 8. Salmon fillet weight changes depending on the salting brine (4-25% NaCl; DS: dry salting without drainage, which has been defined as pickling in this thesis; n = 5) (Gallart-Jornet et al., 2007b).

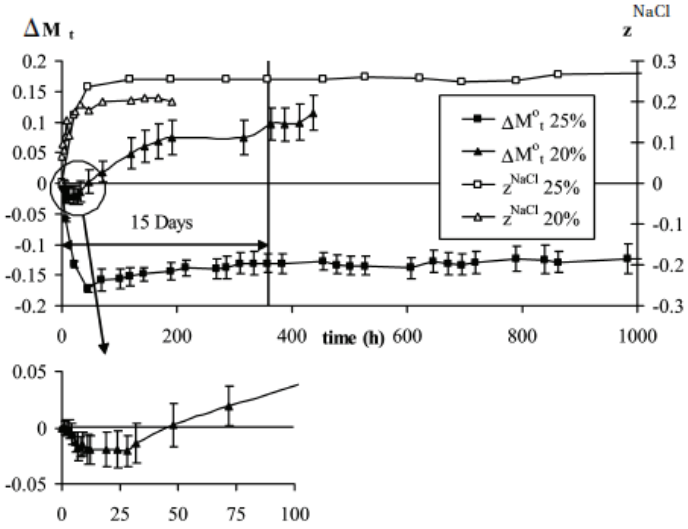


Figure 9. Total weight changes (ΔM), with error bars, and the cod liquid phase NaCl concentration (z^{NaCl}) values through the salting experiments using 20% and 25% brine solutions (Barat et al., 2002).

The weight of different mass transfer mechanisms change with salting time due to the continuous solubilisation/denaturation of proteins and the concomitant changes in muscle structure. Initially, diffusion is the most important mechanism resulting from concentration difference between the muscle and the surrounding brine. At later stages, the salt-induced protein changes probably influence the pressure gradients. It is important to note the gradients existing within the muscle, where salt content decreases from the surface layer to the skin side of the fish (Erikson et al., 2004). After salting and

relocalisation of water in the muscle compartments, the main active forces are due to pressure (gravity) and water activity gradients. Finally, the fluxes of salt and water are reversed during rehydration of the products before consumption.

3.4.1 Mass transfer kinetics during rehydration

The main components transferred during desalting of cod muscle are salt and water, and to some extent nitrogenous compounds. The mass transfer can be considered a solid-liquid extraction. The solutions are the salt ions, the solvent is the water and the solid phase is constituted by the muscle proteins. If the products have been dried after salting, the muscle contains another solid phase which is salt that is crystallised as the muscle is dehydrated (Barat et al., 2004b). The rate of mass transfer is influenced by the condition of the raw material used for salting, salting methods, drying of products after salting, muscle thickness and rehydration methods (Andres et al., 2005a; Barat et al., 2006; Barat et al., 2004a; Barat et al., 2004b; Barat et al., 2004c). The driving forces for the mass transfer in the muscle have been described by component activity gradients (osmosis) and pressure gradients (Barat et al., 2004b). The chemical fluxes are at the highest rate during the first hours of rehydration, after which the rate declines (Figure 10). The rehydration has been reported to take more time than the desalting process, i.e. water uptake and weight continues to increase for some time after equilibrium is reached between the salt concentration in the liquid phase of the muscle and the surrounding solution (Barat et al., 2006; Barat et al., 2004a).

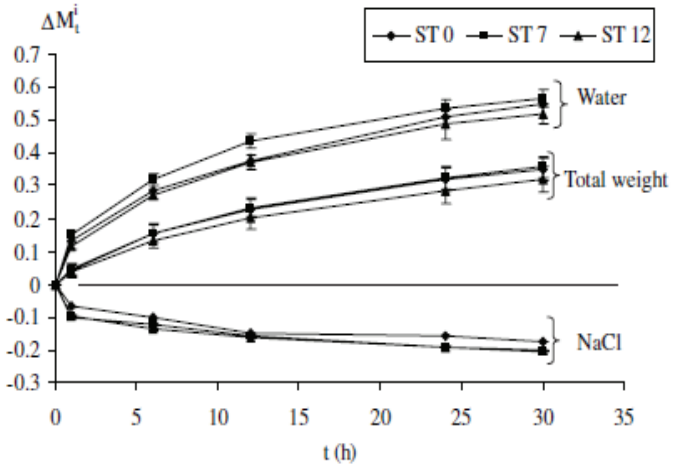


Figure 10. Total water and NaCl weight changes of cod throughout the desalting experiments (ST: storage time of raw material) (Barat et al., 2006).

3.5 The water phase of the muscle

The water content of lean fish such as cod accounts for approximately 80% of the weight of fresh fish. All changes of the water content are of economic importance to producers due to the high proportion of water content of the total mass. It is also a relevant factor with regard to different quality attributes of the desalted and cooked products, such as appearance, texture and juiciness.

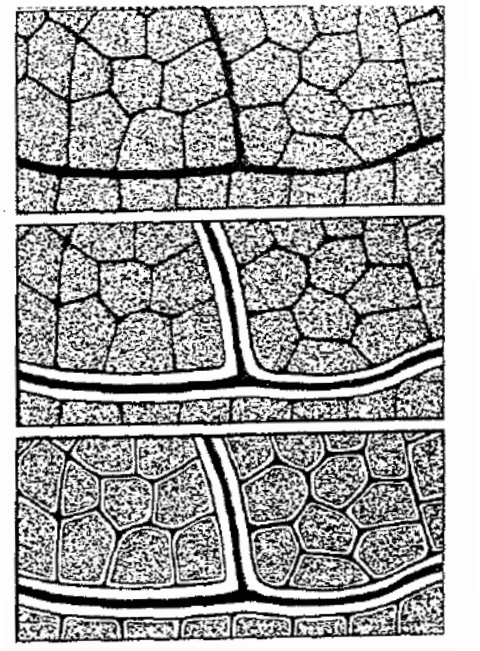
3.5.1 Condition of water in the muscle

In a living muscle, there is a small amount of extracellular water; the majority of the water, known as free water, is found in intracellular locations, mainly within the myofibrils. There it is held by capillary forces between thick and thin filaments and among myosin molecules. This can be compared with a sponge: when dipped in water, the sponge will absorb many times its dry weight water in its narrow channels, even though the water is not bound to the sponge. Only 4-5% of the total water of muscle is thought to be tightly bound to the muscle proteins and not influenced by changes in the structure and charges of proteins (Fennema, 1990; Schnepf, 1989; Warrier, Gore & US, 1975; Zaitsev, Kizevetter, Lagunov, Makarova, Minder & Podsevalov, 1969).

The distribution of water in the muscle and water retention in the postmortem muscle is influenced by biological conditions of the fish, handling after catch, processing and storage methods. The effects of rigor mortis and the degradation process post mortem alter the location of water. Swelling and shrinkage of myofibrillar lattice change the distribution of water within the muscle, i.e. how much is extracellular, between the muscle fibres and the endomysial network or between fibre bundles and the perimysial network (Figure 11). Shrinkage or expansion of the myofilament does not necessarily result in changes in total volume of water in the myofibrillar lattice (Offer, 1988b). It depends on the reason for the volumetric changes of the muscle fibre, e.g. rigor mortis and process-related changes, such as from salting. Alterations can be from only relocalisation of water in the muscle to water being extracted or squeezed out of the muscle resulting from contraction within the muscle, degradation processes, concentration gradients, and/or gravity.

In pre-rigor muscle, cross-linking between the actin and myosin filaments is prevented by high ATP concentration in the muscle, which facilitates the expansion of the filament lattice. During rigor, glycolysis leads to hydrolysis of ATP, formation of lactic acid and lowering of pH in the muscle. Since ATP is necessary to remain in a relaxed state, this leads to irreversible cross-bridges between actin and myosin and shrinkage of the muscle fibrils (Fennema, 1990). Softening of the muscle starting early post mortem is mainly due to proteolytic degradation of key structural muscle proteins. The process includes proteolysis of inter-myofibrillar linkages and of proteins that link the fibrils to the sarcolemma. The connective tissue is detached from the muscle cells which, with further

postmortem aging, can lead to gaping of the muscle. The inter-fibrillar spaces become wider, the Z-discs disintegrate and the thin filaments of the I-band split up (Goll, Robson & Stromer, 1977; Hamm, 1975; Huss, 1995; Offer & Trinick, 1983; Ofstad, Egelandsdal, Kidman, Myklebust, Olsen & Hermansson, 1996).



a) Living muscle and early time *post mortem.*

b) 4-6 hours *post mortem*

c) Rigor (48 hours post mortem) – the perimysial and endomysial networks are shown completely separated from the fibre bundles and fibres which they divide; in reality intermittent contacts are still made (Offer & Cousins, 1992).

Figure 11. Summary of the structural changes occurring in beef muscle post mortem. Each diagram schematically depicts a transverse section of muscle including parts of three fibre bundles separated by perimysium (thick strands). The fibres (tippled) are separated by endomysium (thin lines) (Offer et al., 1992).

The rigor state of the muscle affects the mass transfer and weight changes during salting. Swelling will be restrained only by the holding of the thick and thin filaments by the M- and Z-line proteins respectively (Lawrie, 1998a). It is suggested that the active ATP-driven ionic pumps in pre-rigor muscle work against salt uptake by maintaining concentration gradients across cell membranes. During rigor, contraction of the muscle is believed to reduce brine uptake (Wang, Tang & Correia, 2000; Wang, 1998). With rigor mortis, the intercellular spaces and the permeability of cell membranes increases (Bello, Luft & Pigott, 1981), favouring swelling of post-rigor muscle even though actin and myosin are bound together.

3.5.2 Different terms used for conditions of water in the muscle

The water is separated into different populations according to mobility and how tight the water molecules are bound to the muscle structure (Chou & Morr, 1979 (as cited by Schnepf, 1989)):

- *Constitutional water (<1%)* is the proportion of water which is bound directly to protein molecules by hydrogen bonding. It stabilizes the native structure of the proteins and is important in determining the three-dimensional conformation. This is only a small portion of the water in the muscle; it is located inside the macromolecule and is not available for chemical reaction.
- *Interfacial water (5-15%)* is the proportion which forms hydration shells around the protein molecules. It is bound to the surface of the protein by hydrogen bonding or dipole interaction, depending on the amino acid composition and available polar side chains of the protein. The water may also be associated with the protein molecule via hydrophobic interaction. The interfacial water is not available as a solvent but may take part in certain reactions.
- *Bulk/immobilized water (75-95%)* is found in the spatial arrangement formed by the myofibrillar proteins, where it is mainly held by capillary forces. It is available for chemical reactions and acts as a solvent, but can only be removed by force.

The bulk water is of great importance because of its high proportion in the muscle and because it is strongly affected by processing and storage. It is not homogeneous but should rather be viewed as a continuum. Some of the water is strongly immobilized within the tissue and therefore difficult to remove, whereas part of it can easily be squeezed out by weak forces. Hamm (1975) defined the bulk water as immobilized water that could be categorized according to internal and external factors influencing the water retention of the muscle (Hamm, 1975). Internal factors are, for example, aging of the muscle, where the only force causing drip is gravitational. External factors are assigned to processing and storage methods like salting.

3.6 The muscle structure

The muscle of fish and other higher vertebrates is made up of three major components: proteins, lipids and connective tissue. The functionality and properties of the muscle vary by species and the environment in which the animals live. The following differences between fishes and mammals can be stated. First, the body of a fish is supported by water and does not require strong connective tissues to maintain and support the muscle as in animals living on solid ground. The content of connective tissues in the muscle is only 2-3% in fish compared to 10-15% in mammals. Secondly, fish live in a cold environment, and therefore fish proteins have different properties than warm-blooded animals. Third, the structural arrangement is also markedly different due to different movements of fishes compared with mammals (Foegeding, Lanier & Hultin, 1996; Hultin, 1984). In mammals, the muscle

fibres form bundles parallel to the skeleton, whereas in fish, the muscle fibres are at a sharp angle to the skeleton. The fish muscle is composed of W-shaped segments called myotomes (1-2 cm across), which are connected by collagenous connective tissue, called myosepta. The horizontal septum separates the myotomes into dorsal (or epaxial) myotomes and ventral (or hypaxial) myotomes. The myotomes are composed of long cylindrical cells termed muscle fibres or myofibres. Myofibres are 50-200 μm in diameter and up to several centimetres in length (Figure 12). They form layers that lay parallel to the length of the fish and are bound together and to the skeleton with connective tissue.

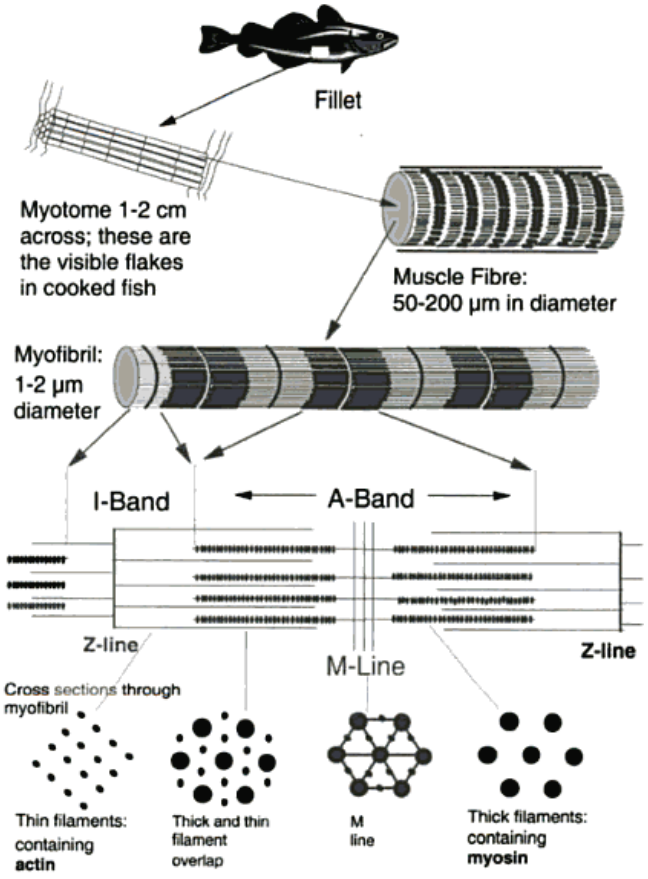


Figure 12. Structural organisation of fish fillet (Goodband, 2002).

Longitudinal analysis of muscle cells shows the arrangement of the myofibrils within the fibre. 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. In the centre of each I-band is a dark line, called the Z-line or Z-disc. The part of the fibril between two Z-lines is the basic contractile unit of the muscle and is called a sarcomere (approximately 2.5 μm long) (Foegeding et al., 1996;

Hultin, 1984). The main components are the thick and thin filaments that constitute the contractile unit of the muscle. In addition, the sarcomere contains a third filament lattice, the longitudinal filaments, which keep the thick and thin filaments in their lateral register.

3.7 Muscle proteins

Muscle proteins are classically divided into three groups based on their solubility in aqueous solution Table 3). Several properties of fish proteins differ significantly from those of mammals. First, fish muscle proteins are less stable than proteins from mammals; they are more sensitive to denaturation by heating and freezing. Secondly, pre-treatment of live fish is impossible as in the case of live animals, due to present catch methods (Connell, 1964; Goll et al., 1977). The main factors influencing muscle structure are the myofibrillar proteins, the cytoskeletal system and the connective tissue (Sikorski, 2001). Therefore, conformational changes in myofibrillar and stromal proteins influence the location and volumetric space for the liquid phase in the muscle. Furthermore, structural alterations influence the mass transfer and liquid retention during processing.

Table 3. Classification of muscle proteins based on their solubility

Muscle proteins	Solubility	Amount for a skeletal muscle[1)]	Amount in a bony fish[2)]
Myofibrillar	Soluble at high salt concentration (0.3-1 M)	50-55%	65%
Sarcoplasmic	Can be extracted with water or dilute salt solution, (soluble at ionic strength of 0.1 or less at neutral pH)	20-30%	25-30%
Stromal	Practically insoluble in aqueous solutions	10-15%	3%

[1)] Ingolfsdottir, 1995, [2)] Huss, 1983

3.7.1 Myofibrillar proteins

Myofibrillar proteins account for 50-55% of the total muscle protein and are the principal structural and functional components of muscle-based foods. They are responsible for the stabilisation of emulsions, binding of water and lipids and formation of gel structures in meat products such as sausages, for example. Myofibrillar proteins play an important role in water-holding capacity, both with regard to water that is tightly bound by proteins through the hydration shell and loosely bound within the muscle structure as the myofibrillar proteins constitute most of the fish microstructure. The amount of water bound by a protein is a function of the amino acid composition and the conformation of the protein. Carboxyl and amino groups are mainly responsible for the binding of the hydration water. In order for the myofibrillar proteins to exhibit desirable properties effectively, a relatively high salt concentration (2-3% sodium chloride) is required in order to extract the proteins (Stefansson, 1994; Goll et al., 1977). Denaturation can occur in several steps; partial loosening of the structure gives a “molten structure” where the secondary structure is maintained. This step is mainly

irreversible. Moreover, denaturation may increase interactions of the protein molecules with each other and their surroundings, leading to aggregation and sometimes gel formation (Tornberg, 2005).

Myosin comprises 50-60% of the myofibrillar contractile proteins. It is the major component of the thick filaments. Other important proteins that support the overall structure of the thick filaments are the C-protein, creatine kinase and proteins of the M-line structure. Creatin kinase is mostly found in soluble form in the sarcoplasm but also plays an important structural role in the M-line. C-protein, F-proteins, H-proteins, X-protein and I-protein are in connection with the A-band (Hultin, 1996).

Actin comprises about 15-30% of the myofibrillar protein of the muscle. It is the major component of the thin filament. 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). In contractile bundles, the actin-bundling protein α-actinin separates each thin filament (~35 nm), allowing thick filaments to fit in between and interact (Hultin, 1996).

Table 4. Myofibrillar proteins of vertebrate skeletal muscle – Thin filament lattice

Sarcomere structure	***Subunits***			
Thin filament lattice	**Relative amount (%)**	**No**	**MW (kDa)**	**Major function**
Thin filaments				
Actin	21-23	1	42	Contraction
Tropomyosin	5	2	35	Regulates contraction
Troponin	5	1	37 (TnT)	Regulates contraction
		1	22 (TnI)	
		1	18 (TnC)	
β-actinin	<1	1	37	Regulates length of thin filament
		1	34	
γ actinin	<1	1	35	Inhibits G-actin
Z-line				
α actinin	1-2	2	90	Connects thin filaments to Z-line
Amorphin	<1	1	85	Forms amorphous matrix of Z-band (?)
Eu actinin	<1	1	42	Contributes to Z-disc density
Z protein	<1	1	55	Lattice structure of Z-line
Z nin	<1	1	40	Lattice structure of Z-line
Capa Z	<1	2	32	Prevents depolymerisation of F-actin (?)
Filamin	<1	2	250	Transversely links myofibrils in Z-disc

(?) Function not definitely known (as cited by Stefansson, 1994, compiled from: Small et al., 1992; Wang, 1985; Pearson and Young, 1989)

Troponin and tropomyosin are other major components of the thin filaments. Tropomyosin is a long, thin, two-stranded alpha-helical rod. The structure is supposed to be stabilised by hydrophobic interactions and tends to aggregate by head-to-tail binding between individual molecules. Each

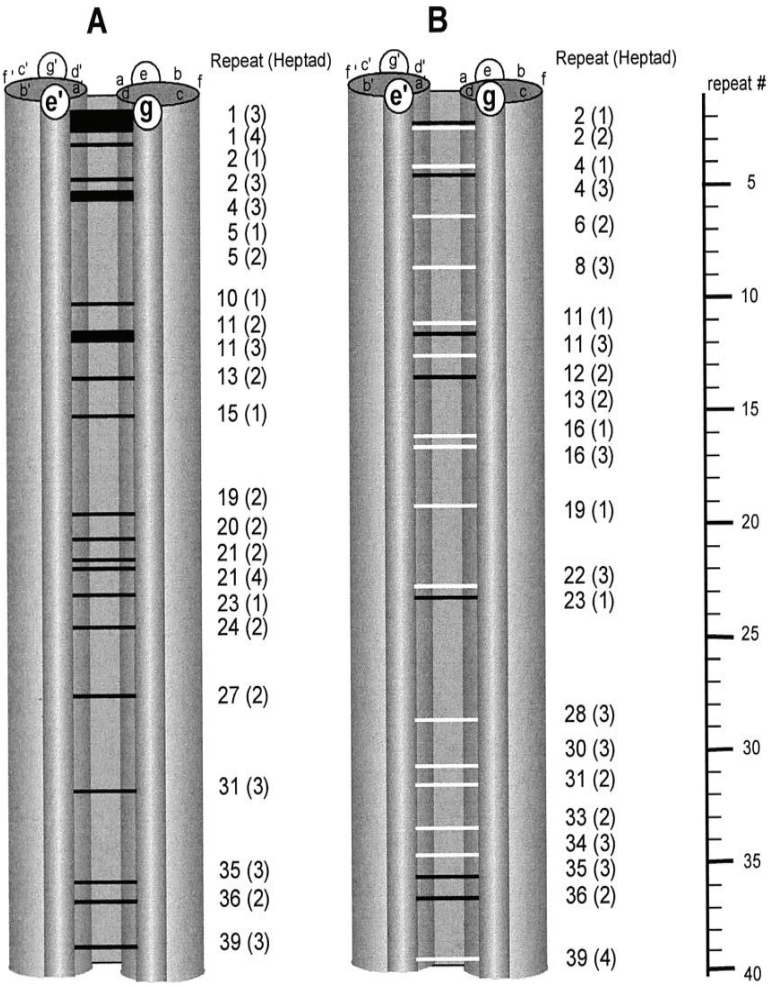
tropomyosin molecule spans seven G-actin monomers and has a troponin molecule bound towards one end of it. Tropomyosin, along with the troponin complex, regulates muscle contraction by regulating the binding of myosin to actin (Foegeding et al., 1996). Troponin is attached to tropomyosin and lies within the groove between actin filaments in muscle tissue.

3.7.1.1 Myosin

Myosin is the major component of the thick filaments, comprising 50-60% of the myofibrillar contractile proteins. It plays an important part in gelling properties and the water-holding capacity of the muscle during salting due to its structural properties. The molecule is highly charged and has some affinity for calcium and magnesium ions (Lawrie, 1998a). It has a high content of the polar, negatively charged amino acids (glutamic and aspartic acids) and of dibasic amino acids. Interaction between the charged amino acids residues in the rod part leads to formation of interhelical salt bridges which, along with hydrophobic interactions, provide the structure and stability of the myosin dimer (Figure 13) (Arrizubieta & Bandman, 1998; Arrizubieta & Bandman, 1999).

The myosin structure has been further defined in accordance to its proteolytic fragments: the light meromyosin (LMM 130-140 kDa) and the heavy meromyosin (HMM 350 kDa) (Figure 14). The light meromyosin (LMM) is almost 100% α-helical in nature, insoluble in water but soluble at ionic strength above 0.3. The HMM is approximately 45% α-helical and is soluble in water (Goll et al., 1977). It can be further split into different subfragments (S1 and S2) by enzymes. The S1 has all the ATP-ase and actin-combining properties of myosin molecule. It contains all the light chains, has less than 20% of the alpha helical structure and is soluble in water. The S2 is about 90% α-helical and insoluble in water. The ATP-ase and actin-combining properties depend upon free SH-groups in the molecule, but the HMM part contains 40-42 SH groups (Goll et al., 1977; Lawrie, 1998a).

The myosin hinge is thought to be the region of the myosin molecule where the HMM section swivels in order to interact with actin. The significant differences in structure of the different parts of the myosin molecule are relevant with regard to solubilisation and denaturation of the myofibrils during salting. The cross bridges in the rod can be disrupted by high ionic strength during salting. The connection between conformational changes in the protein and different salting methods has to be studied in further detail to explain how water-holding capacity of the muscle varies with salting mechanisms.



A. Pattern of potential salt bridges in the adult chicken MyHC isoform. Thicker lines represent multiple salt bridges (two and three salt bridges located in consecutive heptads).

