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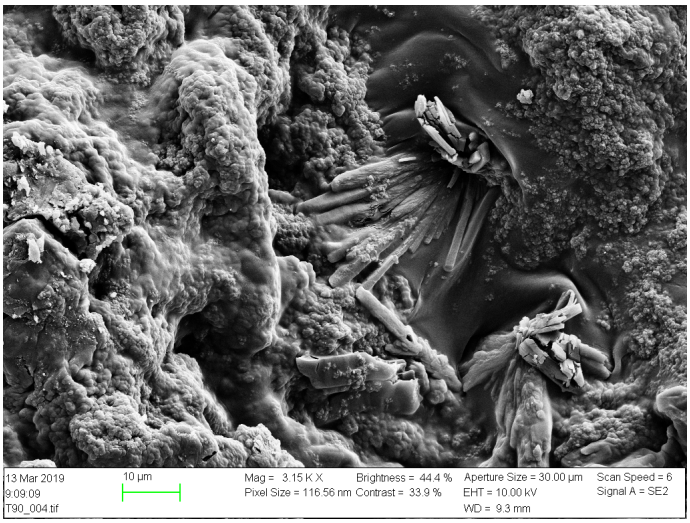
LIQUID LOSSES FROM ATLANTIC COD (*GADUS MORHUA*) DURING HEAT TREATMENT



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on

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Abstract

This thesis concerns a project in cooperation with Nofima, where we consider the mechanisms behind water loss during cooking of Atlantic cod (*Gadus morhua*). The thesis is a closing part of a master program in Biological Chemistry at the University of Stavanger, spring semester of 2019.

During cooking of fish muscle, pressure gradients are formed, and in their presence excess liquid is moved to the surface of the fish, and this expelled liquid is known as cooking loss. In the present study, the cook loss from cod fish muscle was studied as a function of temperature. The comparative study of the cook losses as presented in this thesis yields insight about the content of the liquid which is released and its properties, which in turn can say something about the ability of the cod fish muscle to hold liquid after various heating treatments.

3×2 cm samples from cod loins were vacuum packed together with a glass tube, heat treated in a water bath, and the cook loss was continuously collected in the glass tube. The amount of cook loss collected increased substantially when heating cod muscle at 40 °C and at 70-90 °C. It was observed by scanning electron microscopy that more fibrous proteins were expelled at higher temperatures. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis analysis revealed that these proteins were the myosin heavy chain. Pressure induced water flow experiments were used to obtain the permeability of cod muscle. The permeability was slightly lower at 60 °C and 90°C, compared to the permeability at 40 °C. The decrease in permeability do not correlate well with the substantial increase in water loss at these temperatures.

Thermophysical properties were measured for mass transfer modelling purposes. It was found that the density of the cook loss was larger and that the specific heat capacity was lower, when compared to pure water. The viscosity of the cook losses was found to be complex, but the values were always larger than that of pure water.

The properties that were acquired in this thesis can be further used to improve mathematical models of cooking of cod, which in turn can be used to optimise cooking treatments and prepare healthy fish meals of higher and more stable quality.

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Abbreviations

C_p	Specific heat capacity
CL	Cook loss
CVi	Complex viscosity
DSC	Differential scanning calorimetry
HTP	Heat treatment parameter
kDa	Kilodalton
LHC	Liquid holding capacity
LL	Liquid loss
MF	Myofibrillar
MHC	Myosin heavy chain
MLC	Myosin light chain
Mw	Molecular weight
nm	Nanometer
pI	Isoelectric point
RDE	Residual denaturation enthalpy
SDS-PAGE	Sodium dodecyl sulphate–polyacrylamide gel electrophoresis
SP	Sarcoplasmic
SEM	Scanning electron microscopy
TL	Residual denaturation enthalpy
T_{max}	Transition temperature
μm	Micrometer
WHC	Water holding capacity

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Chapter 1

Introduction

1.1 Cod

Cod is a lean fish containing only about 5% fat (Ofstad et al., 1993). The composition and structure of cod is shown in Figure 1.1. A narrow strip of brown muscle fiber, containing most of the fat, is found along the length, just underneath the cod skin. The remaining muscle fibers are white. These muscle fibers contain about 75% water, 20-22% proteins and ash (Lynum, 1999, pp. 28-30). In the present thesis, the white muscle fibers are analysed, and it has been reported that white muscle alone from cod fish has a uniform composition (Foegeding, Lanier, and Hultin, 1996).

Cod fish, like most fish species, have large coil shaped muscles that extend from the head to the tail. A horizontal connective tissue divides two longitudinal fillets of muscle mass on each side. The muscle mass is divided into muscle segments (myotomas) surrounded by connective tissue membranes (myoseptides). Each muscle segment consists of muscle fascicles made up of dense layers of parallel, thread-like muscle fibers (cells). The muscle fascicles are surrounded by a connective tissue that consists of fiber proteins. The muscle fiber consists of cell juice and repeating units of thick and thin filaments called sarcomeres, that are bundled together to form myofibrils (Provost et al., 2016, p. 303).