B. Interhelical ion pair pattern found in rabbit smooth muscle myosin. Salt bridges at positions common to adult chicken myosin are represented in black, and salt bridges at positions unique to the smooth muscle myosin are represented with white lines. On the right side of each diagram, the individual ion pairs are listed according to the position of the **g** residue. The myosin rod is organised in forty 28-aa repeats, each encompassing four 7-aa heptads and the position of the **g** residue is represented by two numbers with the annotation: repeat (heptad). The first number corresponds to the 28-aa repeat within the rod sequence, and the number in parenthesis corresponds to the heptad number within the 28-aa repeat. For example, ion pair **2(1)** denotes the ion pair between the **g** residue in the first heptad of the second 28-aa repeat and the **e** residue in the next heptad of the opposite strand.

Figure 13. Distribution of potential interhelical salt bridges in the myosin rod. The α-helical coiled-coil structure of the myosin rod is represented by two parallel cylinders, with heptad positions a-g (a*-g*in the opposite strand) shown at the top of each cylinder. Heptad positions g and e* are represented by smaller cylinders. Black bars extending between the two strands of the rod represent g-e* pairs at (i, i*/5) positions occupied by oppositely charged residues constituting potential sites of interhelical salt bridges (Arrizubieta et al., 1998).

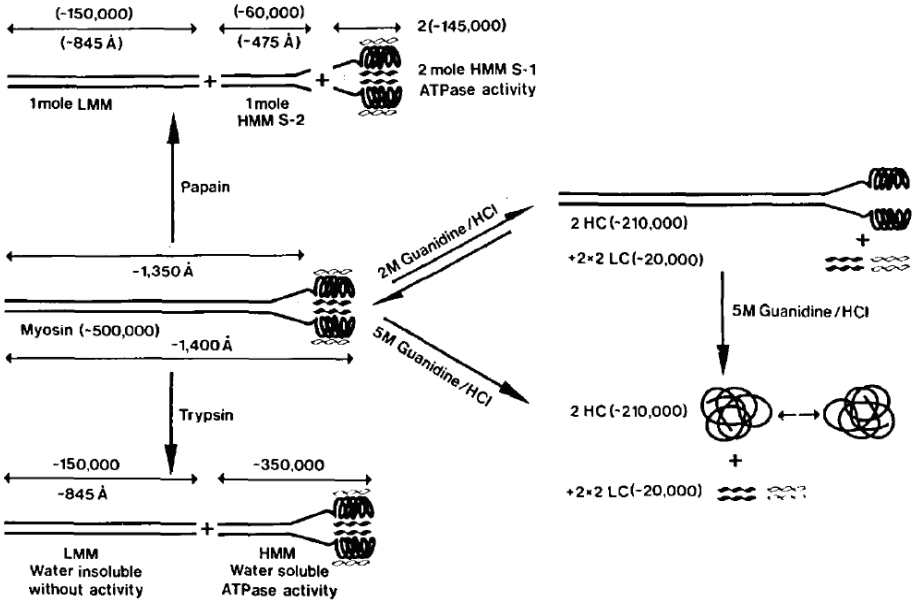


Figure 14. Diagrammatic summary of subunit structure and interactions of myosin: Schematic representation of the proteolysis of myosin with papain and trypsin. Dissociation of light and heavy chains by 2 and 5M guanidine hydrochloride. LMM, light meromyosin; HMM, heavy meromyosin, HMM S-1, heavy meromyosin subfragment 1; HMM S-2, heavy meromyosin subfragment 2; HC, heavy chains, LC, light chains (Medugorac, 1975).

Other important proteins that support the overall structure of the thick filaments are the C-protein, creatine kinase and proteins of the M-line structure. The C-protein appears as several stripes in the striated muscle A-band, binding with titin and the rod part of myosin. Several structural models are suggested in relation with the role of the C-protein in the thick filaments, which is believed to be regulatory and structural. One implies that the C-protein forms a collar around the myosin filament surface (Moolman-Smook et al., 2002). Other explorations indicate that the C-protein is a strand running axially along the myosin filament, parallel to titin binding to those proteins and interacting with actin (Figure 15) (Squire, Luther & Knupp, 2003). The molecule has 3 (to 4) proteolytic fragments, a 14 kDa, a 28 kDa and a 100 kDa (5 kDa and 95 kDa) fragment (Okagaki, Weber, Fischman, Vaughan, Mikawa & Reinach, 1993). The solubility of the C-protein is similar to myosin, indicating that it may play a significant role in the swelling mechanisms of the myofibrillar lattice, influencing the actin-myosin cross linking and depolymerisation of the thick filaments during salting. However, the precise mode of binding of C-protein to the myosin filament has not been determined.

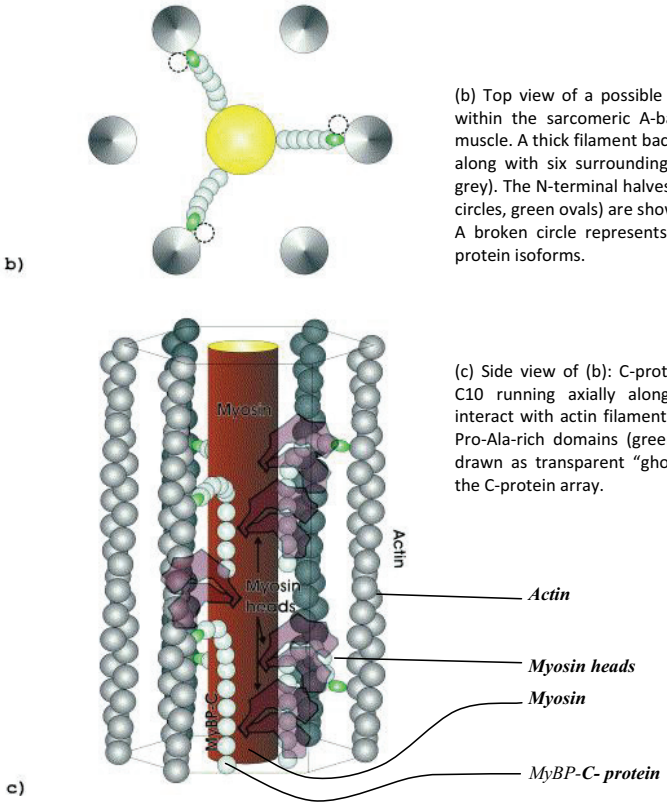


Figure 15. A model suggesting the structural arrangement of the C-protein in the muscle filaments (Squire et al., 2003).

3.7.2 Stromal proteins

Stromal proteins account for insoluble proteins in the sarcomere lattice (Table 5) and in the extracellular matrix (connective tissue). The cytoskeletal proteins hold the myofibrillar proteins organised in the sarcomere. They are found in the Z-disc and the M-line from where they stretch out to hold the thin and thick filaments (titin, nebulin). Part of the cytoskeletal proteins are the costameres: protein filaments that are anchored in the Z-disc and interconnect myofibrils and the myofibrils to sarcolemma (desmin, γ-actin, vinculin) (Figure 16) (Kijowski, 2001). Titin (connectin) and nebulin are components of the longitudinal filaments. Titin is a long, rather insoluble, high molecular weight myofibrillar protein, which composes 8-10% of the total amount. It acts like a spring to hold the thick filaments centred in the sarcomere. Titin regulates elasticity and stiffness of the muscle. Nebulin constitutes about 3-4% of the myofibrillar proteins. It is a long, very insoluble molecule with a molecular weight of about 600 kDa, depending on species, muscle type and age.

Nebulin runs parallel to the thin filaments and is thought to act as a template for thin filament formation and regulate the length of the thin filament.

Table 5. Myofibrillar proteins of vertebrate skeletal muscle – Sarcomeric lattice

Sarcomere structure	*Subunits*		
Exosarcomeric lattice	**Relative amount (%)**	**MW (kDa)**	**Major function**
Transverse intermediate filament			
Desmin	<1	55	Myofibril-myofibril connections; linking of myofibrils to cell membrane
Vimentin	<1	58	Transversely links myofibrils in Z-disc (?)
Synemin	<1	220	Associated with desmin and vimetin (?)
Endosarcomeric lattice			
Longitudinal filament			
Titin	8-10	>2000	Holds thick filaments in lateral register
Nebulin	3-5	600-800	Thin filament regulator

(?) Function not definitely known (as cited by Stefansson, 1994, compiled from: Small et al., 1992; Wang, 1985; Pearson and Young 1989)

The connective tissue consists of various fibres, several different cell types, and amorphous ground substances (carbohydrates, proteins and lipids). The most important proteins in the connective tissue are collagen, elastin, and lipoproteins of the cell membrane. All have a fibrous structure, but collagen is quantitatively predominant (Kijowski, 2001).

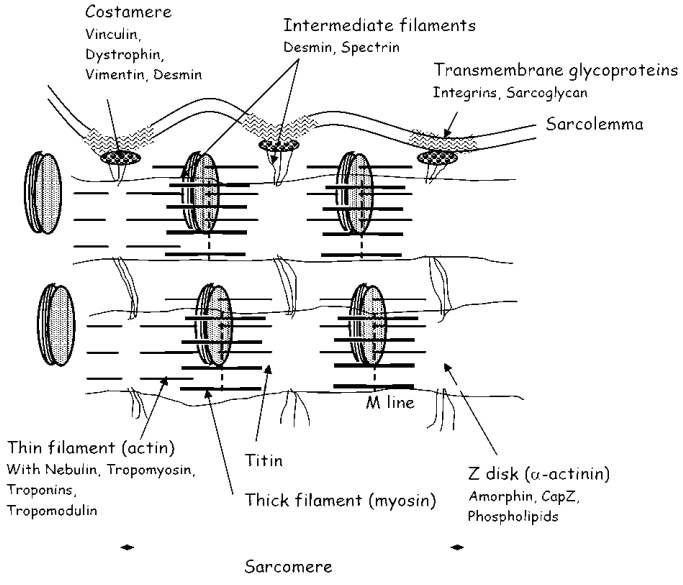


Figure 16. A schematic diagram of myofibrillar organisation (adapted from (Campbell, 1995) (Trinick, 1994) (Delbarre-Ladrat, Chéret, Taylor & Verrez-Bagnis, 2006b).

There are significant differences between the properties and content of connective tissue in fish and in mammals. The characteristics are also influenced by species and natural habitats (Foegeding et al., 1996; Hultin, 1984). The connective tissue of fish is generally more easily solubilised than those of mammals and normal cooking processes destroy it (Ingolfsdottir, 1995; Hultin, 1984). The condition of the connective tissue varies by season; it thickens during periods of sexual maturation and becomes thinner during the intensive-feeding season. Therefore, the cod muscle is rather fragile after spawning and in the beginning of summer, which can result in an increased occurrence of gaping (Love, 1988).

3.7.2.1 Collagen

The collagen molecule tropocollagen is a triple helix, consisting of three peptide chains that can be different or identical, depending on type of collagen (I, II, III, IV, V and VI) found in the helix (Table 6) (Sikorski, 2001). More than 20 types of collagen have been identified in higher vertebrates (Wang, 2002). Each of the polypeptide strands (α-chains) has a conformation of a left-handed helix. These three helixes are twisted together in to a right-handed coiled coil, a collagen microfibril (Figure 17). The fibrils aggregate to form fibres or sheets (Gross, 1964).

Table 6. Types of collagen in meat. I, II, III and V are fibrillar but IV and VI are non-fibrillar (Belitz & Grosch, 1999) (Bonaldo, Russo, Bucciotti, Doliana & Colombatti, 2002) (van der Rest & Garrone, 1991).

Type	Peptide chains[a]	Molecular composition	Occurrence
I	α^1, α^2	$[\alpha^1(I)]_2\ \alpha^2(I)$	Skin, tendons, bones, muscle (epimysium)
II	α^1	$[\alpha^1(II)]_3$	Cartilage
III	α^1	$[\alpha^1(III)]_3$	Fetal skin, cardiovascular system, synovial membranes, inner organs, muscle (perimysium)
IV	α^1, α^2	$[\alpha^1(IV)]_3(?)$[b]	Basal membranes, capsule of lens, glomeruli
		?	Placental membrane, lung, muscle (endomysium)
V	αA, αB, αC (?)	$[\alpha B_2\ \alpha A$ or $(\alpha B)_3 + (\alpha A)_3$ or $(\alpha C)_3$ (?)	Placental membrane, cardiovascular system, lung, muscle (endomysium), secondary component of many tissues
VI	α^1, α^2, α^3	α^1(VI), α^2(VI), α^3 (VI)	Around and between collagen fibres and on surface of cells (Bonaldo et al., 2002)

[a] Since the α chains of various types of collagen differ, they are called α^1(I), α^1(II), α A, etc. ? Not completely elucidated

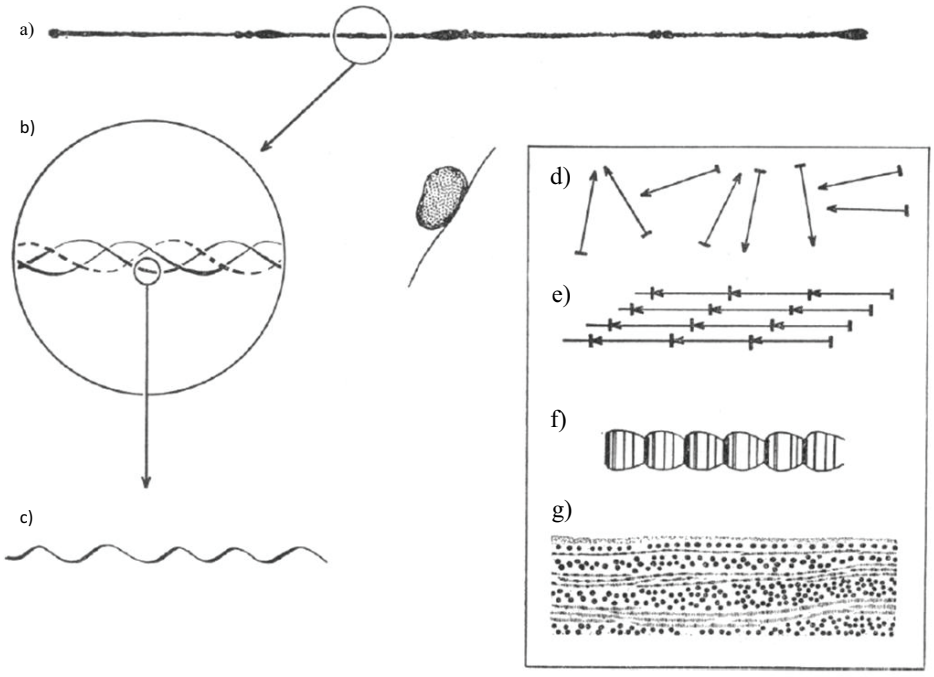


Figure 17. a) A tropocollagen molecule. b) Three chain coiled helix—one of the polypeptide chains (dashed line) is different in amino acid composition from the other two. c) Single chain molecular helix. Tropocollagen units (d) are postulated to come together, overlapping each other in a staggered array (e) by about one-quarter of the total length, thereby giving rise to a collagen fibril (f) with a repeating period of about 1700 A. Collagen fibrils are organised in plywood-like sheets (g) in a variety of tissues (Gross, 1964).

The collagens form a wide range of structures depending on their role in the muscle. They are, however, mainly categorised into two major classes, fibrillar collagens and nonfibrillar collagens (Figure 18), based on the assemblies and other features (Wang, 2002). Collagens I, II, III and V are fibrillar collagens. The non-fibrillar collagens IV and VI form sheets (IV) constituting basement membranes and thin-beaded filaments (VI) that interact with fibrils and muscle cells. Several other types of collagens (e.g. IX, XII, and XIV) connect the collagen fibrils and sheets to other components of the muscle. Collagen VII acts as anchoring fibrils that bind epithelial basement membranes and entrap collagen fibrils from the underlying stromal, gluing these two structures together (van der Rest et al., 1991).

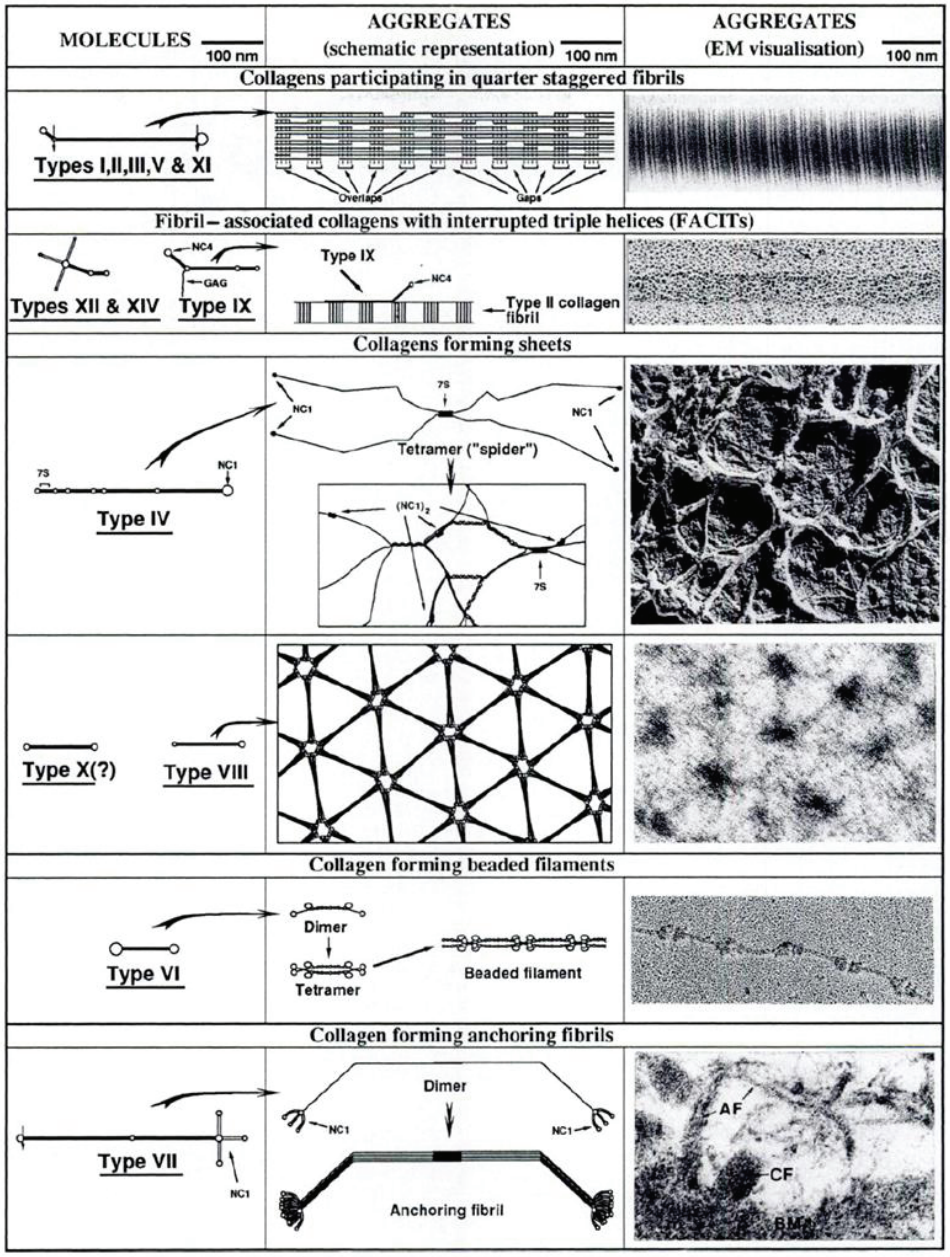


Figure 18. Molecular structures and supramolecular assemblies of collagens. This figure combines schematic scale representations and electron micrographs of molecules and aggregates of various collagen types.
Triple helical (COL) domains are drawn as thick black lines and non-triple helical (NC) domains are represented by double lines or empty circles. The larger circles at the extremities of the molecules correspond to the globules visible on rotary shadowing electron micrographs of isolated intact collagen (or procollagen) molecules. Vertical arrows (drawn on the molecules depicted on the upper and lower panels) indicate the sites of action of the processing proteinases (van der Rest et al., 1991).

Intra- and intermolecular cross-links are essential for the high mechanical strength of collagen. These cross-links are covalent and of three kinds: disulphide (collagens III and IV), divalent (between lysine or hydroxylysine aldehydes) and more complex bonds where more than two α-chains are joined (mature cross-links). The stability of the collagen fibres depends mainly on intermolecular cross-links, such as aldimine, oxoimino and mature cross-links like pyridinoline (Bailey, Peach & Fowler, 1970; Lawrie, 1998b). The ratio of different cross-links varies significantly depending upon the origin of the muscle tissue (Tanzer, 1973). In fish, pyridinoline has been found to be related to solubility and mechanical strength of collagen (Ando, Makino, Tsukamasa, Makinodan & Miyosh, 2001; Ando et al., 2006).

Collagen can be extracted by neutral salt solutions or under acidic conditions. Neutral salt-soluble collagen dissolves into three peptide chains, the α-components that have similar molecular weights (~100 kDa) (Figure 19). Acid-soluble collagen dissociates into at least two components, α- and β-collagen. The β-collagen is composed of two α-chains bound together with an alkali-labile bond. The molecular weight is approximately 200 kDa. The third type, γ-collagen consists of three α-chains, linked together with covalent bonds (Klomklao, Benjakul, Visessanguan, Kishimura & Simpson, 2006; Tristram, Worrall & C., 1965).

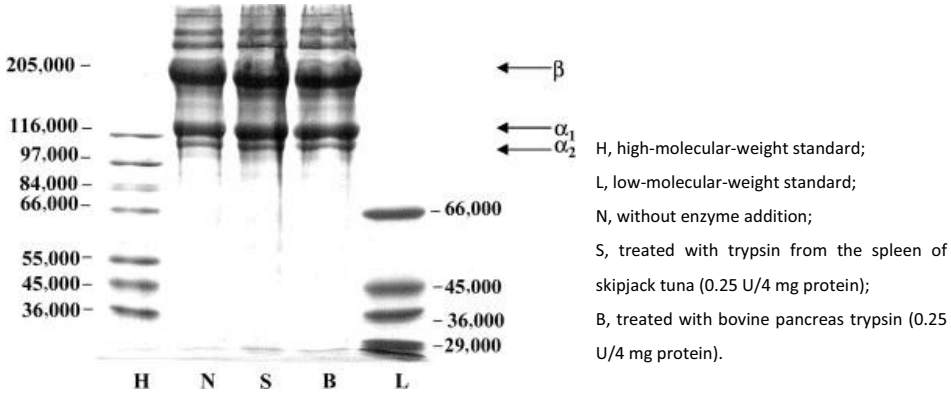


Figure 19. Peptide mapping of sardine collagen hydrolysed by purified trypsin from the spleen of skipjack tuna and trypsin from bovine pancreas. Reaction was conducted at 55 °C for 60 minutes (Klomklao et al., 2006).

3.7.2.2 Collagen in fish muscle

Collagen has an important role in the structural arrangement of fish muscle. Thin collagenous tissue membranes (myocommata or myosepta) divide the muscle into segments known as myotomes (1-2 cm across). The myotomes are composed of muscle fibres that run parallel to the long axis of the fish, terminating at the boundary of the myocommata. Around each muscle fibre is a connective sheet known as endomyosium, and each fibre bundle is surrounded by a collagenous tissue known as perimysium (Foegeding et al., 1996; Hultin, 1984). The collagen content varies by species as well as the condition of the fish, depending on maturation and feeding. It is lower in fish muscle than in mammals, ranging from 1.6% to 12.4% of the crude protein, i.e. 0.3-2.2% of wet weight of the muscle (Sato, Yoshinaka, Sato & Shimizu, 1986). Collagen is relatively low in a bony fish (2-5%) like cod, where it makes up 1.5% of the total protein content and the aerial fraction of the total muscle area accounts for 3% (Table 7). Twenty-five percent of the total connective tissue is found in the endomysial sheets.

Table 7. The amount distribution of connective tissue content in swimming muscle of cod, evaluation by light microscopical morphometric analysis (*Gadus morhua*) (Kryvi, Totland, Ulriksen & Slinde, 1985).

Ratio of total protein content (%)	Areal fraction of total muscle area (%)		Thickness of endomysial sheet (μ)		Areal fraction of endomysial sheet (%)	
Collagen	Connective tissue	Myocommatal conn. tissue	Red fibres	White fibres	Red muscle	White muscle
1.5	3	2.3	0.30	0.16	2.3	0.5

In fish muscle, collagens I and V are the main types found in the extracellular matrix (Ando, 1999; Sato et al., 1998). The relative concentration of type V to type I is higher in the endomysium fraction than in the myocommata fraction. However, both types are less soluble in the endomysium fraction (Sato, Yoshinaka, Sato & Tomita, 1989). In cod muscle, collagen III has also been found in the myocommata and the endomysium (Table 8). Type IV is reported to form a thin non-ordered sheath around individual muscle fibres. Type IV and talin (an attachment protein) are present in a significant amount in the junction between the myofibres and the myocommata (Brüggemann & Lawson, 2005).

Table 8. Distribution and types of connective tissue in cod (Brüggemann et al., 2005).

Collagen type	Localization	Shape
I	Myocommata, Endomysium	Fibrous
III	Myocommata, Endomysium	Fibrous
V	Myocommata, Endomysium	Fibrous
IV	Basement membrane (at the myotendinous junction of the myocommata)	Network forming
VI	Myocommata	Beaded

3.8 Weakening of muscle structure during postmortem aging

Structural constraints to volumetric changes (swelling) become weaker during postmortem aging of the muscle (Table 9 and Table 10). The permeability of cell membranes increases, and there is a breakage of the Z-line and/or of the thin filament to the Z-line and a breakdown of the connective tissue (Bello et al., 1981). Postmortem aging of the muscle is believed to result from combined effects of the enzymes and other changes in the muscle, like pH-drop, sarcoplasmic Ca^{2+} increase, osmotic pressure rise and oxidative processes (Delbarre-Ladrat, Chéret, Taylor & Verrez-Bagnis, 2006a). This favours the swelling of post-rigor muscle even though actin and myosin are bound together.

Table 9. Postmortem events, factors and mechanisms suggested as responsible for flesh degradation and loss of quality during chilled storage of sea bass (Delbarre-Ladrat et al., 2006b).

Changes encountered post mortem	Suggested factors	Mechanism for loss of quality
Metabolic:		
Nucleotides breakdown	Nucleotide-degrading enzymes	
Lipid hydrolysis and oxidation	Phospholipases, oxidation status in cell	Loss of flavour
Drop of pH	Glycogen degradation and lactic acid accumulation, protein detoriation	Protein deterioration, enzyme regulation
Increase in osmotic pressure		Increased susceptibility to proteolysis
Ca^{2+} increase	Increase in osmotic pressure and pH fall (protein deterioration)	Calcium theory: weakening of Z-discs, degradation of titin and nebulin
Increase in nitric oxide and free radicals		Increased susceptibility to proteolysis
Myofibrillar structure:		
α-actinin release	Calpains, cathepsins D and L	
α-actinin proteolysis	Calpains, cathepsins B, D and L	
Titin degradation	Calcium *per se*	
Nebulin proteolysis	Calcium *per se*	Z-disc weakening
Myosin proteolysis (stable in sea bass)	Calpains, cathepsins B, D and L	Z-disc weakening
	Calpains	Myofibril destruction
Tropomyosin delocalisation	Cathepsins L	Myofibril destruction
Tropomyosin proteolysis	Cathepsins B and L	Myofibril destruction
Troponin T proteolysis (stable in sea bass)	Cathepsins B and L	Myofibril destruction
30 kDa fragment appearance (not in sea bass)	Cathepsins L	
Actin degradation	Calcium *per se,* calpains, cath. B and L	Myofibril destruction
Transversal structure:		
Desmin degradation in vitro (stable in sea bass muscle)		Intermediate filament and myofibril destruction
Sarcolemma anchorage:		
Dystrophin degradation	Calpains	Costamere degradation

Postmortem degradation of the muscle occurs both within the extra- and intracellular matrix. Degradation of fine collagen fibres leads to detachment within the muscle fibre from the myocommata and weakening of the pericellular connective tissue (Hernández-Herrero, Duflos, Malle & Bouquelet,

2003). Within the cell, the main changes are believed to result from proteolytic degradation of minor components linking structural units together rather than direct breakdown of the myofibrils (Ólafsdóttir et al., 1997). As an example, titin and nebulin are highly sensitive to proteolysis during the rigor mortis period. Other structural proteins, like α-actinin, are very resistant to proteolysis, but are released from their structural compartments during post-mortem aging of the Z-disc region (Astier, Labbe, Roustan & Benyamin, 1991; Delbarre-Ladrat et al., 2006a).

The degradation processes are influenced by the biological condition of the raw material and handling and processing methods after catch (Table 10). The enzyme activity is affected by changes in temperature, ionic strength and type of ion. Certain alterations lead to increased access of enzymes to substrate, such as degradation in the muscle and increases in substrate concentration, for example by dehydration or partial freezing.

Table 10. Summary of autolytic changes in chilled fish (Huss, 1995).

Enzyme(s)	Substrate	Changes Encountered	Prevention/Inhibition
Glycolytic enzymes	glycogen	production of lactic acid, pH of tissue drops, loss of water-holding capacity in muscle, high temperature rigor may result in gaping	pre-rigor stress must be avoided fish should be allowed to pass through rigor at temperatures as close to 0°C as is practically possible
Autolytic enzymes, involved in nucleotide breakdown	ATP ADP AMP IMP	loss of fresh fish flavour, gradual production of bitterness with Hx (later stages)	same as above rough handling or crushing accelerates breakdown
Cathepsins	proteins, peptides	softening of tissue, making processing difficult or impossible	rough handling during storage and discharge
Chymotrypsin, trypsin, carboxy-peptidases	proteins, peptides	autolysis of visceral cavity in pelagics (belly-bursting)	problem increased with freezing/ thawing or long-term chill storage
Calpain	myofibrillar proteins	softening, molt-induced softening in crustaceans	removal of calcium thus preventing activation?
Collagenases	connective tissue	gaping of fillets, softening of the muscle	connective tissue degradation related to time and temperature of chilled storage
TMAO demethylase	TMAO	formaldehyde-induced toughening of frozen gadoid fish	store fish at temperature ≤-30°C ; physical abuse and freezing thawing accelerate formaldehyde-induced toughening

3.9 Effects of salt on muscle proteins

First of all, it is important to keep in mind that biochemical changes due to microbial and enzymatic activity are not completely hindered during salting. The activity changes based on different effects of salt on microbes and enzymes; in some cases the activity is only paused, whereas in other cases it is stimulated. Therefore, alterations in muscle proteins during heavy salting of cod are influenced by complex and coupled effects of biochemical processes and changes in ionic strength.

3.9.1 Effects of salt on enzyme activity

The effect of salt on enzyme activity depends on the nature of the different enzymes found in fish muscle. The reaction rate of cathepsin has been found to decrease with increasing salt concentration (Reddi, Constantanides & Dymaza, 1972). The alterations depend on fish species and processes applied. During heavy salting of cod, the activity of cathepsin B/L remains unchanged, whereas the activity of acidic proteases declines as the salt concentration in the muscle increases (Stoknes, Walde & Synnes, 2005). The activity of trypsin (Figure 20), collagenase and elastase (Table 11) is stimulated by increasing salt concentration (14-17% NaCl g/100g sample). During the first 5 days of salting, there is a 15- to 24-fold increase in the activity depending on the salting method applied. The increase is higher in fish that is only pickle salted during 5 days compared to a combination of brining and pickling (Stoknes et al., 2005). During the dry salting step that follows (30 days) the activity decreases again.

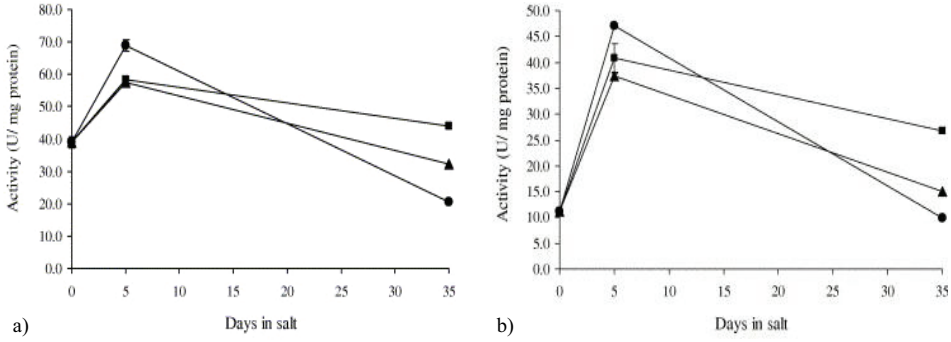


Figure 20. a) Trypsin-like and b) chymotrypsin-like activity in cod muscle that was subjected to three different salting methods (Stoknes et al., 2005):

Circle (A): 24 h soaking in brine of 20.5% NaCl (20 °Be), then pickled for 4 days before dry salting for 30 days;
Triangle (B): 24 h soaking in brine of 25% NaCl (24 °Be), then pickled for 4 days before dry salting for 30 days;
Square (C): The fish was pickled for 5 days before dry salting for 30 days.

Changes in elastase activity are opposite to collagenase. The activity tends to be higher in muscle brined in 20% brine followed by pickling for 4 days. In other respects, the elastase activity follows the

same pattern as collagenase. Before salting, the activity is low, but it increases with the amount of time in salt and decreases again during dry salting. The amount of elastin in the muscle is minor compared to collagen. Therefore, the effects of collagenase are more important than those of elastase with regard to structural changes in the muscle. The activity of cathepsin B/L is low and does not change much with salting time (Stoknes et al., 2005).

Table 11. Salt content and enzymatic activity of cod muscle subjected to different salting methods (Stoknes et al., 2005).

		Days in salt			
	Salt treatment	0	1	5	35
Concentration of NaCl (%)	A	0.3 ± 0.1	4.4 ± 0.1	15.3 ± 0.4	18.2 ± 0.3
(g/100g)	B	0.3 ± 0.1	3.3 ± 0.5	14.4 ± 0.6	15.7 ± 0.5
	C	0.3 ± 0.1	n.d.	13.8 ± 0.5	19.2 ± 0.6
Cathepsin B/L	A	2.0 ± 0.2	2.4 ± 0.7	1.8 ± 0.4	0.7 ± 0.0
(U/mg protein)	B	2.0 ± 0.2	n.d.	2.0 ± 0.5	2.2 ± 0.1
	C	2.0 ± 0.2	n.d.	2.8 ± 0.4	1.5 ± 0.1
Collagenase	A	1.5 ± 0.0	0.9 ± 0.0	22.0 ± 1.5	0.6 ± 0.0
(U/mg protein)	B	1.5 ± 0.0	n.d.	23.1 ± 3.2	8.6 ± 1.2
	C	1.5 ± 0.0	n.d.	36.0 ± 2.6	8.3 ± 0.8
Elastase	A	2.3 ± 0.2	3.3 ± 0.4	13.7 ± 1.3	1.0 ± 0.3
(U/mg protein)	B	2.3 ± 0.2	n.d.	9.7 ± 3.2	3.3 ± 0.3
	C	2.3 ± 0.2	n.d.	8.5 ± 0.2	5.5 ± 0.3

A: 24 h soaking in brine of 20.5% NaCl (20 °Be), then pickled for 4 days before dry salting for 30 days;
B: 24 h soaking in brine of 25% NaCl (24 °Be), then pickled for 4 days before dry salting for 30 days;
C: The fish was pickled for 5 days before dry salting for 30 days.

The variation in enzyme activity of the salted cod muscle may be related to differences in parameters such as water-holding capacity and weight yields, depending on salting methods applied. The enzymes do also play an important role in developing the characteristic flavour and texture of bacalao.

3.9.2 Swelling and shrinkage of the myofibrils

Swelling is a mechanism that occurs during pre-salting with injection and brining (depending on brining concentration), whereas shrinkage results from pickling and dry salting due to the extreme changes in chemical content. Swelling and shrinkage of myofibrils or muscle fibres alter the distribution of water within the muscle. However, the alterations do not necessarily result in changes in total volume for water in the myofibrillar lattice (Offer, 1988b).

3.9.2.1 Salting-in of muscle proteins (<1M)

Salting-in or swelling of the muscle is coupled with the solubility of the myofibrillar proteins. Cod muscle proteins are soluble in water and at very low ionic strength (≤0.0003), at neutral pH. When the salt concentration is slightly increased (0.025-0.150), the solubility decreases to a minimum, which is

approximately at the physiological ionic strength in the muscle. The salt ions neutralise the protein charges independently of the nature of the salt. At a higher concentration (>0.1M), the solubility increases again and reaches its maximum at approximately 0.8-1.0M. The main changes occur in the range of 0.6M to 0.8M when the A-band becomes partly solubilised. The changes in solubility are believed to result from decreased protein-protein interactions, protein-solvent interactions, conformational changes, polymerisation and depolymerisation of proteins (Fennema, 1990; Morrisey, Mulvihill & O´Neill, 1987; Offer, 1988a; Stefansson & Hultin, 1994). The "salting-in" effects are mainly dependent on the ionic strength but are generally independent of the ion type (Stefansson et al., 1994; Von Hippel & Schleich, 1969).

The swelling of the muscle depends on the spacing between filaments, which is determined by balance between the following: the long-range electrostatic repulsive forces between charged filaments (the thick filaments), restraining forces, osmotic pressure and mechanical forces (cell membrane) (Offer, 1988a). The spacing of filaments grows larger with increasing ionic strength (0.15-0.8M) due to the binding of salt ions (Cl^-) to oppositely charged amino acids residues (Figure 21) and the rupture of intra- and intermolecular bonds. Consequently, more water molecules flow between the polymers. Furthermore, water-protein bonds become stronger than protein-protein bonds, and more water is bound in the muscle (Offer et al., 1983; Schmidt et al., 2008).

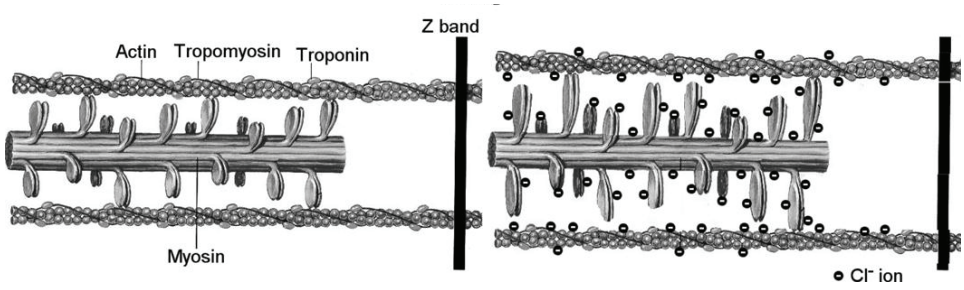
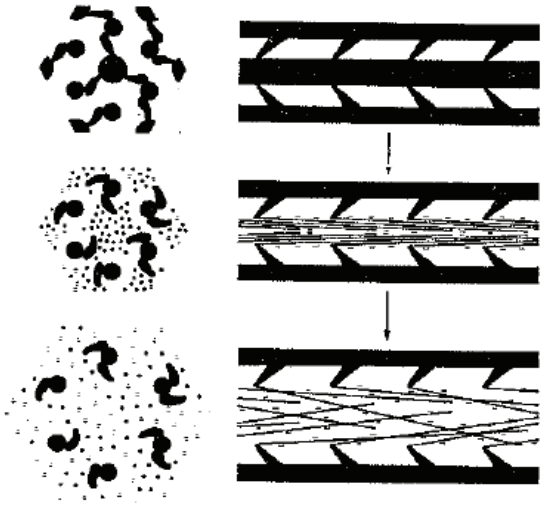


Figure 21. Illustration of the formation of the complex Cl^- -myofibrillar proteins, which increases the matrix potential of the meat (Schmidt et al., 2008).

The myofibrillar structure and the actomyosin complex become weaker with rises in salt content. Addition of phosphates leads to dissociation of the complex (Offer, 1988a). The thick filaments are depolymerised by solubilisation of myosin shafts at salt concentrations of >0.5M, allowing further swelling of the muscle (Figure 22) (Offer et al., 1983; Schmidt et al., 2008).



a) Rigor muscle before salt treatment. The left-hand diagram represents a thin transverse section through the filament lattice, the smaller circles representing thin filaments, and the larger circle at the centre representing a thick filament backbone. The curved, pear-shaped objects represent cross-bridges. The right-hand diagram represents a longitudinal section through one thick filament and two thin filaments with connecting cross-bridges.
b) Structure formed by depolymerisation of the thick filament backbone, but before swelling. In the transverse section shown in the left-hand diagram, the dots represent the tails of the myosin molecules (33 in any transverse section of a filament). It is supposed that the motion of the tails is considerably restricted by the presence of neighbouring decorated thin filaments, and that the attached myosin heads tend to prevent the tails from approaching the surface of the thin filaments.
c) Structure formed after swelling. The myosin tails are now able to move through a larger angle (Offer, 1988a).

Figure 22. Mechanism of swelling of myofibrils in salt (Offer, 1988a).

3.9.2.2 Salting-out of muscle proteins (>1M)

Protein solubility decreases at a higher salt concentration (>1M); these effects have been termed "salting-out" effects. Different mechanisms have been discussed in relation to salting-out effects (Baldwin, 1996), i.e. that the salt-induced effects are only through alterations of water structure (Arakawa & Timasheff, 1982; Robinson & Jencks, 1965), whereas others assume that salt binds directly to the proteins and at the same time influences hydrophobic interactions through ordering of water molecules around the binding site (Von Hippel et al., 1969). Stefansson et al. (1994) explained the declining solubility of cod muscle proteins at concentrations of >1M by losses of stable hydrophilic surfaces of the proteins. At the same time, the exposed hydrophobic areas of the proteins were believed to interact, leading to aggregation and precipitation of proteins. Moreover, the repulsive forces between proteins decreased and protein-protein bonds became stronger than the solvent-solute interactions. When the proteins aggregate, the water holding capacity of the muscle declines. Duerr & Dyer (1952) showed that loss of protein solubility was accompanied by a loss of water as the salt concentration reached about 8-10% in the muscle. These changes are believed to be accompanied by shrinkage of the muscle (Barat et al., 2003; Duerr et al., 1952; Offer et al., 1983; Offer, 1988a; Stefansson et al., 1994; Xiong, 2000).

The salting-out effects are strongly dependent on the ion type. The relative effectiveness of different ions (at constant ionic strength) to promote salting-out of proteins follows the so-called Hofmeister series:

Anions: $SO_4^{2-} < F^- < CH_3COO^- < Cl^- < Br^- < NO_3^- < I^- < ClO_4^- < SCN^-$

Cations: $NH_4^{4+} < K^+ < Na^+ < Li^+ < Mg^{2+} < Ca^{2+}$

The ions on the left promote salting-out of the proteins, loss of solubility, aggregation and stabilisation of the native conformation. The ions on the right promote unfolding, dissociation, and salting-in of the proteins (Damodaran, 1996; Stefansson, 1994; Ragnarsson, 1988). The function of the salt is dependent on pH: the lower the pH, the less salt is necessary to reduce the water-holding capacity of the muscle due to the shift towards lower pH in the isoelectric point of the proteins (Hamm, 1972).

3.9.3 Solubility of collagen and effects of salting

Solubility of proteins is known to be lowest at the isoelectric point of proteins. Two pH values have been reported in relation to the isoelectric point of collagens, 4.7 and 7.8 (Highberger, 1939), which makes it possible to obtain different fractions under acidic or alkaline conditions with the aid of salt precipitation (Figure 23). For comparison, it can be mentioned that the isoelectric point of cod myosin is at pH 5.3 and of actin at pH 4.7-4.8 (Connell, 1954). Differences in solubility have been used for classification of collagen, such as acid soluble (ASC), pepsin soluble (PSC) and insoluble. In salmon, the ratio of each fraction has been reported to be 6%, 93% and 1%, respectively (Eckhoff, Aidos, Hemre & Lie, 1998). In addition, collagen fractions can be isolated in salt solutions (Gross, 1958; Gross, Highberger & Schmitt, 1955; Neklyudov, Berdutina, Ivankin, Mitaleva & Evstaf'eva, 2003).

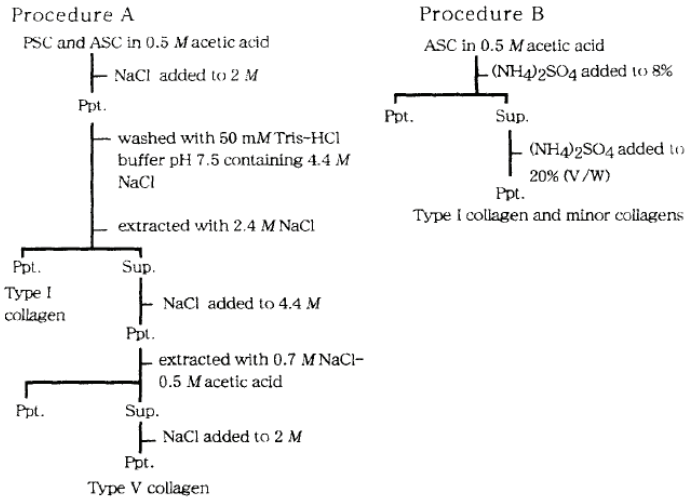


Figure 23. Diagram of fractionation procedures of acid-soluble (ASC) and pepsin-solubilised (PSC) collagens by sequential salt precipitation (Ppt: precipitate, Sup: Supernatant) (Sato et al., 1997).

The solubility of the ASC fraction is highest in the pH range of 1 to 4-5 but declines rapidly at more alkaline conditions (Figure 24). The solubility remains relatively constant at salt concentrations up to 4% (0.7M) (Figure 25). At higher concentrations, the solubility declined rapidly due to increasing hydrophobic interactions and protein aggregation (Bae, Osatomi, Yoshida, Osako, Yamaguchi & Hara, 2008).

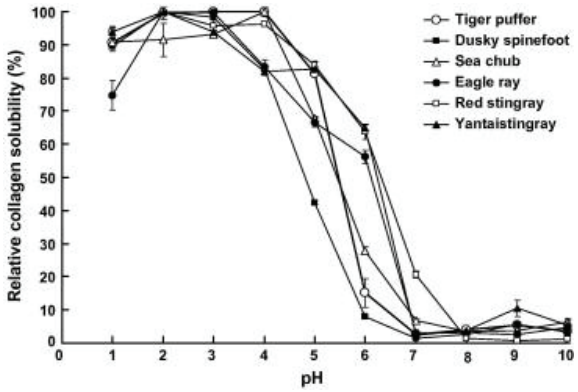


Figure 24. Relative solubility of ASC from the skins of tiger puffer and underutilised fishes at various pHs. Values are means ± standard deviation (*n* = 3) (Bae et al., 2008).

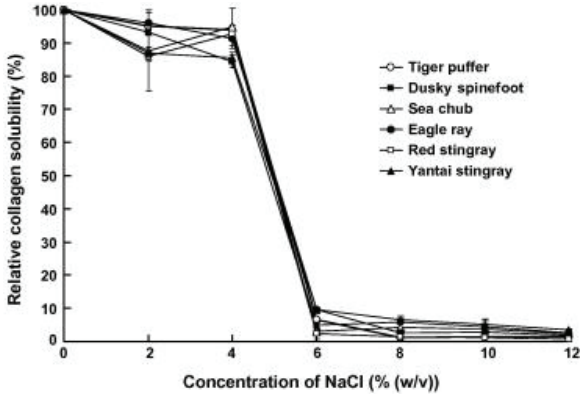


Figure 25. Effect of NaCl concentration on the solubility collagens (ASCs) that were extracted (50 mM acetic acid) from the skins of several underutilised fishes. Values are means ± standard deviation (*n* = 3) (Bae et al., 2008).

The maximum solubility of collagen seems to be obtained at similar levels as by myofibrillar proteins (Stefansson et al., 1994), both for the ASC fraction and of collagen fractions obtained by water-salt extraction (Neklyudov et al., 2003) (Figure 26 and Figure 27). The salt-soluble fractions have been reported to have good water-binding properties (Neklyudov et al., 2003). Therefore, solubilisation and

swelling of collagen may partly be the reason for increased weight yields of muscle food at low salt concentrations.

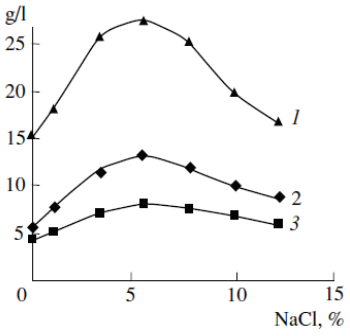


Figure 26. Dependence of the content of cattle collagen in solution (g/l) on the concentration of NaCl. Collagen was extracted from (1) subcutaneous tissue, 50°C; (2) tendon, 50°C; (3) subcutaneous tissue (Neklyudov et al., 2003).

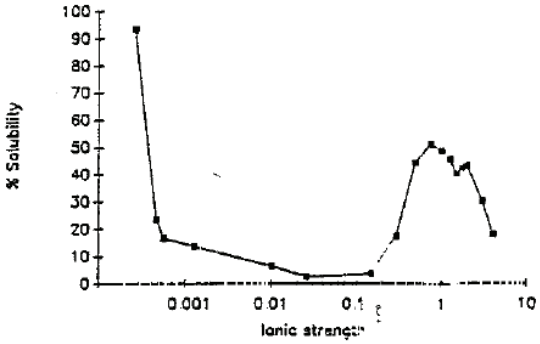


Figure 27. Effect of ionic strength on solubility of myofibrillar proteins from cod muscle. Solubility is expressed relative to total protein in the washed muscle (Stefansson et al., 1994). (1M NaCl = 5.85% NaCl)

Solubility and other properties such as heat stability of collagen are affected by amino acid composition, animal age, species and tissue type (Table 12). The salt solubility of collagen from skin has shown to be higher than that of collagen from muscle, in the case of pig and bovine collagen. The opposite is true for cod and Pollack. Cod skin collagen has shown to be completely solubilised in a 0.45M salt solution at 45°C (Table 12) (Sadowska & Kotlowski, 1999). Fish species living in cold environments have lower contents of hyproxyproline and exhibit lower thermal stability than those from fish living in warm environments (Bae et al., 2008).

Table 12. Soluble collagen content in diluted salt and acid solutions (Sadowska et al., 1999; Sikorski, 2001).

		Collagen solubility in percent of total collagen	
Animal species	**Tissue**	**In salt (0.45M NaCl at 45°C)**	**In acid (0.5M acetic acid)**
Pig	Skin	1.7	1.4
	Muscle	0.7	2.7
Bovine	Skin	1.6	1.9
	Muscle	0.6	2.6
Cod	Skin	1.5	86.0
	Muscle	4.2	76.3
Pollack	Skin	5.2	60.3
	Muscle	5.1	55.0

Alterations in thermal stability of collagen in relation to changes in salt concentrations have been described as follows. At low concentrations (0.02-0.2M), neutral salts bind to collagens and shift thermal transitions to lower temperatures, indicating conformational changes in the collagen molecule. It has been suggested that destabilisation of the triple collagen helix may be due to electrostatic

interactions. Ionisable and hydrophobic side chains are believed to be grouped in patches along the triple helix (Brown, Farrell & Wildermuth, 2000; Hulmes, Miller, Parry, Piez & Woodhead-Galloway, 1973). At higher concentrations the thermal stability increases, which is expressed by higher denaturation temperatures and enthalpy. This may result from electrostatic and hydrophobic interactions, as well as from hydrogen bonds and the electron-withdrawing character of hyproxyproline (Brown et al., 2000; Holmgren, Taylor, Bretscher & Raines, 1998). In studies of calf skin, two peaks were observed which overlapped to a great extent at 1M NaCl. In addition, the T_{max} in DSC investigations was shifted to higher temperatures (Figure 28a). The results showed that the denaturation at approximately 35°C was reversible, whereas denaturation at 40°C was more sensitive to heating (Komsa-Penkova, Koynova, Kostov & Tenchov, 1996). The denaturation temperatures (T_{max}) are affected by the type of anion in sodium salts used, whereas the denaturation depends more on the concentration of salt when chloride salts are added to collagen. Three concentration regions are clearly distinguished during salt denaturation of collagen: 1) a salt-independent drop of the transition temperature at low salt concentrations (20mM), 2) a salt-dependent change (predominantly decrease of the enthalpy) at intermediate concentrations, and 3) an increase of T_{max} at high concentrations (Komsa-Penkova et al., 1996).

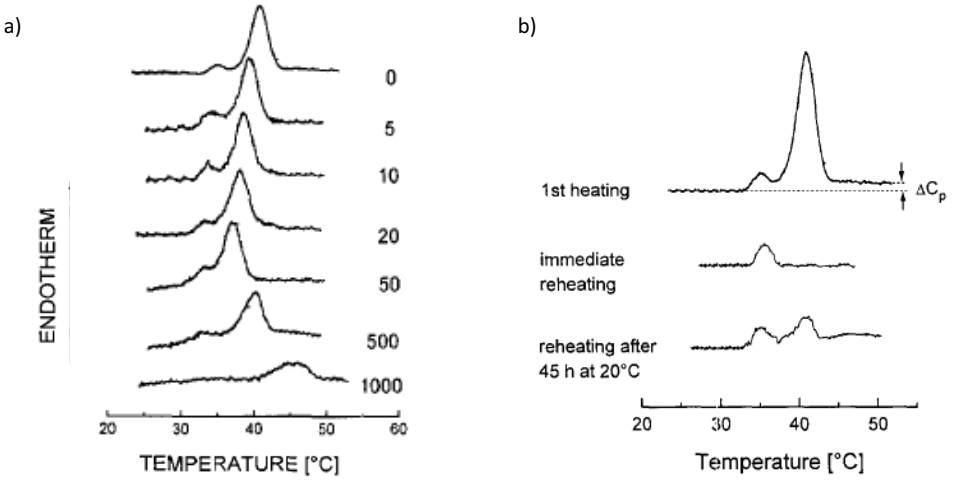


Figure 28. a) Heating thermograms of calf skin collagen type I (0.5 mg/ml) in 0.05 M acetic acid: a) in the presence of NaCl of concentrations in mM as indicated; b) recorded during first heating, immediate reheating of the sample and reheating of the sample after 45 h storage at 20°C (Komsa-Penkova et al., 1996).

The collagen in herring skin seems to be very resistant to high salt concentrations, whereas the conformation of myofibrillar proteins was significantly affected, according to DSC-profiles (Schubring, 1999). However, the muscle collagen in the gadoids has shown to be more susceptible to salt-induced changes in the skin collagen (Sadowska et al., 1999). Information about the effects of heavy salting of cod collagen was not found.

4 Materials and Methods

In this section methods are listed and discussed, but more detailed descriptions can be found in Papers I-VI (in appendix). Non-laboratory work was carried out at processing plants under the same conditions as used for commercial production.

4.1 Raw material and processing

Cod (*Gadus morhua*) was used for all studies. It was caught by long line from commercial fishing boats. The fish was bled and gutted immediately after catch. It was washed and then iced in tubs, where it remained until processed. To reduce the variability in raw material, fish of similar size were chosen from the same batch, i.e. catch, time from death, handling after catch, processing equipment and processing methods were the same.

The fish was filleted after rigor mortis (3-4 days after catch). After filleting, fillets were divided into groups of 45-50 and each fillet identified with a numbered plastic tag. The fillets were weighed individually before salting and again after each processing step. The salting and curing procedures could be divided into three basic steps: pre-salting, dry (pile) salting and storage after packing (Figure 29). The aim of the work was to evaluate the effects of different pre-salting procedures on the characteristics of salted and rehydrated cod fillets. The pre-salting procedures varied by different methods applied and by the composition of the brine used for injection and brining.

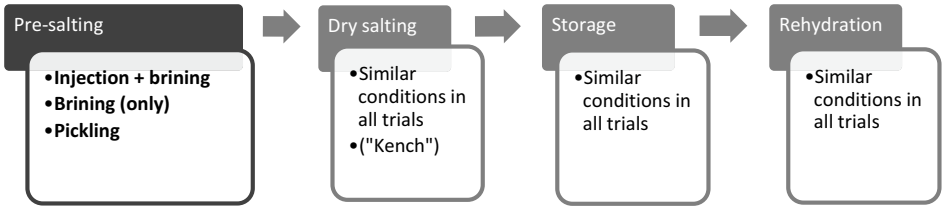


Figure 29. Graphical presentation of the main process and subject in the study, which was the variation of the pre-salting step: a) injection and brine salting, b) brine salting, c) pickling, that were compared to a single dry salting (kench) step without any pre-salting.

The pre-salting methods used were injection, brining and pickling, which are briefly described here:

- Injection was carried out by (FOMACO FMG 64/256F, FOMACO Food Machinery Company, Koga, Denmark) to obtain about 12-15% weight gain of the original fresh weight. Injection was always followed by brining.
- Brining was carried out by immersing fillets into brine for 2 days. The composition of brine used was different for injection and brining and varied with trials, as will be described below.
- Pickling involved stacking the fillets with alternating layers of salt in closed tubs for 3 days. The liquid drawn from the fillets by the salt formed saturated brine, which was not allowed to drain away.
- Dry salting followed brining and pickling. The fillets were piled into tubs with alternating layers of salt, and the liquid formed was allowed to drain away. The duration of this step was approximately 3 weeks, during which time the temperature was kept at 3-5°C.

Simulation of the old kench salting method was used as a reference method in Papers IV and V to evaluate effects of pre-salting. It was carried out by dry salting the fillets without any pre-salting. Restacking was omitted, the pressure was lower due to reduced height of stacks and the duration was shorter than in the old method. After dry salting, the fillets were packed into waxed cardboard boxes and kept at 0-2°C and approximately 78% RH. Finally, the fillets were rehydrated and desalted to levels suitable for cooking and consumption. The fillets were submerged in water at 4 ± 1°C for approximately 4 days, generally with one water change.

4.2 Experimental design

The experimental setup is described in the following pages, and in the papers (I to VI), which are presented in the appendix. The effects of different salting procedures were evaluated by varying the pre-salting methods applied, the salt (S) concentration in brine and with/without addition of phosphates (P). Different parameters were analysed during the process (Table 13).

Table 13. Parameters evaluated in the different papers, as affected by different salting procedures and heavy salting of the cod muscle.

Paper	Yields	Proximate analysis	Phosphate content	NPN	Water retention	Commercial quality rating	Sensory analysis	SDS-PAGE DSC	Rheology Microstructure
I	x	x	x		x	x	(x)		
II		x						x	
III	x	x		x	x	x			
IV	x	x		x	x				
V								x	
VI	x	x		x	x				x

4.2.1 Paper I

Cod fillets were salted in a traditional way, with and without the addition of polyphosphates. After 3 weeks of storage, the fillets were rehydrated and desalted. Changes in weight, water-holding capacity and chemical content were followed throughout the process. In addition, the commercial quality of salted fillets was evaluated and sensory analysis was performed on rehydrated fillets.

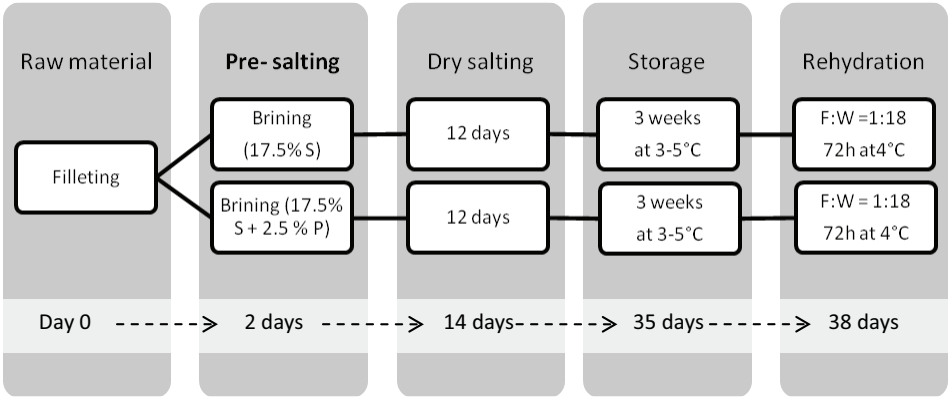


Figure 30. Experiment I in Paper I (F:W = Ratio of fish to water).

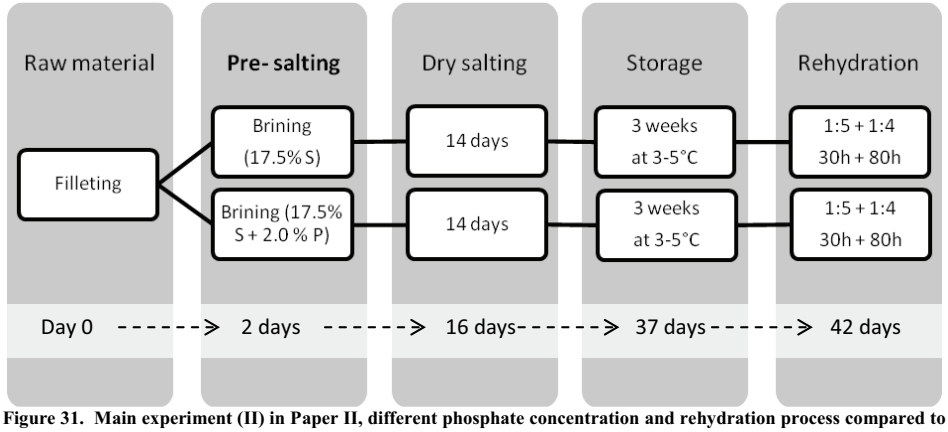


Figure 31. Main experiment (II) in Paper II, different phosphate concentration and rehydration process compared to trial I. Rehydration was carried out at 4 ± 1°C, with water change after 30 hours.

4.2.2 Paper II

The effects of salt curing and rehydration on conformational stability and degradation of muscle proteins were studied using SDS-PAGE and DSC. Traditional salting procedures were used, starting with brining in 17.5% brine, followed by 14 days of dry salting and 3 weeks of storage (Figure 31).

4.2.3 Paper III

The influence of different brine concentrations on yields, chemical content, water-holding capacity, and commercial quality of the fish fillets was evaluated (Figure 32). Additionally, the effect of different brine salting methods was studied: traditional brine salting, maintaining a constant brine concentration, and increasing the salt concentration gradually during brining (Figure 33 and Figure 34).

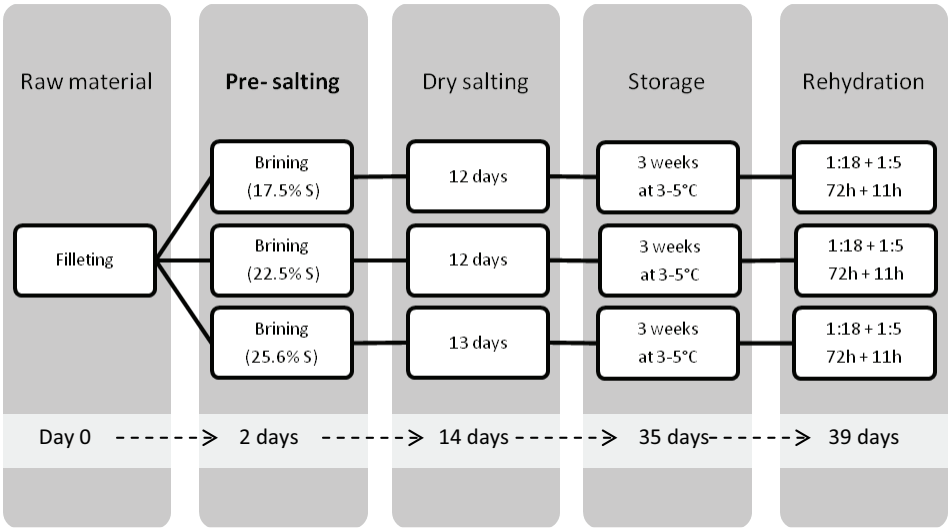


Figure 32. Pre-experiments (I) in Paper III, where different brine concentrations were tested.

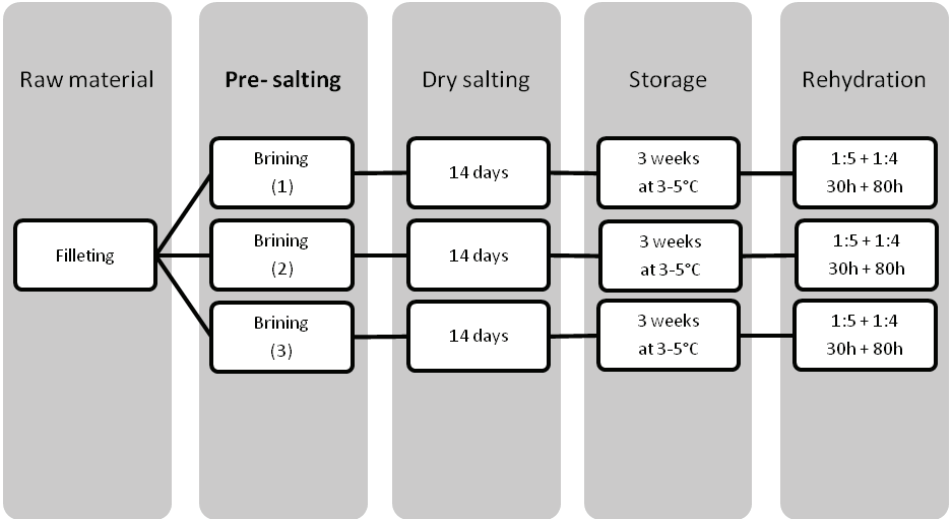


Figure 33. Main experiment (II) in Paper III, where different brining procedures (1, 2 and 3) were used in the pre-salting step.

During traditional brining (1), the brine concentration decreased due to salt uptake and an opposite flux of water from the muscle. Salt was added three times during salting to compensate for salt lost during brining (2) and to increase the brine concentration gradually during brining (3).

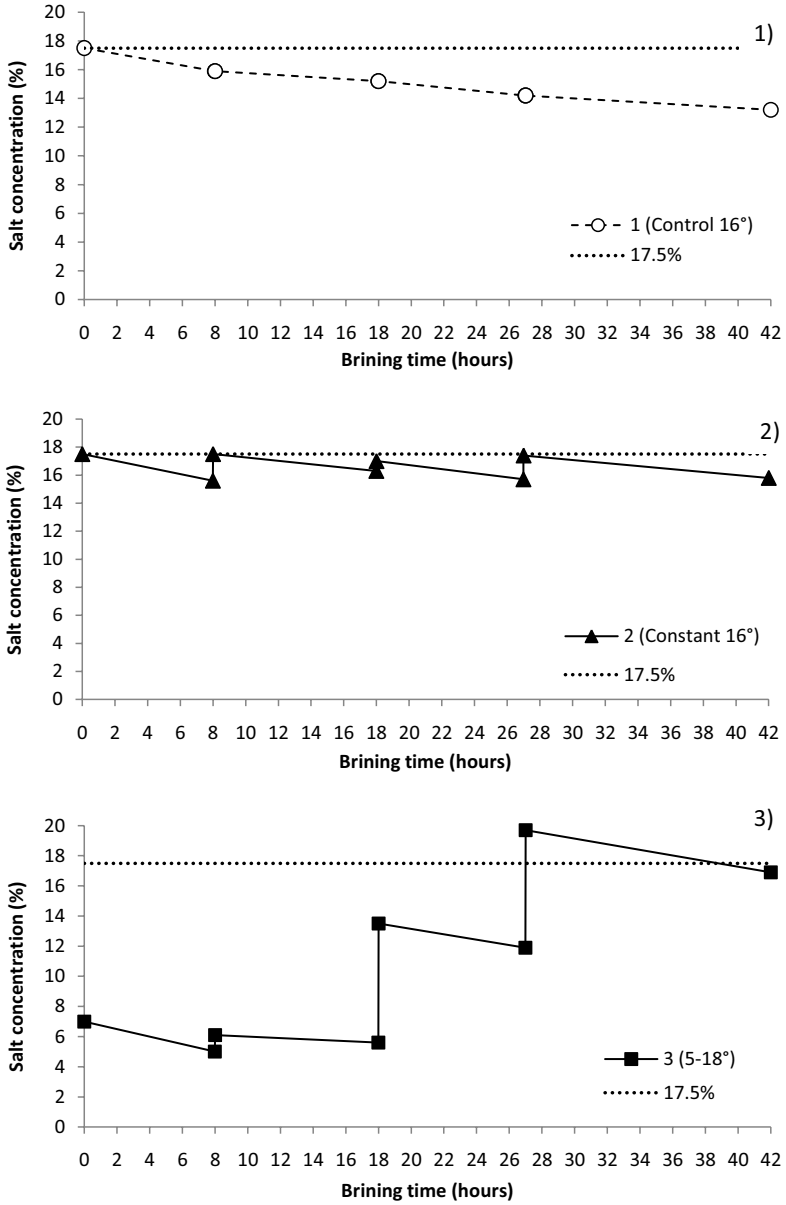


Figure 34. Changes in salt concentration in brine during brine salting by: 1) increasing the salt concentration gradually during brining, 2) maintaining a constant brine concentration and 3) traditional brine salting where no salt was added during brining.

4.2.4 Paper IV

In this paper, the effect of different pre-salting methods (injection and brining, brining only, and pickling) on yield and chemical composition of salted cod fillets was compared to a single kench salting step. Changes in weight, water-holding capacity and chemical contents of fillets were followed throughout the process (Figure 35). The data obtained was used to calculate weight yields, yields of chemical components and ratios of water to protein. In addition, weight yields of cooked rehydrated samples were evaluated.

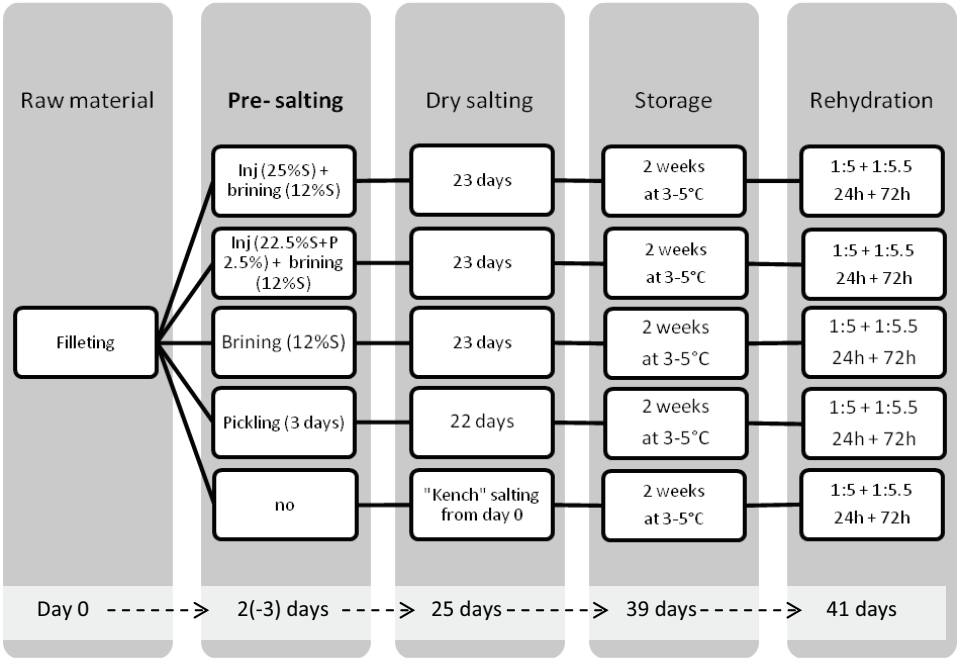


Figure 35. Experimental design in Paper IV, where different brining procedures were used in the pre-salting step.

4.2.5 Paper V

Samples from fillets salted according to the procedures in Paper IV (Figure 35) were analysed by SDS-PAGE and DSC. The aim was mainly to observe differences in denaturation, conformational changes and aggregation of myofibrillar and stromal proteins during salting and rehydration. The results were evaluated in relation to changes in chemical content and yields of the salted and rehydrated products, which varied with salting procedures.

4.2.6 Paper VI

In this study, micro-structural and rheological changes in salted and rehydrated cod fillets were followed. Pre-salting methods were varied: injection and brining versus brining only were applied (Figure 36). In addition, the influences of chilling methods on board were evaluated by using fish stored in liquid ice and plate ice for salting.

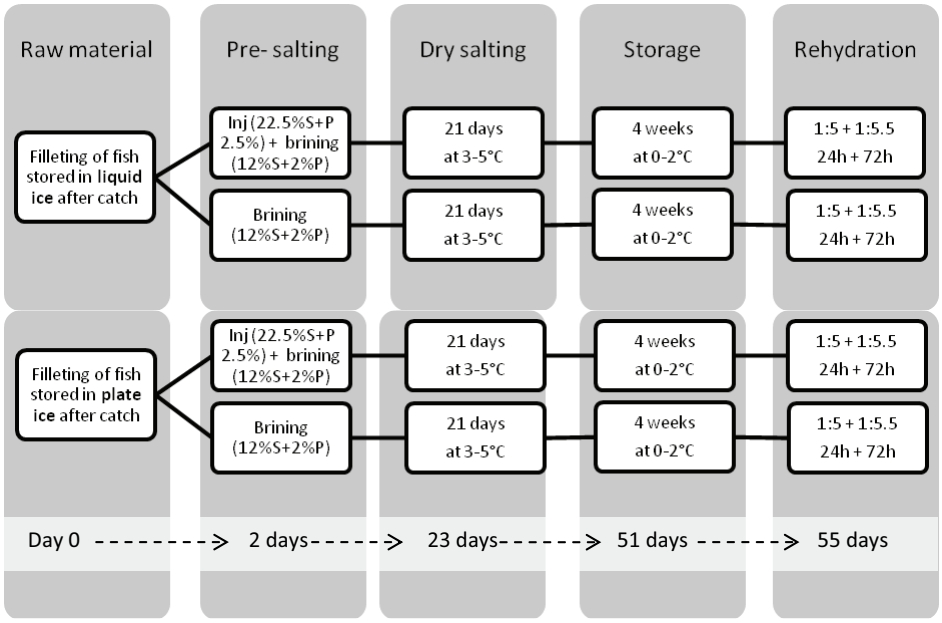


Figure 36. Experimental design in Paper VI, where effects of injection compared to only brining during pre-salting were evaluated. A different rehydration process was used for the rheological samples, where 3 cm wide slices were soaked in water (1:16) for 36 hours. The method represented in the scheme was used for whole fillets, which were evaluated by microstructural analysis.

4.3 Parameters analysed

The different methods used were carried out using known laboratory methods, from which references can be found in the different papers. Therefore, the methods are discussed in general rather than describing the exact methodology. Modifications of the methods or adjustments of measurement settings will, however, be described.

4.3.1 Weight yield

The weight yield (%) is an essential economic factor for producers, but it is also a relevant indicator of the water-holding capacity of the muscle. When this variable is used, it is important to consider what the reference value is and how weight gains are evaluated. In this study, the weight of fillets directly

after filleting and a minor trimming was used as a reference weight. It was chosen in order to focus only on the influence of the pre-salting procedures. Producers might also want to include the processing yield by using the weight of the gutted fish as removed from the ice before processing. It must also be kept in mind that some weight changes occur during storage of the raw material after catch.

The total weight yield (Y_t^o) was calculated after each step (t) in the process and compared to the initial weight of the fillets before pre-salting (M_0^o). The equation used was slightly modified from other studies (Andres et al., 2005; Barat et al., 2003; Gallart-Jornet et al., 2007a), where the initial value represented no changes, whereas it was 100 in this study. Values >100 in this study indicated a weight increase of the fillets and values <100 a weight reduction:

$$Y_t^o = \left(\frac{M_t^o}{M_0^o} \right) * 100 \tag{4.1}$$

The values obtained during salting (and storage) represented the curing yield. Additionally, the proportional weight changes (ΔM_B^o) during each step (A to B) of the process were calculated, as in the rehydration step:

$$\Delta M_B^o = \left(\frac{M_B^o - M_A^o}{M_A^o} \right) * 100 \tag{4.2}$$

A and B = weights before and after rehydration, respectively

Multiplication of the curing yield and the rehydration yield also gives the total yield of the process used in the study or the same results as eq. 4.1, when the weight after rehydration was divided by the weight of the fresh fillets. A higher curing yield usually means that the rehydration yield becomes lower. This is very important for producers and exporters of salted fish to keep in mind when changes in salting procedures are made.

4.3.2 Proximate analysis

Moisture content (g/100g) was calculated from the weight loss during drying at 103 ± 2°C for 4 hours (ISO-6496, 1999). Salt content was determined using the Volhard method (AOAC, 1990). The total protein content of the fish muscle and brine was calculated from the total nitrogen content (TN *6.25) and analysed using the Kjeldahl method (ISO-5983, 2005). The pH of the muscle was measured by

inserting a combined glass electrode (SE 104, Portamess 913 pH, Knick, Berlin, Germany) directly into the cod mince.

4.3.3 Non-protein nitrogen

Trichloroacetic acid (TCA)-soluble nitrogen was used to estimate quantities of non-protein nitrogen (NPN) (Thorarinsdottir, Arason, Bogason & Kristbergsson, 2004). The difference between TN and NPN was used to calculate the approximate amount of nitrogen bound in proteins (PN). The method involved precipitation of the protein fraction with an addition of 10% trichloroacetic acid (TCA) and centrifugation (Gudmundsdottir, 1995). Then the soluble nitrogen in the supernatant was analysed by Kjeldahl. The NPN fraction contains various nitrogenous compounds, such as free amino acids, small peptides, trimethylamine oxide, (TMAO), trimethylamine (TMA), creatine, creatinine, nucleotides and low molecular weight organic compounds from the muscular plasma (Dambergs,1964) (Velankar & Govindan, 1958). The ratio of NPN in gadoids and flatfishes is generally in the range of 7-14% of the total nitrogen (Simidu, 1961, Huss 2002), but it is influenced by the handling and processing methods of fish. During salting, the levels of free amino acids and peptides rise due to the proteolytic degradation of proteins during curing (Gudmundsdottir, 1995). However, the NPN compounds are also partly lost in the liquid phase and extracted from the muscle during salting and rehydration processes (Thorarinsdottir et al., 2004).

4.3.4 Phosphate content

Phosphates are known to have a chelating effect on metals and therefore a reducing effect on oxidation. The use of phosphates in bacalao production has been a matter of debate, since it is only allowed in frozen fish products (maximum of 5 g P_2O_5/kg of the product (phosphorus pentoxide (P_2O_5) $\approx 2.29 \times$P; orthophosphate $PO_4^{3-} \approx 3.06$ P)). However, it has been practised to some extent because of the positive effects on appearance and on weight yields of the products (Lindkvist et al., 2008).

The phosphorus (P) content of the muscle was analysed by colorimetry as phosphate vanadomolybdate (Hanson, 1950; Sutton & Ogilvie, 1967) using a spectrometric method, which is based on the reaction of orthophosphate (PO_4^{3-}) in an acidic solution with ammonium molbybdate and ammonium vanadate in nitric acid. The absorbance of the solution prepared was determined at 420 nm and compared to a calibration curve of a series of standard solutions (vanado-molybdate reagent).

The phosphate content was presented in the form of phosphorus oxide (P_2O_5/g sample = P * 2.2914 / g sample). To report the phosphorus content as PO_4^{3-}, the P content must be multiplied by 3.07.

The range of phosphorus content (0.026-1.1%) in fish is large, which makes it difficult to determine the amount of added phosphates in food products by simple quantitative methods (Lawrie, 1998a; Lee, Hendricks & Cornforth, 1998; Unal, Erdogdu, Ekiz & Ozdemir, 2004). The identification of different phosphates types by more advanced methods is also of limited use in products like salted cod due to degradation of the added phosphates in the muscle. The added phosphates are hydrolysed to pyro- and finally orthophosphates (PO_4^{3-}) through action of muscle phosphatases. The degradation rate is influenced by different factors: phosphate type, other ingredients, muscle species, nature of enzymes, condition of the raw material, processing methods, storage time and temperature (Belton, Packer & Southon, 1987; Hamm & Neraal, 1977a; Hamm & Neraal, 1977b; Hamm & Neraal, 1977c; Hamm & Neraal, 1977d; Hamm & Neraal, 1977e; Sutton, 1973).

4.3.5 Yield of chemical components

The yield or recovery of the different chemical components (Yx_t^C), salt (NaCl), water, protein and non-protein nitrogen was calculated from changes in weight of the fillets (M) and weight fractions (x) of each component (C) with time (t), compared with the initial weight of each component in the fillet before salting (0):

$$Yx_t^C = \left(\frac{M_t^o * x_t^C}{M_0^o * x_0^C} \right) * 100 \tag{4.3}$$

In previous studies (Andres et al., 2005, Barat et al., 2003, Gallart-Jornet et al., 2007a), the changes were shown as a difference in chemical content (ΔM_t^C) compared to the fresh fillet:

$$\Delta M_t^C = \left(\frac{M_t^o * x_t^C - M_0^o * x_0^C}{M_0^o * x_0^C} \right) * 100 \tag{4.4}$$

This is easiest to explain by showing an example. The curing yield is 80% of salted fillet and its protein content increases from 18% in the fresh fillet to 21% in the salted fillets. This would mean that the weight of a 500 g fillet is reduced to 400 g during salting. The protein mass would decrease from 90 g to 84 g. Therefore the protein yield (Yx_t^C) would be 93.3%, which is equivalent to 16.8% protein content in the fresh fillets. The ΔM_t^C would be -1.2, or the percentage point difference in protein content using the protein content in fresh fillet as a reference value. The results shown are the same in both cases, and it is only a question of presentation. It was considered to be an advantage to show the changes as proportional (Yx_t^C) rather than as a difference between values.

4.3.6 Salt concentration in liquid phase of the muscle

The proportion between analysed water (x^w) and salt (x^{NaCl}) content in the muscle was calculated after each step of the process (Andres et al., 2005, Barat et al., 2003, Gallart-Jornet et al., 2007a) as follows:

$$z^{NaCl} = \left(\frac{x^{NaCl}}{x^w + x^{NaCl}} \right) * 100 \quad (4.5)$$

This ratio gives information about the saturation of the water or liquid phase in the muscle and water activity. Ions to some extent may be bound to proteins, and in the water phase there are also other soluble compounds that affect the water activity.

4.3.7 Water activity

The water activity (a_w) represents the ratio of water vapour pressure in any food system to the water vapour pressure of pure water at the same temperature. Water activity plays an important role in the preservation of food and the control of microbial growth, especially pathogens. The water activity of the cod samples were evaluated in a Novasina a_w Center measuring instrument (Novasina AG, Axair Ltd., Pfäffikon, Switzerland) at 25°C. The relative equilibrium humidity in % ERH was determined, which is directly correlated with the water activity in accordance with the following formula: a_w = ERH/100.

4.3.8 Water retention of the muscle

Different techniques are used to evaluate the distribution and condition of water in the muscle (Fennema, 1990). In this study, the variables concerning water-holding capacity during salting and rehydration were water yield, water-holding capacity (centrifugation), water-to-protein ratio (W/P), and cooking yield. *The water yield* was used to show proportional changes in water content. Higher values indicate higher water retention/uptake of the muscle when comparing different treatments at the same stage in the process. The relationship to other variables like salt content must be considered at the same time, since it is an essential factor with regard to protein swelling/contraction. *The water holding capacity (WHC)* was evaluated by centrifugation of the minced samples. The weight lost during centrifugation ($\Delta m_{centrifuged}$) was evaluated as water loss. WHC was calculated as the ratio of the water retained in the sample, compared to the mass of water before centrifugation ($m_t * x_t^w$).

$$WHC = \left(\frac{m_t * x_t^w - \Delta m_{centrifuged}}{m_t * x_t^w} \right) * 100 \quad (4.6)$$

The ratio of water to protein was calculated from weight fractions of water (x^w) and protein ($x^{\Pr}$) after each step. There are always some variations in water and protein content of the fish for biological reasons, such as age, sex and condition of the fish. Seasonal variation in the fish condition is highly important. This ratio can be expected to vary from 4.1 to 5.5, using extremes in the range of chemical content of cod (Fennema, 1990; Schnepf, 1989; Warrier et al., 1975; Zaitsev et al., 1969). If, for example, the water content of the cod muscle is 81% and the protein content is 17.5%, this ratio is 4.6. Here, the protein content is based on evaluation of TN*6.25. Different values are obtained for PN *6.25 = (TN-NPN) * 6.25. The results for the different calculations were presented in Papers IV and VI.

$$W/\Pr = \left(\frac{x^w}{x^{\Pr}} \right) \quad (4.7)$$

The cooking yield is related to the amount of liquid lost by the muscle during heating as a result of protein denaturation. In some cases an external force is applied, but that was not performed in this study. It is usually presented as the weight retained after cooking:

$$Cooking\,yield\,(\%) = \left(\frac{M_B^o - M_A^o}{M_A^o} \right) * 100 \quad (4.8)$$

A and B = weights before and after cooking

In water-rich food systems like cod muscle (80%), the main weight reduction is due to water loss. The cooking yield can also be calculated as water retained after cooking using the water content of the uncooked muscle as a reference value:

$$Cooking yield_w\,(\%) = \left(\frac{m_t * x_t^w - \Delta m_{cooking}}{m_t * x_t^w} \right) * 100 \quad (4.9)$$

4.3.9 Commercial quality rating

The quality of the final product depends on many factors, such as the condition of the raw material used, processing method and equipment and, finally, the methods used for salting, storage and rehydration (Lindkvist et al., 2008; Thorarinsdottir, Arason, Bogason & Kristbergsson, 2001; van Klaveren et al., 1965). The salted products are rated according to size and commercial quality at packaging, for example, as PORT (400 and 800 kg units) and SPIG (25 kg units). PORT refers to export to Portugal and SPIG to Spain, Italy and Greece. Some basic rules for quality rating are shown in this chapter, but the experience and training of the grader are essential factors. The appearance or colour of the fish is of great importance. Higher standards are used for SPIG fish; it is supposed to be whiter and thicker than the PORT fish. Within the SPIG category are three quality grades, I, II and III:
SPIG I: Fillets should be thick, with light appearance but no defects (PORT AB may have small defects and a slightly darker colour).
SPIG II: Fillets are similar to SPIG I, except that minor defects are allowed.
SPIG III: Fillets are allowed to have more gaping than fish in SPIG II and minor defects due to heading and filleting machines.
Three quality grades are usually used for assessing the PORT fish, A, B and C:
PORT A: Fillets that are light in colour, thick, without blood stains; only minor gaping is allowed (gaping may appear as openings or ruptures between the myotomes because of the weakening of the connective tissue).
PORT B: Fillets that are not of grade A quality because of small defects. The colour is darker and the fillets are thinner or with long gaps along the fillets.
PORT C: Fillets that have quality defects, like gaping or other apparent mechanical defects in the fish flesh. The colour of the fillets is too dark to be graded as B quality. Fillets that have been washed due to slight red discoloration of the fillets (caused by the growth of halophilic bacteria).
PORT AB: Fillets of A and B grades can be packaged together. The maximum ratio of B fillets is 50% in each unit.
PORT CD and E: Grades D and E are sometimes also used. Then C and D fillets are packaged together. Fillets of grade D are of lower quality than C due to stronger gaping, discoloration or other visible defects. Fillets of grade E have great defects but are yet suitable for human consumption. The parts of the fillets with visible defects can be cut away.
Fillets that have yellow staining, which is caused by metal-catalysed oxidation of fat, must be sorted from fillets of other quality grades. The metals, such as copper and iron, are present as impurities in the salt used (Anonymous, 1990).

4.3.10 Sensory analysis

The procedure of sensory analysis and results from the first trial are discussed in Paper I. Results from other trials will not be presented in this thesis. The method used was mainly Quantitative Descriptive Analysis (QDA), introduced by Stone and Sidel (1985). This is a method that is useful for defining and evaluating organoleptic characteristics of salted fish. In this study, the following 29 attributes evaluated were related to appearance (light/dark colour, heterogeneity, flakiness), odour (characteristic, sweet/boiled milk, sea, butter, earth/boiled potatoes, tablecloth, sour, chlorine, TMA, sulphur), flavour (ripening, salt, sweet, sea-like, butter, earth, sour, TMA, pungent, chlorine, frozen storage) and texture (softness, juiciness, tenderness, rubber, clammy) (Magnusson, Sveinsdottir, Lauzon, Thorkelsdottir & Martinsdottir, 2006).

4.3.11 Electrophoresis (SDS-PAGE)

Polyacrylamide gel electrophoresis (PAGE) is a technique in which proteins can be separated in an acrylamide gel in relation to their charge and size. When a surface active agent, like sodium dodecyl sulfate (SDS), is added to the sample, it acts as a denaturant and a solubilising agent, unfolding the peptide chains and converting them to a rod-like shape. The SDS binds to the proteins and cancels out the intrinsic charge of the proteins giving them the charge of SDS (Pomeranz and Meloan, 1987). The separation by electrophoresis in the SDS-PAGE system will then only be based on molecular weight but not charge. SDS-PAGE can be used both as a qualitative and quantitative method in the identification of different proteins. SDS-PAGE has been used for identification of different muscle proteins and their subunits in fresh muscle and also to estimate the effects of storage and processing on the stability of proteins (Bechtel and Parrish, 1983, Garcie, Diez and Zumalacarregue, 1997).

4.3.12 Differential scanning calorimetry (DSC)

Differential scanning calorimeter (DSC) is a powerful technique available for studying the thermodynamics of protein stability and can provide a basic understanding of protein denaturation under various processing conditions. Proteins may be analysed in situ without solubilisation of the muscle tissue. Hastings, Rodger, Park, Matthews and Anderson (1985) studied the stability of muscle proteins using DSC. The myosin molecule was found to be unstable and easily and irreversibly denaturated during both handling and processing. Actin, on the other hand, was more stable, and its denaturation was largely reversible except when denaturation was conducted with heat. Fish muscle proteins are less stable than mammalian proteins against physical processes such as freezing and frozen storage. Changes in protein structure during DSC analysis have often been referred to as “transition” changes, with the basic DSC profile of muscle proteins based on a pattern representing myosin, actin, sarcoplasmic proteins and collagen peaks (Figure 37).

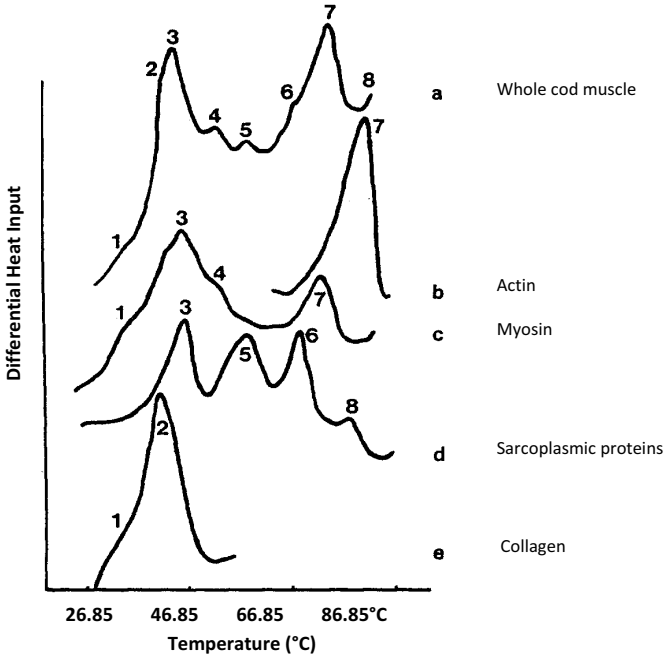


Figure 37. DSC thermograms of cod proteins (temperature scale converted from °K to °C) (Hastings et al., 1985).

One or more transitions were displayed for myosin, which is a multi-domain protein, depending on external factors such as pH and ionic strength (Hastings, et al., 1985; Wright et al., 1977). Collagen transitions in fresh cod appear as a strong peak (no. 2) at approximately 40°C and a smaller one at ~30°C (the shoulder no. 1) (Hastings, Rodger, Park, Matthews & Anderson, 1985). DSC patterns of myosin depend on the macrostructure of the myosin molecule. Formation of filaments, similar to the thick filaments in muscle, lends extra stability to a particular region of the myosin molecule and results in higher transition temperatures (Wright et al., 1977), whereas purification may lead to decreased heat stability. By comparing the thermograms of myosin with those of isolated myosin sub-fragment, Wright and Wilding (1984) were able to demonstrate that the three major transitions associated with the thermal denaturation of rabbit myosin could be assigned to different regions of the myosin molecule: the helical tail, the hinge-region and the globular heads (1, 3 and 7 for myosin in Figure 37).

Addition of salt affects the stability of proteins, and DSC analysis can therefore be used to observe how the protein transitions are displayed in the DSC thermograms (Figure 38). The effects of salt treatment on herring muscle showed that the transition temperatures were decreased by 5-10 K and the

peak areas were also decreased by the salt addition (Hastings et al., 1985). Dialysis results only in partial recovery of the peak areas and restoration of the original transition temperatures.

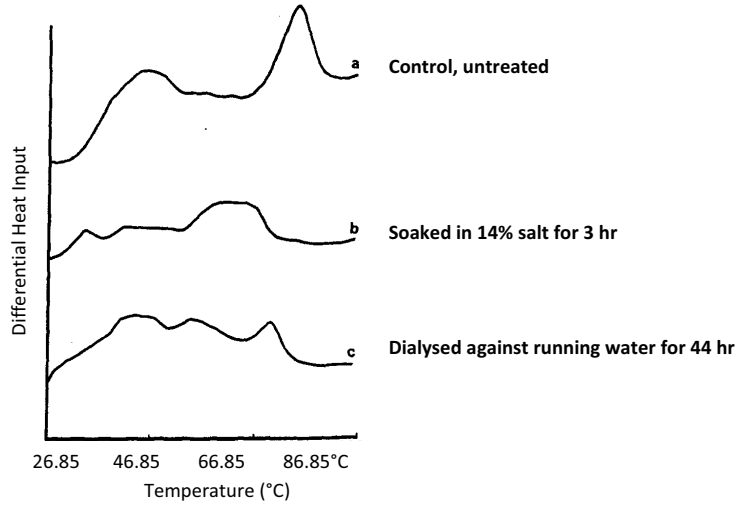


Figure 38. DSC thermograms of herring muscle (Hastings et al., 1985).

4.3.13 Microstructural analysis

Light microscopy and image analysing techniques have been used to study effects of process and light salting on the microstructure in meat and fish products (Aursand, Veliyulin, Böcker, Ofstad, Rustad & Erikson, 2009; Böcker, Ofstad, Bertram, Egelandsdal & Kohler, 2006; Sigurgisladottir, Ingvarsdottir, Torrissen, Cardinal & Hafsteinsson, 2000; Sigurgisladottir, Sigurdardottir, Torrissen, Vallet & Hafsteinsson, 2000; Sigurgísladóttir, Sigurðardóttir, Ingvarsdóttir, Torrissen & Hafsteinsson, 2001; Straadt, Rasmussen, Andersen & Bertram, 2007). The technique provides valuable indications about the location of water in the muscle, expressed by the ratio of intercellular spaces and cross-sectional area of muscle fibres.

The disadvantage of the method is that differences in sampling location on fillet and analysis samples can easily cause false variation. In addition, the biological variation between individuals is high. In this study, only a limited number of samples were taken. Therefore these results should only be considered as preliminary. Paired comparison was used to reduce effects of natural variation in the fish: the left fillet from the fish was used for injection, whereas the right one was measured in its fresh stage as a reference fillet. Paired comparison was also carried out at different stages of the process to evaluate the effect of injection. Finally, the microstructure of dry-salted fillets to fresh fillets was also compared.

4.3.14 Rheological properties

An oscillatory test was used in this study to provide information about differences in rheological properties of the salted cod with regard to injection in the pre-salting step. The obtained parameters in the oscillation measurements were the storage or elastic modulus (G′), the viscous modulus (G″) and phase shift in degrees (PD). G′ represents the energy stored and subsequently released per cycle of deformation per unit volume. It is stored in the elastic bounds of the sample and gives information about the amount of structure present in a material. It is the ratio of in-phase stress to strain. G″ represents the viscous part or the amount of energy dissipated as heat in the sample per cycle of deformation per unit volume. It is the ratio of out-of-phase stress to strain. Storage and loss modulus are connected by the following equation: tan δ (PD) = G''/G'. If G' is higher than the loss or viscous modulus (G″), the material can be regarded as mainly elastic (δ < 45°), and when δ = 0° the material is perfectly elastic. A material is equally elastic and viscous at δ = 45° and perfectly viscous if δ = 90°. The relative amounts of elastic and viscous behaviour may vary with frequency and can therefore be used to determine at which time scales a material will react more as an elastic body and when it is more viscous.

4.3.15 Rheological measurement

In this study, oscillation stress sweep measurements were used to evaluate the rheologcial properties of heavy salted cod, with a StressTech rheometer (Rheologia Insturments, Lund, Sweden). The oscillation was performed at a constant frequency of 1 Hz, increasing the stress from 1-1000 Pa in 35 logarithmic steps. It was not possible to run the measurements up to 1000 Pa on all samples, due to slipping, i.e. for many samples the probe started to rotate with increasing stress, but the exact value where this was observed varied between samples.

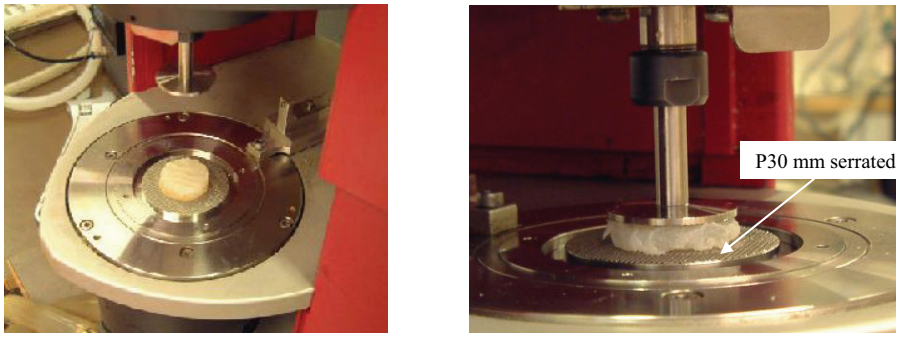


Figure 39. Measurements on salted fish samples in a StressTech rheometer

The rotor or probe used was “P30 mm serrated” (moving surface). The samples were placed on a plate (stationary surface) at room temperature (21°C) (Figure 39). The samples were cut out of the skinned fillets using a sharp cylindrical “knife” to obtain round samples with a diameter of 30mm. These samples were sliced horizontally to obtain 2-3 samples with a height of 5 mm (Figure 40 and Figure 41). Samples from two fillets were used to evaluate the effects of sampling position on salted fish (before rehydration), both with regard to position (A, C, D, and E) on the fillets and different layers, i.e. next to the skin (S), middle layer (M) and next to surface (Y). Samples C, D and E were collected and analysed 4 days later than A. The slicing of the samples was parallel to the direction of fibres in the muscle, increasing the risk of slipping during measurements, as opposed to slicing the samples perpendicular to the fibres. However, the latter was not possible since the fillets were not thick enough.

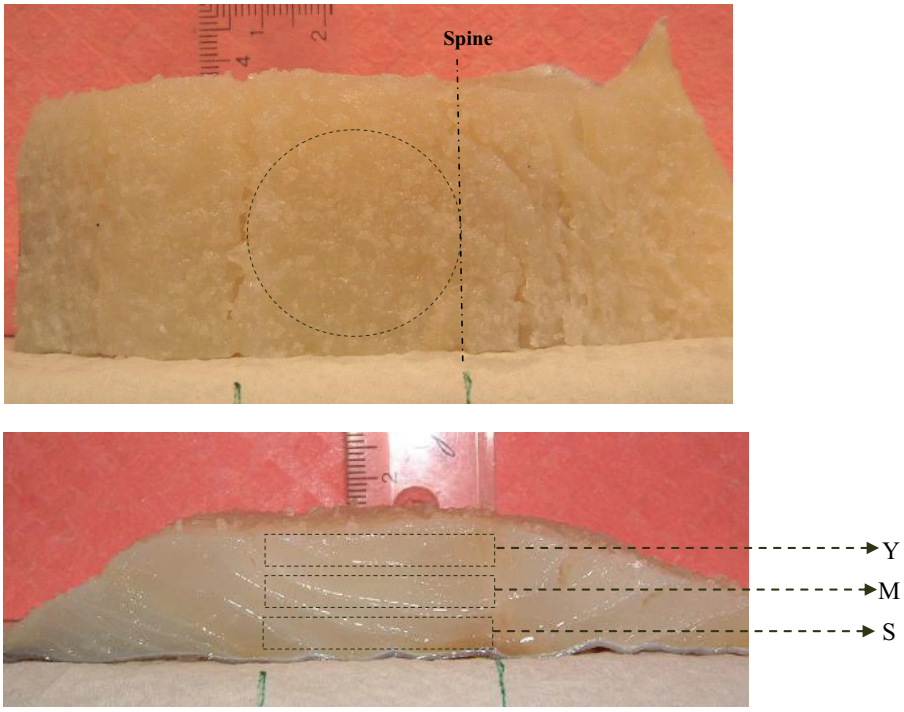


Figure 40. Sampling position on fillet shown on salted fish. Samples were collected from 2-3 layers (S, M, Y) after skinning.

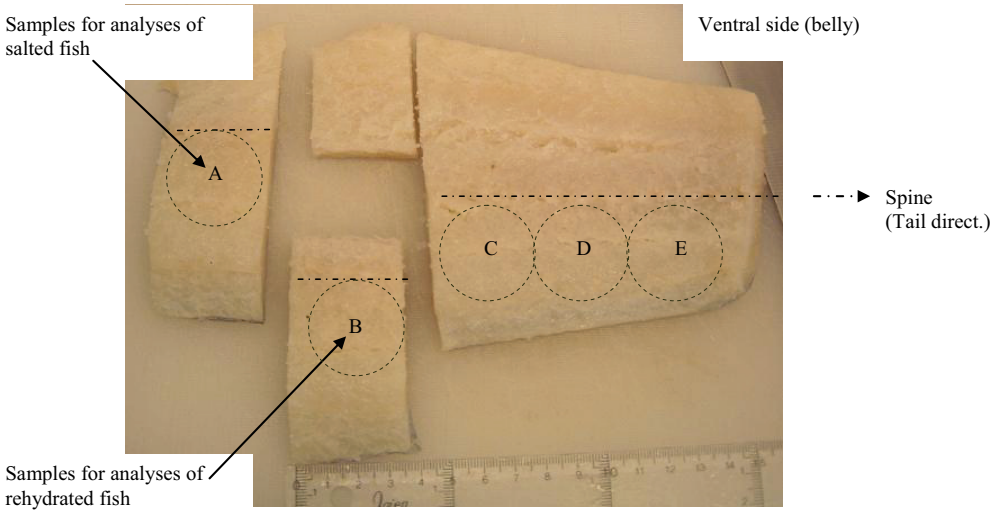


Figure 41. Sampling from heavy salted cod fillets, where the rheological properties were measured before (A) and after rehydration (B). Additionally, samples C-E were collected to test the effects of position on rheological properties (results for C-E are not reported).

4.4 Statistical analysis

Statistical analysis was carried out by Microsoft Excel 8.00 (Microsoft Inc., Redmond, California, U.S.A.) and NCSS 2000 (NCSS, Kaysville, Utah, U.S.A.). Regression statistics: correlation coefficient (r), degree of explanation (r^2) and p-values between different variables were calculated. Comparison of different variables with regard to salting procedures was carried out by Anova and Duncan's test. Multivariate analysis of the data (principal component analysis (PCA)) was conducted with the statistical program Unscrambler (Version 7.5, CAMO ASA, Oslo, Norway). However, the results did not add to the results presented in figures and tables with statistics from other tests. Therefore the PCA plots are not presented in papers or in the thesis.

5 Results and Discussion

The characteristics of salted cod fillets were significantly affected by the different salting procedures applied. Injected fillets were distinct from the others throughout the entire process despite the salt saturation of the liquid phase (LP) of the muscle. Therefore, it was suggested that the pathway of protein solubilisation, denaturation and aggregation was the main reason for the differences obtained in water retention and weight yields.

5.1 Weight yields

5.1.1 The effect of injection as a pre-salting step

The highest weight yields were obtained by the use of a pre-salting step, which involved both injection and brining (Figure 42). The second highest yield was obtained by brining only. Pickling did not improve yields compared to solely dry salting (kenching) the fillets.

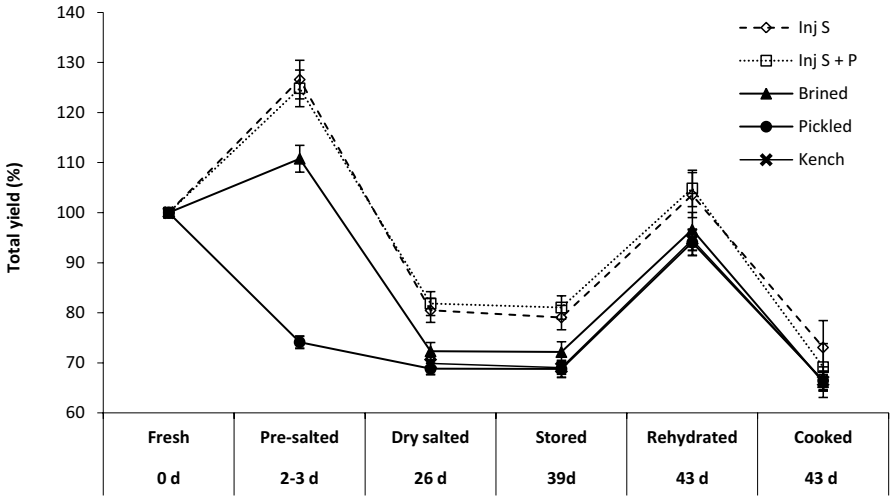


Figure 42. Changes in weight yield during heavy salting, rehydration and cooking of cod fillets. The fillets were stored dry salted for two weeks before rehydration; the total process took 43 days (ave ± stdev; n = 37-40 during salting, 13-23 during rehydration, 3 for cooking) (Paper IV).

The weight gained as a result of injection was relatively well maintained throughout the entire process (Figure 42). During dry salting, the weight losses in injected and brined fillets were not higher than in fillets that were only brined during the pre-salting step (Table 14). However, after cooking, the yield of injected fillets was slightly higher ($p>0.05$) than that of other groups, 69% versus 66%, respectively. It is also important to consider weight changes obtained in different parts of the process.

Usually, the products are exported as salted, and rehydration is carried out by retailers or consumers. Therefore, it is important for producers to keep in mind that higher weight yields after salting often lead to lower increases in weight during rehydration. This was confirmed in our experiment.

Table 14. Weight changes (during separate processing steps during heavy salting and rehydration of cod fillets (ave ± stdev; n = 37-40 during salting, 13-23 during storage and rehydration, 3 for cooking).

Group	Pre-salting (%)	Dry salting (%)	Storage (%)	Total salted (%)	Rehydra-tion yield (%)	Cooking yield (%)	Total rehydr. and cook. (%)
Inj S	$27^{a} \pm 4$	$-36^{a} \pm 2$	$-1.1^{a} \pm 1.6$	-21 ± 1	$31^{a} \pm 3$	$-35^{a} \pm 2$	$21^{a, b} \pm 2$
Inj S + P	$25^{b} \pm 4$	$-34^{b} \pm 2$	$-1.0^{a} \pm 1.7$	-19 ± 2	$29^{a} \pm 2$	$-34^{a, b} \pm 3$	$20^{a} \pm 3$
Brined	$11^{c} \pm 3$	$-35^{b} \pm 1$	$-0.3^{a} \pm 1.6$	-28 ± 2	$34^{b} \pm 3$	$-34^{a, b} \pm 1$	$23^{a, b, c} \pm 1$
Pickled	$-26^{d} \pm 1$	$-7^{c} \pm 2$	$-0.8^{a} \pm 1.4$	$-31^{a} \pm 1$	$37^{c} \pm 3$	$-29^{b} \pm 3$	$24^{b, c} \pm 3$
Kench		$-30^{d} \pm 1$	$-1.4^{a} \pm 1.2$	$-31^{a} \pm 1$	$37^{c} \pm 2$	$-30^{b} \pm 1$	$25^{c} \pm 1$

[a-d] Sharing the same lower-case letter in each column indicates the results were not significantly different (p> 0.05).

A similar tendency was observed for the cooking yield. The total weight changes during rehydration and cooking were negatively correlated to the salting yield (R^2 = 0.92; p = 0.01). Reduction in rehydration and cooking yields may lead to a negative experience for the end consumer. On the other hand, changes in salting procedures have improved the quality of the products, which counteracts the lower rehydration yields.

5.1.2 The effect of brine concentration

The salt concentration of the brine influenced the weight changes during brining (Paper III). An inverse relationship (R = -0.9, R^2 = 0.8; p = 0.29) was found between brine concentration (17.5%, 22.5% and 25.6%) and weight yields after brining (108%, 106%, and 99%, respectively. This was in harmony with previous studies on meat and fish (Andres et al., 2005b; Barat et al., 2002; Gallart-Jornet et al., 2007a; Gallart-Jornet et al., 2007b; Larsen & Elvevolla, 2008; Schmidt et al., 2008). However, the weight gained by the use of lower salt concentrations did not improve the final weight yield after dry salting and rehydration. Even when starting with brine of 7.5% salt concentration, no significant differences were obtained after brining compared to starting with 17.5% brine (Figure 44). Two procedures were carried out, one in which the brine concentration decreased gradually during brining, the other in which salt was added during brining to compensate for dilution effects from salt uptake and liquid diffusing from the muscle during brining of the fillets (Paper III). It should be noted that the salt content of the fillets after brining was in a critical range (9.7-12.7%) with regard to protein aggregation and loss of water retention of the muscle. Therefore, a more reliable comparison of yields was obtained after further salting and rehydration. This was performed in order to determine whether the lower concentrations would increase weight yields throughout the entire process. That was not the case. Differences seemed to level out during dry salting and were not significant after rehydration. The initial concentration of the brine was assumed to have been too high, resulting in salting-out

effects. To obtain weight gains throughout the entire process, the brine concentration would probably have to be even lower than 7.5%, as presented by Callow (1947). He used 2% brine, which seemed to result in irreversible effects on proteins and maintenance of high weight gains even after reaching high salt concentrations.

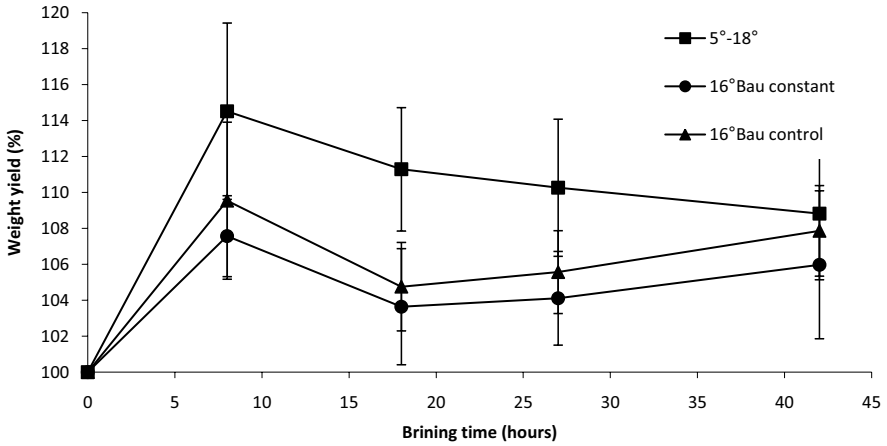


Figure 43. Weight yield (%) of cod fillets (n = 35-46) during brine salting, where the salt concentration of the brine was increased gradually during brining (5-18°); simulated to constant 16° or decreased with time (control 16°) (Paper III).

5.1.3 The use of phosphates

The phosphate blend and dosages used in this study increased the yield of salted but not of rehydrated products (Papers I and IV), possibly due to degradation of the phosphate during the processes and extraction during rehydration. The weight gain was mainly obtained when pre-salting was carried out by brining only (Paper I). The results supported the previous findings of improved yields of salted products by addition of phosphates (Arnesen & Dagbjartsson, 1973; Arnesen & Dagbjartsson, 1974). However, in studies by Arnesen and Dagbjartsson (1973, 1974), the effects on the weight yields of rehydrated products were not evaluated. The use of injection with regard to weight yields was far more effective than the addition of phosphates (Papers I and IV). This was believed to result from the low salt content (2-3%) obtained in the muscle after injection, resulting in salting-in effects and swelling of proteins. Phosphates have been reported to decrease the salt concentration (NaCl) needed to reach maximum swelling, which has been explained by effects on ionic strength. On the other hand, the addition of phosphates is not believed to increase the maximum swelling of the proteins (Paterson, Parrish, Stromer & Stromer, 1988). Therefore, their use can be more effective in light salted (~2%) products, where the salt concentration is limited and below levels to reach the maximum. It should be pointed out that the addition of phosphates in heavy salted products has not been approved, although it is believed to be used in the industry (Lindkvist et al., 2008).

5.2 The flux of different components during processing

During brining, increases in salt content were negatively correlated to changes in water content of the muscle (Paper II), which was in agreement with previous studies (Barat et al., 2003; Crean, 1961a; Duerr et al., 1952; Gallart-Jornet et al., 2007a). The rate of the chemical changes was linear during brining (Figure 44), but at rehydration the chemical fluxes occurred at relatively higher rates during the first hours (Figure 45) (Paper III). The same behaviour has been observed in other studies (Andres et al., 2005a; Barat et al., 2004b; Crean, 1961b).

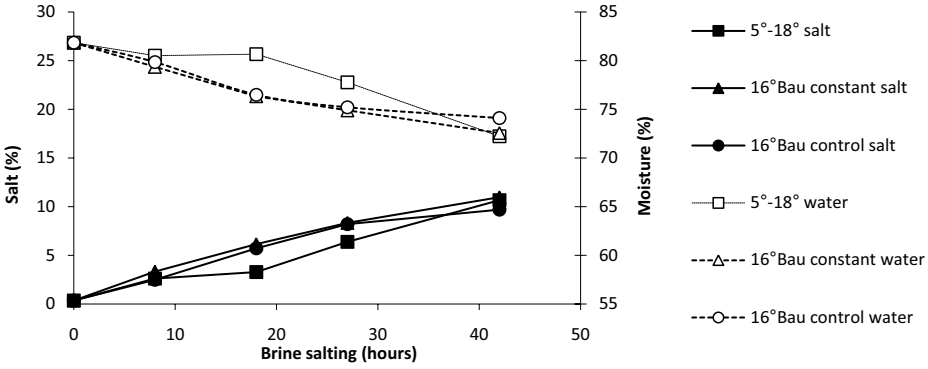


Figure 44. Mean water and salt content in cod fillets (n = 3) during brine salting (Paper III).

One of the reasons for faster changes at the beginning of the rehydration step is that the ratio of water to fish is higher than during brining. Therefore, the initial driving forces originating from concentration gradients were stronger than in the brining process. In addition, the muscle structure of salted products was suggested to swell more easily during rehydration than brining, due to the degradation/solubilisation of swelling constraints during salting.

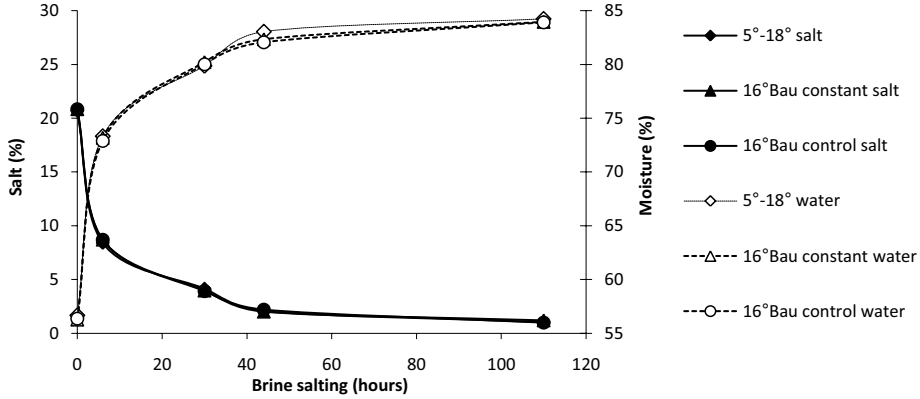


Figure 45. Mean water and salt content in cod fillets (n = 3) during rehydration (Paper III).

In Paper IV, different pre-salting procedures were compared with regard to the salt content in the fillets after the processing steps used: injection and brining, brining, or pickling. The most extreme changes in salt content were obtained by pickling, resulting in a salt content of about 19% (Table 15), which resulted in protein aggregation and dehydration of the muscle. The average salt content in injected and brine salted fillets was slightly higher than the maximum level reported for swelling of muscle proteins (Offer et al., 1983). The salt content after injection alone was not analysed, but was estimated to be in the range of 2-3%, based on the weight uptake by injection and concentration of the brine used for injection. This is near the initial salt concentration used by Callow (1947), where he showed that a substantially higher weight uptake was achieved even after using such high salt concentrations of 20-25% in the brine in the following processing steps. Thus it appears that similar phenomena are achieved when using injection and brining as a pre-salting step. In dry-salted fillets, the salt content exceeded 20%, regardless of the methods applied. During rehydration and desalting, the salt content decreased again to 1.3-1.7%.

Table 15. Salt content (%) as a fraction of weight (x^{NaCl}) of fresh, salted and rehydrated cod fillets. The fillets were stored for two weeks from pile (dry) salting to rehydration; the total process took 43 days.

Group	Pre-salted	Dry salted	Rehydrated
Inj S	6.2	21.4	1.3
Inj S + P	5.8	21.4	1.7
Brined	5.1	20.4	1.4
Pickled	19.2	20.6	1.3
Kench		20.6	1.3

5.2.1 Salt concentration in water phase of the muscle

The salt concentration in the water or liquid phase (LP) of the muscle (z^{NaCl}) was a better indicator of how far salt content in the muscle was from equilibrium with the surrounding brine. However, this variable only included salt and water in the muscle. It did not take into account the proportion of salt that was bound to proteins, or other components soluble in the muscle liquid, like nitrogenous compounds, which were likely to influence concentration gradients. During pre-salting, the z^{NaCl} increased from 0.4% in fresh fillets to 6.0-7.2% in injected and brined fillets and to 24.9% in pickled fillets (Table 16), which was slightly less than salt saturation of brine (26.3% at 0°C). The z^{NaCl} of the brined fillets was lower than the salt concentration in the brine after pre-salting, indicating that equilibrium between the LP and the brine had not been reached. After dry salting, the z^{NaCl} was in the range of 26.1-27.0% in all groups.

The water activity (a_w) also showed that the LP was nearly salt saturated after pickling and that changes during the following dry-salting step were relatively small (Table 16). On the other hand, a_w of brined fillets (0.98) was similar to that of the fresh fillets (0.99). It was only slightly decreased

(0.95-0.96) by adding injection prior to brining. After dry salting, the water activity was in the range of 0.65-0.72, which was slightly lower than values reported for saturated brine (0.75) (Doe et al., 1982). These results show that the increase in water content in injected fillets was accompanied by higher salt content, and therefore the shelf life of injected fillets should be comparable to those produced through other methods.

Table 16. Salt concentration (%) in the liquid phase (z^{NaCl}) and water activity (a_w) of salted and rehydrated cod fillets. The fillets were stored for two weeks from pile (dry) salting to rehydration; the total process took 43 days (Paper IV).

	z^{NaCl}			a_w		
Group	**Pre-salted**	**Dry salted**	**Rehydrated**	**Pre-salted**	**Dry salted**	**Rehydrated**
Inj S	7.2	26.6	1.5	0.95	0.65	0.97
Inj S + P	6.7	26.5	2.0	0.96	0.69	0.98
Brined	6.0	26.1	1.7	0.98	0.72	0.99
Pickled	24.9	27.0	1.6	0.74	0.72	0.98
Kench		26.8	1.6		0.72	0.99

During rehydration, the fillets were desalted to 1.3-1.7% salt content, and water activity increased in all groups to similar levels as in the fresh fillets. This indicates that the shelf life of desalted products would be relatively short compared to the salted stage.

5.2.2 Phosphate content

Quantitative analysis of phosphates could not be used to verify the presence of the added phosphates in salted cod (Paper I). The polyphosphates were added to the cod muscle by injection and brining (Papers I, IV, V, VI). The phosphate content of the fresh fillets was 4.41 mg P_2O_5/g sample (Paper I) (Table 17). It was significantly increased ($p<0.05$) in the phosphate containing brine. However, during further salting, the difference compared to the control became insignificant. After rehydration, similar amounts of phosphate were observed in both groups ($p>0.05$). This was believed to result from a degradation and extraction of phosphates from the muscle during the process. That is probably the reason for no observed effects of phosphates on weight yields after rehydration.

Table 17. Phosphate content (mg P_2O_5 /g sample) in raw material and cured fillets (Paper I).

	Raw material	After brine salting	After storage	After rehydration
P-test (II)	4.41 ± 0.42	5.98 ± 1.02	4.17 ± 0.40	1.02 ± 0.26
Control (II)	4.41 ± 0.42	3.33 ± 0.86	3.39 ± 0.49	0.99 ± 0.24

5.2.3 Protein content

The main changes in protein content were due to mass transfer of water and salt to/from the muscle and the surrounding brine/salt/rehydration water (Figure 46). The proportion of proteins increased as the muscle was dehydrated during salting but decreased again during rehydration. Losses during the process were mainly due to extraction of non-protein nitrogen from the muscle, as a comparison of TN*6.25 and PN*6.25 demonstrated (Paper IV and Figure 41). Similar values were obtained for both variables after rehydration.

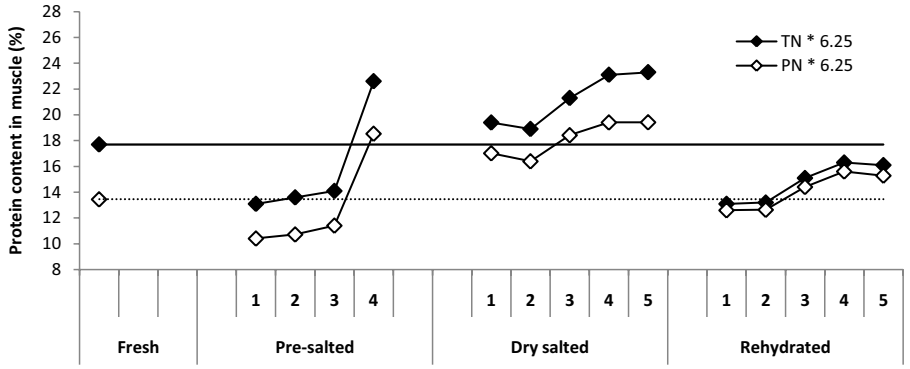


Figure 46. Protein content in the cod fillets at different stages in the process, i.e. in fresh fish, after pre-salting, dry salting and rehydration. It was evaluated as 6.25*total nitrogen (TN) and 6.25*protein nitrogen (PN=TN-NPN). Different salting procedures were used: 1) Inj S, (2) Inj S+P, (3) Brined, (4) Pickled, (5) Kench salted (Paper IV).

The use of different brine concentrations did not seem to influence the proportion of NPN lost either during brining or later in the process (Figure 47). It is notable that the percentage non-protein nitrogen decreased from about 13% in the raw fish to about 2% in the rehydrated fillet.

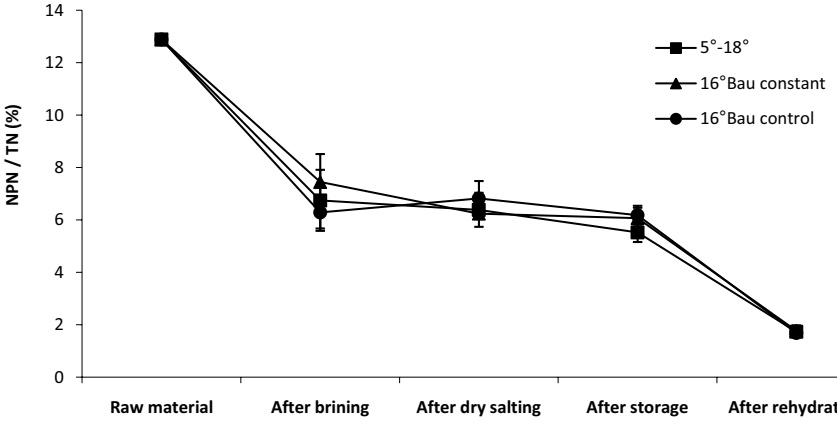


Figure 47. Non protein nitrogen expressed as a ratio of total nitrogen in fresh, salted and rehydrated cod fillets (ave +/-stdev; n=3) (Trial described in paper III).

The PN*6.25 of injected fillets was slightly lower than in fillets processed by other salting procedures (Figure 46). This is partly due to higher water yields and partly due to a stronger tendency towards protein solubilisation and extraction at the lower salt contents in the muscle obtained by injection compared to other methods. This will be discussed further in the following section.

5.3 Yield of chemical components

The water yields were coupled with weight yields (Paper IV) due to the fact that water is the largest proportional component of cod muscle. Quantitative changes in water content were expressed by water yield, i.e. the water content at each stage was compared to the initial amount in fresh fish. Values <100% indicated water loss, values >100% water uptake. The water-holding capacity of the muscle indicated the water retention of the muscle under application of external force at each sampling point.

5.3.1 Water retention of the muscle

The water yield was highest in injected and brine-salted fillets through salting and rehydration (Figure 48). The gain obtained by brining alone compared to pickling or a single dry salting step levelled off during rehydration (Paper IV). Pickling resulted in strong dehydration of the muscle during the pre-salting step, whereas water uptake and weight increase was obtained by injection and brining.

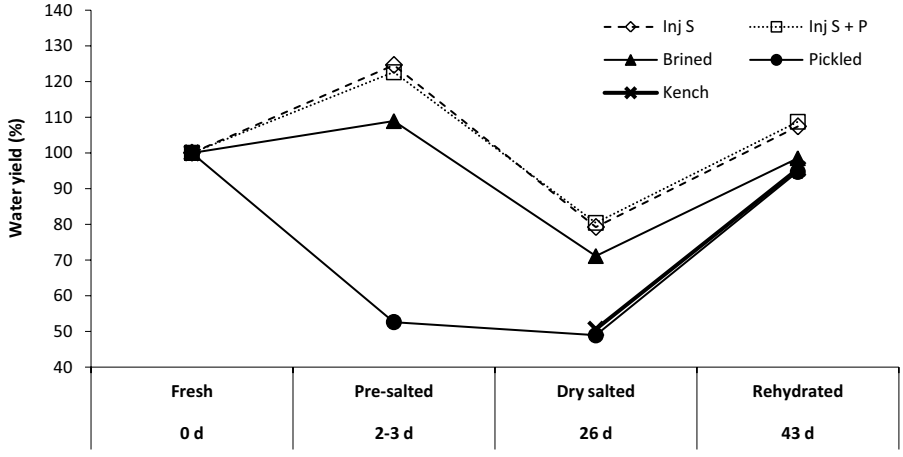


Figure 48. Water yield of cod fillets during salting and after rehydration. The fillets were stored for two weeks from dry salting to rehydration (Paper IV).

The high water retention of the injected fillets could also be seen by the ratio of water to proteins (Figure 49). The results indicated that higher proportions were present in injected and brined fillets after salting and rehydration. After rehydration, the ratio was lower in pickled and kench salted fillets when compared to fresh fish, but slightly higher in injected and brined fillets. The ratio was evaluated with regard to the different calculations of protein content, based on total nitrogen (W/TN*6.25) and nitrogen estimated to be bound in proteins (W/PN*6.25). The differences in W/PR ratios were most pronounced in the fresh fish and after pre-salting, where only 25-30% of the NPN had been extracted from the fillets. The values became more similar during the process due to the increasing losses of NPN with time.

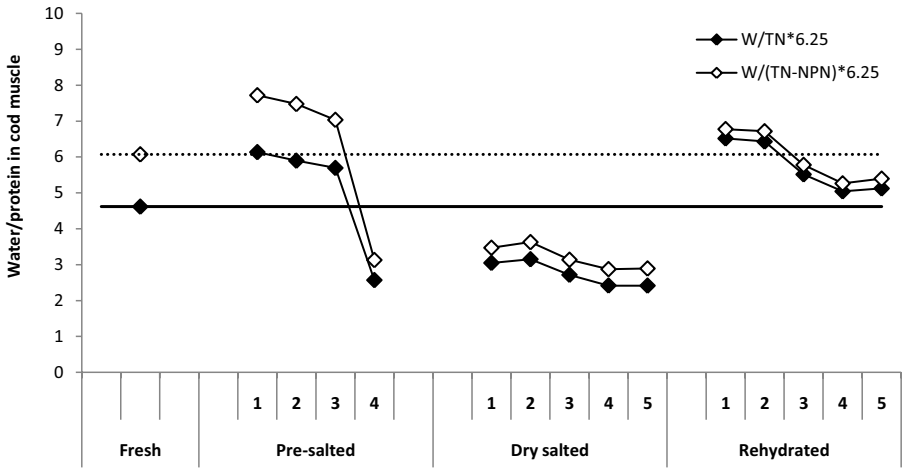


Figure 49. The ratio of water/protein in the cod fillets at different stages in the process, i.e. in fresh fish, after pre-salting, dry salting and rehydration. The protein content was evaluated as 6.25*TN and 6.25*PN. Different salting procedures were used: 1) Inj S, (2) Inj S+P, (3) Brined, (4) Pickled, (5) Kench salted (Paper IV).

The water-holding capacity (WHC) of the dry-salted fillets was inversely related to water yield in the dry-salted fillets. Therefore, a lower water-holding capacity was obtained for injected fillets compared to other salting methods (Figure 50). This indicated that the additional water was relatively loosely bound in the muscle. It was in harmony with microstructural results, which indicated that higher portions of extracellular water were present in the injected fillets compared to brined fillets. The addition of phosphates did not improve the water-holding capacity (Paper I), neither did the different brine concentrations influence the water-holding capacity (Paper III). It was only after brining that lower values were observed for fillets brined at concentration >20% (data not shown).

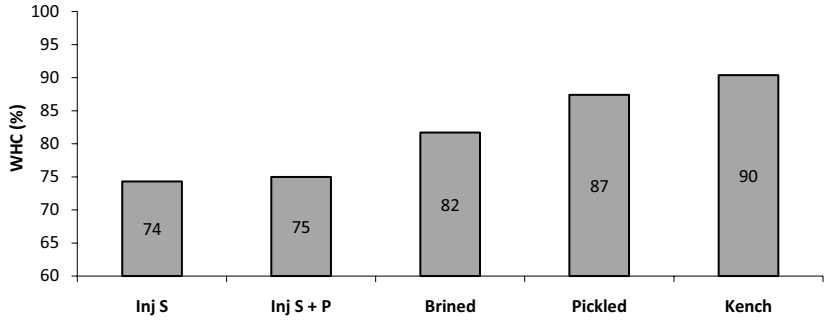


Figure 50. Water-holding capacity of dry-salted cod fillets. The WHC of the fresh fish was 84% (Paper IV).

5.3.2 Yields of protein and non-protein nitrogen

Losses of nitrogenous compounds during the process were mainly due to extraction of non-protein nitrogen (Figure 51) (Papers II and IV). As an example, the NPN yield in the trial described in Paper IV was in the range of 70-85% after pre-salting, 45-64% after dry salting and 12-18% after rehydration. Higher losses were obtained by the wet-salting procedures, especially by injection. The NPN content declined gradually through the process. In the trial described in Paper III, higher losses were obtained during brining and rehydration (Figure 47). This can be explained by different procedures of brining and rehydration between the two trials. In the trial in Paper III, the fillets were weighed several times during brining and rehydration; at the same time, the brine was stirred. This process led to increased mass transfer during the two processing steps. However, the end values in rehydrated products were similar in both cases.

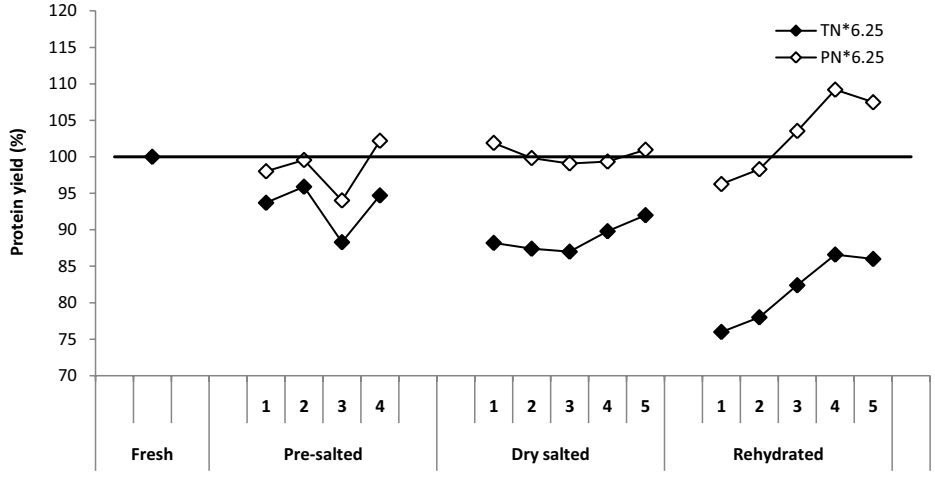


Figure 51. Protein yield in the cod fillets at different stages in the process, i.e. in fresh fish, after pre-salting, dry salting and rehydration. It was evaluated as 6.25*TN and 6.25*PN. Different salting procedures were used: 1) Inj S, (2) Inj S+P, (3) Brined, (4) Pickled, (5) Kench salted (Paper IV).

Overall, higher contents of nitrogenous compounds were extracted by the wet-salting methods (injection and brining), which was in harmony with recent findings in Norwegian studies on different salting methods (Gildberg, Akse & Martinsen, 2009). Higher losses of NPN were supposed to result from higher solubility and extraction of NPN compared to proteins. The stronger swelling of injected fillets and larger intercellular spaces made it easier for smaller components to migrate out of the fillets. Higher losses of proteins may have been related to salt-induced effects on solubility of these proteins, which is higher at low salt concentrations (<1M) (Callow, 1931 (as cited by Lawrie 1998); Callow, 1947; Duerr & Dyer, 1950; Duerr et al., 1952; Stefansson et al., 1994). On the other hand, salting-out effects and aggregation of proteins was obtained by pickle/dry salting methods. According to Martinez-Alvarez and Gomez-Guillen (2005), the myofibrillar proteins (myosin and actin) may have been extracted to some extent as a tendency towards lower yields (PN*6.25), in the injected and brine-salted fillets indicated. When protein yield exceeded 100%, it was supposed to result from individual variation in protein content and weight yields.

5.4 Mass transfer

The mass transfer mechanisms and chemical fluxes in the muscle were markedly influenced by salting procedures. Brining in saturated brine, pickling and kench salting led to strong dehydration of the muscle and a reduction in weight during the first days of salting. On the other hand, injection and brining (<22.5% NaCl) led to weight increases. The mass transfer was explained by gradients between the muscle and the surrounding brine/salt/rehydration water. In addition, mass transfer occurred within the muscle due to gradients between the surface layer and inner parts of the muscle.

The use of injection as a first step in the process resulted in higher uptake of both salt and water during salting (Paper IV). The effects of injection were believed to result from interactions of different factors. First of all, the initiation of salt uptake was distinct from the other methods as described in the review of the literature. From the injection sites, the salt was supposed to diffuse quickly to other parts of the muscle due to concentration gradients, whereas water in the muscle migrated in the opposite direction due to osmotic forces. The increased salt concentration in the middle of the injected muscle reduced the salt concentration gradients between the muscle and the surrounding brine. This decreased the diffusion rate of salt during brining compared to fillets that were only brined.

The forces acting on mass transfer during brining were believed to change with time, from initially being mainly related to concentration gradients between the muscle and the brine, to increasing pressure gradients. The pressure gradients were influenced by salt-induced protein, solubilisation, denaturation and aggregation. In addition, enzymatic degradation of structural components (Stoknes

et al., 2005) may have influenced the degree of swelling and shrinkage within the muscle. During pickling and kench salting, pressure-related mechanisms probably played a larger role than concentration gradients due to the higher concentrations of salt and thereby the stronger aggregation of proteins.

In the trial described in Paper III, in which different brine concentrations were used, the mass transfer was supposed to be related to different gradients during salting. In saturated brine, larger volumes of water were transported from the muscle to the brine due to the osmotic pressure. Salt diffused in the opposite direction due to the differences in salt concentration between the brine and liquid phase in the muscle. Strong salting-out effects on proteins were believed to increase pressure gradients during brining in a manner similar to pickling and kench salting. As myofibrils were salted out, the water was expelled to the extracellular matrix and transported further through the muscle to the surrounding brine/salt.

5.5 The effects of brine concentration on muscle swelling

The relationship between weight yields and z^{NaCl} was observed at different brine concentrations (Figure 52). The weight yields started to drop at approximately 0.5-0.7M salt concentration in the liquid phase of the muscle (Figure 52), which was near the salt levels reported for the maximum swelling of muscle (0.8-1M) (Hamm, 1972; Knight et al., 1988; Offer et al., 1983; Offer, 1988b). It should be pointed out that 0.5-0.7M was the average salt content in the muscle. Expected gradients with regard to thickness were supposed to influence the degree of conformational changes in proteins in different parts of the fillets. In addition, the timing of weighing and sampling may have been prior to/after the maximum swelling of the muscle and highest weight yields.

Injection reduced the effects of thickness of the muscle. The estimated increase in salt content to ~0.3-0.5M obtained by injection alone solubilised proteins and favoured swelling of the myofibrils. Binding of salt ions to the myosin, depolymerisation of the rods, and increased distance between solubilised tails were believed to facilitate salt-protein, rather than protein-protein, interactions (Offer et al., 1983; Offer, 1988a).

In brining alone, the initial salt concentrations influenced the swelling of the muscle, most notably in fillets soaked in 7.5% brine (5°-18°baume), but the brine concentrations were increased during brining by the addition of salt (Figure 52). After the maximum swelling (8 hours), the weight yield decreased as salt was added at each weighing. In the two other groups (16°Bau 17.5%), the muscle did not swell to the same extent. A decline in weight yields above 0.7M was also observed. However, the yields

started to increase again after 18 hours of brining or at approximately 1.2M. These changes may have resulted from salt gradients in the muscle, i.e. the inner parts swelled later causing additional uptake of the brine. Rises in weight yields after a period of decline have also been observed in other studies on cod (Barat et al., 2002), salmon (Gallart-Jornet et al., 2007b) and chicken muscle (Schmidt et al., 2008). This was explained by depolymerisation and swelling of the myofibrils. In salting of pork ham, it has been stated that the diffusion rates decreased during hydration of proteins but increased again as the muscle fibres shrink >1-1.4M (Ockerman, 2003). Ockerman (2003) explained this as a result of changes in extracellular spaces; during swelling of the myofibrils, the diffusion rates in the muscle decreased as the extracellular spaces/channels became narrower. The opposite was believed to occur when salting-out resulted in shrinkage of the muscle fibres. Similar findings have been reported by Schmidt et al. (2008), who further stated that the transfer between hydration and dehydration of the muscle was at 2.2M (13% NaCl).

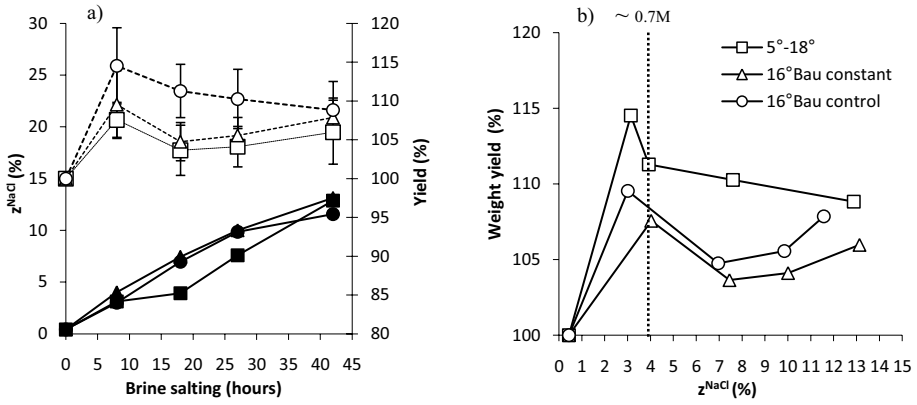


Figure 52. a) Changes in salt content (filled markers) and weight yield (non-filled markers) with brining time. b) The relationship between weight yields and salt content during brining at different brine concentrations. (Data from trial described in Paper III.)

During rehydration, the solubility of the proteins increased with decreasing salt content of the muscle. The protein network expanded again, absorbing water. Results from the rehydration showed rapid changes in weight yields until at approximately 0.8M NaCl (4.7%) in the liquid phase of the muscle (Figure 53). At this concentration, the fibres swelled. However, the conformational changes in muscle proteins were supposed to be different from the brining stage due to irreversible aggregation of the proteins during dry salting. The increase in yield tended to decelerate in the range of 0.8M to approximately 0.4M (2.6%). After 0.4M salt concentration was reached, the weight increased slightly, which was suggested to result from concentration gradients in the muscle. As during brining, the chemical changes were supposed to occur first in the surface layers of the fillets, and at a later stage the inner parts were desalted and rehydrated.

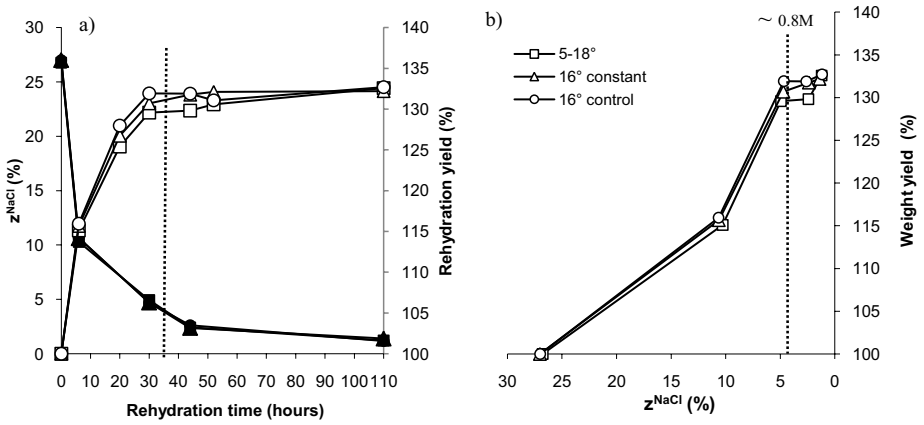


Figure 53. a) Changes in salt content (filled markers) and weight yield (non-filled markers) during rehydration. b) The relationship between weight yields and salt content during brining at different brine concentrations. Dashed lines indicate water changes. The obtained z-value yielded approximately 0.8M NaCl. (Data from trial described in Paper III.)

5.6 Effects of salting procedures on muscle proteins

5.6.1 Myofibrillar proteins

In general, the heavy salting of the cod fillets led to irreversible changes in the myofibrillar proteins, especially in the myosin molecule. Actin was more resistant against increasing salt contents in the muscle. A strong reduction in the myosin heavy chain (MHC) parallel to an increase in 120-140 kDa and 70-80 kDa subfragments was observed. This may have resulted from proteolytic degradation of the rod part of myosin into the HMM and LMM fragments (Paper II). However, another explanation for the observed decrease of MHC is a gross aggregation of myosin when brining and pickling were used as pre-salting methods (Paper V). These aggregates were not solubilised by either the SDS or mercaptoethanol added in the SDS electrophoresis. The degree of aggregation of myosin during salting seemed to increase in the following order with regard to the different pre-salting methods: injection + brining < brining < pickling (Paper V).

Electrophoresis and DSC indicated that the myosin was less aggregated in the injected fillets after pre-salting than by brining only, and this trend was also obtained after rehydration (Paper V). The quick and relatively even increase in the salt content through the whole fillet obtained by injection alone resulted in a relatively low salt concentration below 0.8M, favouring swelling of the myofibrils. Binding of salt ions to the myosin, depolymerisation of the rods, and increased distance between solubilised tails were believed to facilitate salt-protein, rather than protein-protein, interactions (Offer et al., 1983; Offer, 1988a). However, the salt content of brine-salted fillets was lower on average, and

the initial salt concentration of brining was higher for the superficial parts of the fillet, giving rise to a higher degree of myosin aggregation during pre-salting compared to fillets that were injected prior to brining.

During pickling, the proteins were rapidly denaturated and aggregated due to the extreme changes in water and salt content during pre-salting (Paper IV). In addition, the enzymatic degradation was believed to be stimulated by the high initial salt contents (Stoknes et al., 2005). Similar changes were assumed to occur during the first days of kench salting and during the dry salting. The proteins experienced strong salting-out effects, which led to aggregation of the muscle proteins and loss of water retention. The strongest aggregation of proteins during salting was believed to occur in pickled and kench salted fillets, according to the DSC and SDS-PAGE results (Paper V).

Analysis of the rehydrated fillets indicated that the functional characteristics of the myosin rod were better retained when injection and brining were used for pre-salting compared to pickling. The results from electrophoresis also indicated that higher yields of the myosin heavy chain were obtained by injection than brining alone (Papers V and VI).

5.6.2 Collagen

The DSC and microstructural analysis indicated that the connective tissue in cod fillets was affected by the different pre-salting methods and in general by the heavy salting (Papers V and VI). With regard to the DSC-profiles, the main changes were supposed to result from alterations in endo- and perimysium during salting, since the myocommata was separated from the muscle during sampling (Paper V). The two transitions assigned to denaturation of connective tissue were labelled as no. 1 and 2 in the fresh fish (Figure 54). Peak no. 1 has been reported to represent one of the transitions from myosin and collagen (Hastings et al., 1985). In the present study the changes observed in both peaks 1 (33-37°C) and 2 (42.5°C) were assumed to be mainly related to alterations in collagen. Peak no. 2 was markedly reduced during salting, whereas no. 1 could be seen throughout the process, mainly in the groups that were injected and brined.

After rehydration, the enthalpy (peak 1) seemed to be higher in brined muscle, especially in relation to the myosin transitions (Figure 55). This indicated that the collagen was more heat labile in injected and brined muscle compared to only brined fillets. The lower energy needed for denaturation of collagen in injected fillets may indicate stronger degradation of collagen cross-links, favouring stronger swelling of the collagen fibres. Larger intercellular spaces could be related to swelling of collagenous material. This might partly explain the higher water yields obtained by injection (Papers IV and VI).

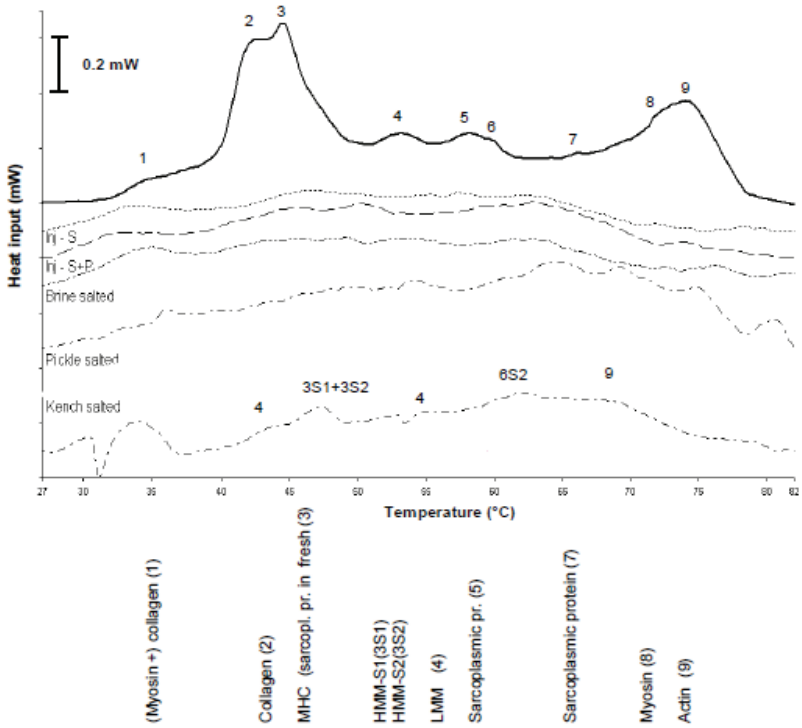


Figure 54. DSC thermograms of rehydrated cod muscle. Different salting methods were applied during the pre-salting step (Paper V). The DSC thermogram of the fresh fillet is inserted at the top of the graph.

The effects of the salt curing on the connective tissue were believed to involve the rupture or weakening of collagen cross-links, possibly altering the stability of the helical structure of the molecule. Salt solubilisation or rupture of cross-links within structural networks and between structural components and muscle cells was believed to cause the greatest differences between the pre-salting methods. Disintegration of the muscle structure started already during postmortem aging of the muscle, including degradation of proteoglycans and glycoproteins. These changes are important for the spatial organization of the collagen fibres and in anchoring cells in the extracellular matrix (Bremner & Hallett, 1985; Ofstad, Olsen, Taylor & Hannesson, 2006). The enzyme activity was supposed to change during the first days of salting, depending on the nature of enzyme but not to be hindered. Indeed, the activity of trypsin, collagenase and elastase has shown to be stimulated during at 14-17% salt concentrations (Stoknes et al., 2005). Therefore, the changes observed may be due to coupled effects of salt and enzyme activity.

A relationship between solubility of fish muscular collagen and pyridinoline concentration has been reported. Pyridinoline forms intra- and/or intermolecular cross-links in the connective tissue and is believed to affect the heat stability of collagen (Ando et al., 2001; Ando et al., 2006). Furthermore, it

has been shown that a lower transition peak (34-35°C) of the calf skin collagen was relatively stable against heating, whereas the second one (39-41°C) was significantly affected (Komsa-Penkova et al., 1996). Increase in salt content (0.5M NaCl) shifted the transition to higher temperatures and lower enthalpy as in the present study. At 1M concentration, only one broad peak with T_{max} at approximately 45°C was observed, indicating salting-out and aggregation of the collagen. Recent studies on solubility of cattle and porcine collagen from tendon and skin support the results of Komsa-Penkova et al. (1996). The maximum solubility was obtained at 1M NaCl or at similar levels as for the myofibrillar proteins. Studies on Nile tilapia collagen extracted under acidic conditions have also showed a relationship between salt concentration and collagen solubility. The solubility remained high at levels up to 2% (0.3M), declined rapidly in the range of 2 to 4% (0.7M), and remained low at levels above 4% (Zeng, Zhang, Lin, Yang, Hong & Jiang, 2009). On the contrary, the effects of heavy salting on collagen in herring skin have been reported to be minor, whereas strong denaturation of actin and myosin was observed, similar to our findings. In untreated muscle, the transition for collagen in skin was located at a similar temperature region (T_{max} 40-50°C) in the transition curves as collagen in red and white muscle (Schubring, 1999).

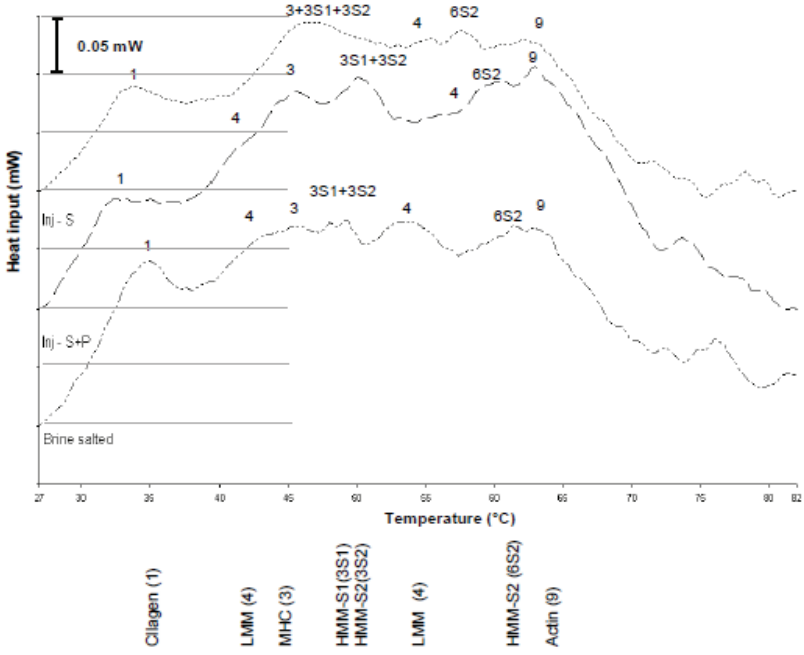


Figure 55. DSC thermograms of rehydrated cod muscle, only groups that were injected and brine salted or only brine salted during the pre-salting step (Paper V).

According to this study, the role of the connective tissue in water retention of the muscle may be underestimated. Possible solubilisation/degradation of collagen with increasing salt concentration of the muscle during pre-salting may play a role in the swelling of the muscle and higher weight yields obtained for the injected muscle. However, the functional properties of connective tissue with regard to different salt treatments in fish have not garnered much interest until now. Further investigations are needed to identify the exact function of collagen in water retention of the muscle.

5.7 Salt-induced changes in microstructure of the muscle

The microstructure of the muscle was significantly affected by the salting process (Figure 56 and Figure 57) (Paper VI). The first sampling was carried out after injection/before brining. Comparison to the paired fresh fillet showed that injection alone led to a slight increase ($p=0.106$) in the intercellular spaces, but the cross-sectional area was less affected ($p=0.326$). After brining, similar intercellular spaces and cross-sectional areas were obtained for injected and brined versus only brined fillets. During dry salting, the cross-sectional area of fibres decreased, whereas the intercellular spaces increased. At this point, the effect of the pre-salting methods (injection + brining versus brining only) was the strongest. The intercellular spaces tended to be larger in injected and brined fillets compared to only brined ones. The opposite was observed for the cross-sectional area of the cells. During rehydration of the fillets, the fibres swelled again up to a similar area as in the fresh fish. Intercellular spaces decreased to some extent but remained larger than in the fresh muscle. After brining and rehydration, similar intercellular spaces were obtained for both salting methods.

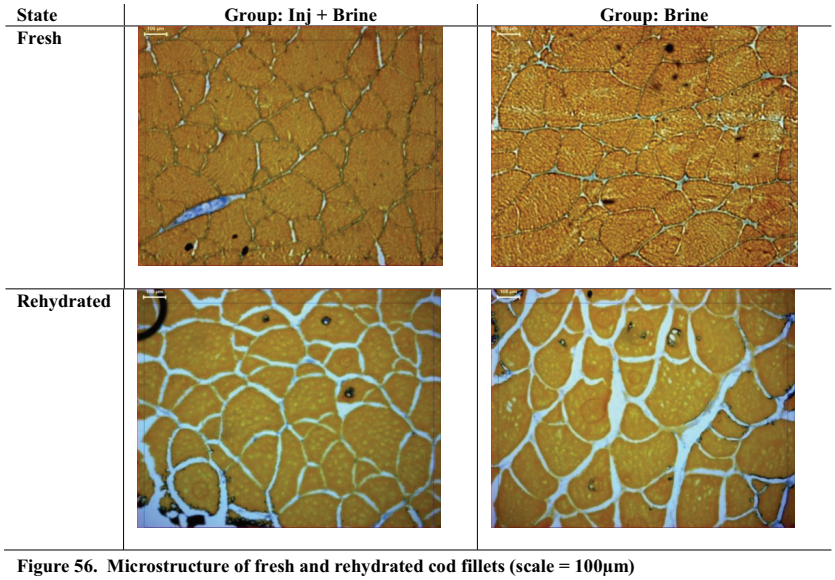


Figure 56. Microstructure of fresh and rehydrated cod fillets (scale = 100µm)

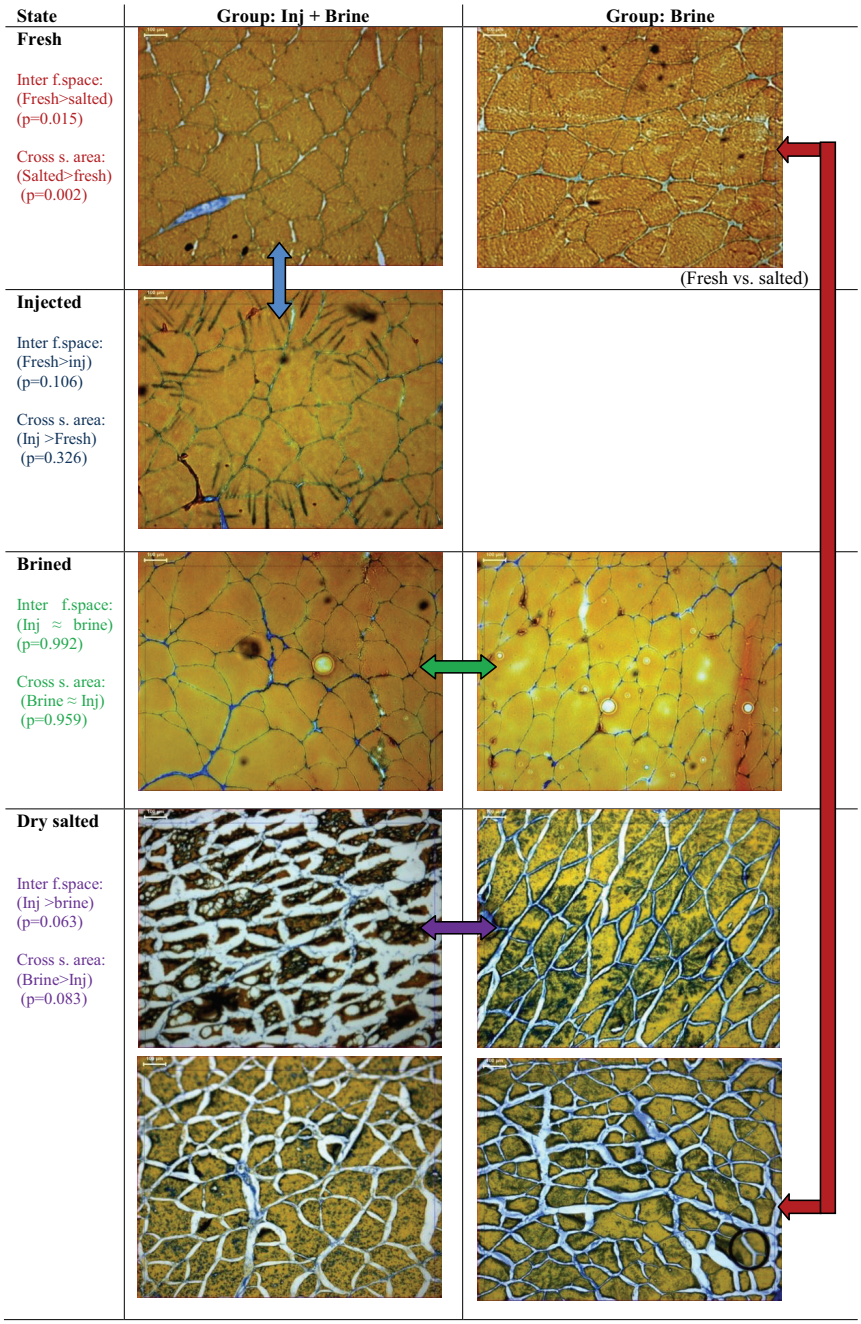


Figure 57. Microstructure of cod fillets, from fresh to salted state (scale = 100µm). Arrows connecting figures represent fillets from the same individual. Values for the paired fillets were compared with a two-tailed t-test and statistical significance is shown on the left side of the figures (3-4 images were used per fillet).

Visual comparison of the microstructural images showed clearly the changes induced by the salting and rehydration process. In addition, the shape of the cells seemed to be irreversibly altered. In the salted products, the connective tissue appeared as fine blue threads "floating" in the intercellular spaces in the salted products. After rehydration, the connective tissue could hardly be detected, indicating the collagen was significantly affected by the salting process.

5.7.1 Rheological properties

The rheological properties were significantly affected by the salting procedures and between the salted and rehydrated stage of the muscle (Paper VI). Statistical analysis of the storage modules (G′) and phase angle (PD) in salted and rehydrated fillets showed that the condition of the fillets (salted versus rehydrated) had the strongest effects, followed by sampling location (skin side versus middle part of fillets) and pre-salting method (brining versus injection and brining). The G′ was higher in salted compared to rehydrated samples, due to aggregation of proteins and shrinkage of muscle fibres (Paper V). The PD was higher for salted compared to rehydrated fillets, indicating that the salted fillets are more brittle even though they have a higher elasticity.

In salted samples, the injection decreased the G′ of the skin layer ($p=0.01$) but effects of pre-salting methods were not significant for the middle layer. During rehydration, the G′ decreased as the muscle swelled and the protein matrix became less compact. The PD decreased at the same time, indicating increasing elasticity of the muscle. The effect of the injection was significant ($p<0.05$) when data from both sampling locations was analysed together, decreasing the G′. When data for the different sampling locations was analysed separately, the influences of injection on G′ in the skin layer ($p<0.1$) and in the middle layer ($p<0.21$) were not as strong. The brine-salted fillets tended to deform at lower stress than the injected ones, and G′ decreased more rapidly, approaching the values obtained for injected fillets at ≥100 Pa. The structure or gel-containing compartments of the muscle seemed to be more brittle compared to injected muscle. This was in harmony with the higher elasticity (lower PD) of the injected fillets both in the skin ($p<0.01$) and the middle layer ($p<0.008$).

During the application of the oscillating stress the strain of different components including the sarcomeric lattice and the network found in the myocommata, perimysium and endomysium, was measured. In addition, muscle fibre-connective tissue junctions were among the factors believed to influence the strain obtained. The direction of the muscle fibres was perpendicular to the myocommata (Figure 58). Therefore, the force acted longitudinally on myofibres, whereas it acted transversely on at least part of the collagen network, and vice versa. This makes interpretation of the data complex, but with aid from the microstructural analysis, DSC and SDS-PAGE (Paper V), an attempt was made to explain to changes observed.

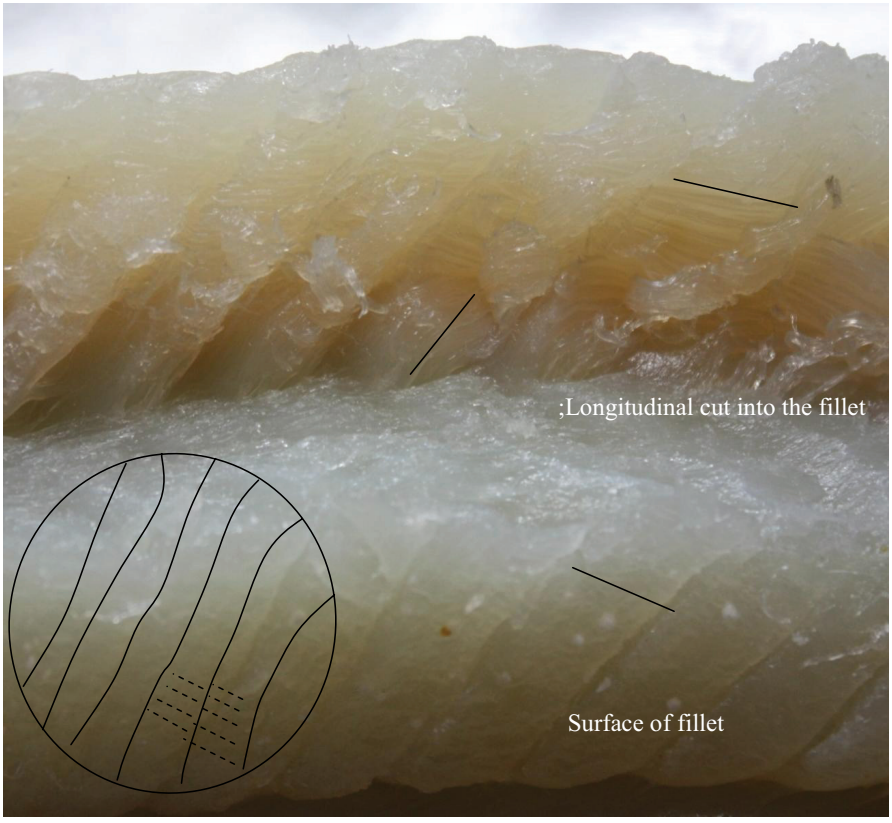


Figure 58. Myotomes (~1-1.5cm thick) in the loin of rehydrated cod fillet; black lines show the direction of muscle fibres. The circle represents a sample used for rheological analysis, collected from the muscle from the loin and the thickest part of the tail. The thickness of the myotomes decreased from head to tail.

The effects of pre-salting methods on G′ and PD were supposed to result from conformational changes in both myofibrillar proteins and structural components like collagen in the connective tissue. The higher G′ of the brined fillets was related to stronger degradation and aggregation of the myofibrillar proteins during salting. In addition, the alterations in the collagen were different compared to injected muscle, as was observed in DSC-analysis (Paper V). The conformational stability of the collagen was relatively stronger in brine-salted fillets during heating. On the other hand, solubilisation of collagen cross-links and stronger swelling during pre-salting was believed to increase the elasticity of the injected fillets. There was less degradation of MHC when the pre-salting step began with injection. Therefore, the functional characteristics of the myosin rod related to gelling and water retention were better retained compared to when only brining was used.

5.8 Commercial quality and sensory analysis

Commercial quality rating was performed after dry salting and storage of the fillets. No significant correlation could be stated between the results from the quality rating and the concentration of brine (Paper III), but it seemed that fillets salted in brine with lower salt concentration (16°Baume) had better appearance. Higher brine concentration (>20%) led to a darker colour of the fillets, which was suggested to result from the strong denaturation and aggregation of the proteins in the surface layer of the fillets. Phosphates have been claimed to improve appearance and thereby the commercial quality of the products. In this study contradictory results were obtained, where phosphates did not improve quality in products described in Paper I but the opposite was observed in the trial described in Paper IV. The condition of the raw material and salting procedures may have influenced the effects of phosphates on the quality. In Paper I, the phosphates were added by brining but by injection in Paper IV. In addition, different phosphate blends were used in the two papers.

In the trial described in Paper I, the phosphate-treated fillets were difficult to classify into specific quality groups and could be described as having a wetter or rawer surface with a white precipitation forming small dots excreting from the flesh, in comparison to the control group. This was presumably due to stronger extraction of proteins from the fillets, which then precipitated on the surface. The proportion of fillets in class A was higher in the control group than in the test group, indicating a negative effect on quality by the polyphosphate. The difference between the test group and the control group was greater in the preliminary experiment, where the concentration of polyphosphate in the brine was 2.5% compared to 2% in the main experiment. Again, this indicated a negative effect of the polyphosphates used. These negative quality attributes noted in the dry-salted fish were not observed in the sensory analysis after cooking.

6 Conclusions

Pre-salting is an effective method of controlling changes in the weight and chemical composition of heavily salted cod fillets. In particular, injection increases both salt and water content in the fillets, thereby increasing weight yields. The weight gain is well retained throughout brining, dry salting and rehydration compared to the other pre-salting methods, brining only and pickling. However, it should be mentioned that the higher curing yields reduced the weight uptake during rehydration. It is important to bear in mind that the different parts of the value chain are performed by different parties (Paper IV).

The brine concentrations (≥20%) influenced weight changes during brining, but no significant differences were observed after dry salting and rehydration (Paper II). However, it is recommended to use a brine of ≤20% due to effects on commercial quality of the fillets. The use of saturated brine resulted in a darker colour of the fillets. Added phosphates in quantities and types used in this study improved yield after salting but not after rehydration. The observed effects on quality were contradictory, having both negative (Paper I) and positive effects (Paper IV). However, the visual decrease in quality manifested by the quality rating was not observed in the sensory attributes after cooking of the salted cod (Paper II). Further studies are needed to evaluate the effects of different types of polyphosphates used at various concentrations.

The salting process significantly decreased the heat stability of both myosin and actin. The protein denaturated at lower temperatures and needed less energy input. However, the decrease in water content during dry salting and storage seemed to shift the transition temperatures slightly back to the higher temperatures. The enthalpy represented by peak areas was significantly smaller after the salting and rehydration steps. The conformational stability of actin and myosin was less than in the fresh material. The actin molecule appeared to be more resistant to the heavy salting according to the SDS-PAGE analysis, although the molecule clearly underwent some conformational changes (Paper III). The myosin heavy chain (MHC) was the most vulnerable to salt-induced changes (Papers III and V). The gradual disappearance of the MHC band observed by SDS-PAGE was accompanied with increases in protein bands that may have represented increasing quantities of HMM and LMM fragments of myosin resulting from proteolytic activity. Another explanation for the decline in MHC is that it may have been salted-out and that the aggregates had molecular weights above the range visible on the gels (Paper V).

The different pre-salting methods affected the structural changes in myofibrillar proteins and collagen. Pickling and kench salting resulted in stronger aggregation of the proteins, whereas injection and brining were milder with regard to these irreversible changes of the proteins. The myosin heavy chain

appeared more on the SDS electrophoresis in injected fillets after rehydration than by other pre-salting methods used, indicating a lesser degree of aggregation even after rehydration. Therefore, the functional characteristics of the myosin rod were believed to be better retained in the injection type of pre-salting. Increases in lower molecular weight components were believed to result from proteolysis during salting.

Salt-induced alterations in the extracellular matrix seem to play a role in water retention and weight yields of heavy salted cod fillets. After dry salting, larger intercellular spaces occurred in injected and brined muscle compared with only brined muscle, but after rehydration this difference had vanished. The differences in microstructural and rheological properties obtained were believed to result from the lower salt concentration in the muscle after injection in comparison with brining only, inducing solubilisation of cross-links in the extracellular matrix and reducing shrinkage of the muscle during further salting. According to this, higher weight yields of salted products are presumed to be related to higher proportions of water in the intercellular spaces of injected muscle.

The drawback of the wet salting methods (injection and brining) has been related to higher losses of dry material. However, the main reduction was due to loss of non-protein nitrogen, although to some extent muscle proteins were extracted during the process (Papers II, IV and V).

7 Further perspectives

The effects of different salting procedures on the commercial quality of salted products and organoleptic properties of rehydrated products are examples of further studies in this area.

The connective tissue and the role of collagenous material is of great interest with regard to the heavy salting processes and should be a subject of a new study in relation to different salt concentrations.

Injection has considerable effects on the water-holding capacity of the muscle due to its advantages in distributing the brine immediately through the muscle. However, further studies of different salt concentrations and effects of different equipment and injection settings may be necessary. The method must be applied carefully, as increased pumping may damage the muscle structure, and there is a great risk of microbial contamination by the recycling of the brine in the equipment.

The use of microstructural analysis and imaging played an important role in this study in allowing visualisation of the effects of different treatments on the muscle. The application of such techniques is recommended in further studies.

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