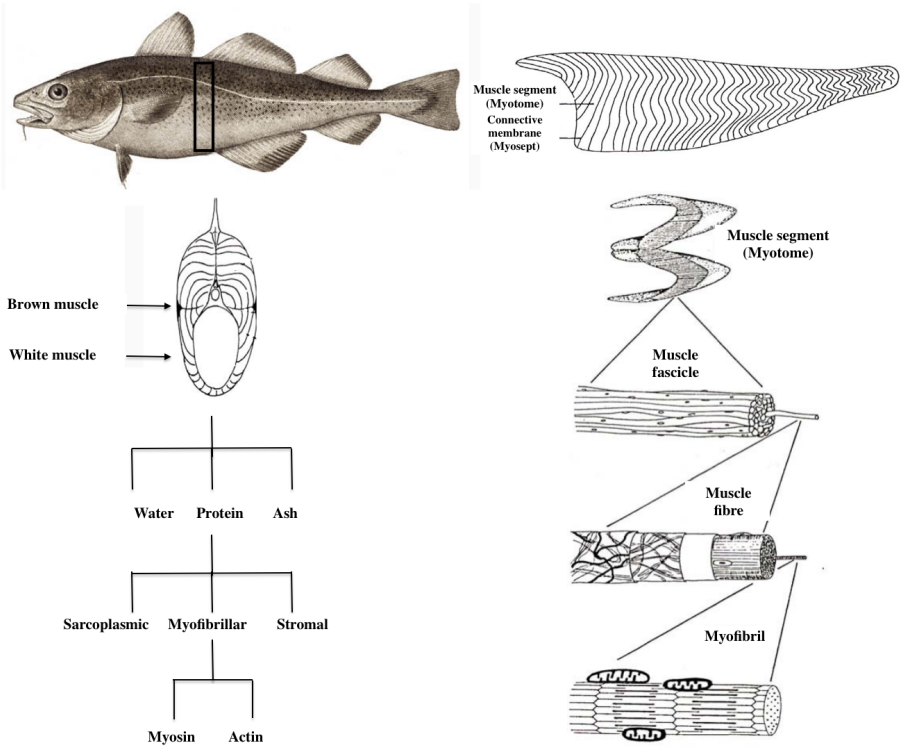


FIGURE 1.1: The composition and structure of cod. Adapted from Lynum (1999).

1.2 Muscle

A muscle is composed of muscle fibers surrounded by a sheath of connective tissue. The muscle fibers are located parallel to one another and are organised into fascicles, which in turn are surrounded by a layer of connective tissue (Provost et al., 2016, p. 278). Collagen is the main structural component of the connective tissue (55–95% of the dry matter content) and is composed of tropocollagen monomers with a molecular weight of 300 kDa (Tornberg, 2005). Collagen is made of three long, insoluble helical protein chains, each wrapped around three other collagen strands to form a triple helix. Fish possess considerably less total collagen and less cross-links than beef or pork (Provost et al., 2016, p. 282). Fish collagen is formed from the same triple helix, however the makeup of some amino acids differs in concentration, and this impacts the stability of the collagen. Collagen is unique among most proteins because in its native state, collagen is an insoluble fibrous protein and when denatured the protein loses some of its three-dimensional structure and becomes water soluble (Provost et al., 2016, p. 300).

The muscle fiber is usually between 20 and 100 μm in diameter and may be several centimeters in length (Provost et al., 2016, p. 275). Each fiber is made up of many smaller membraneless parallel fibers called myofibrils, which are usually 1 to 2 μm in diameter. Myofibrils are surrounded by the sarcoplasm,

which contain soluble, sarcoplasmic proteins. The soluble proteins constitute a very significant portion of the proteins of the cell, usually from 25 to 30% of the total proteins. Most of these soluble proteins are enzymes, principally glycolytic enzymes (Foegeding, Lanier, and Hultin, 1996, p. 897). About 100 different globular proteins are known to be present, having a relatively low molecular weight ranging from 17- 92 kDa (Tornberg, 2005).

1.2.1 Myofibrils and its proteins

The myofibrillar proteins make up more than 55% of the total muscle proteins, in which myosin and actin account for 75-80%(Kristinsson and Hultin, 2003). Thick filaments are approximately 1.5 um in length and contains about 300 molecules of the protein myosin (Murray, 1995, p. 85). In cod muscle, myosin is a hexamer consisting of two 220 kDa heavy chains (MHC), each non-covalently attached to two 18-25 kDa light chains (MLC) (Kristinsson and Hultin, 2003). The two heavy chains form the rod portion and a large part of the myosin head. MLCs bind to the globular region of myosin heavy chain.

Thin filaments are composed of two helical strands of fibrous actin (F-actin), each 1.2 μm long (Murray, 1995, p. 85), formed from longitudinal polymerization of globular actin (G-form, Mw 47 kDa)(Tornberg, 2005). Two strands, which lie in the actin helical grooves, are composed of tropomyosin (Murray, 1995, p. 85). In solution at low ionic strength actin exists in the monomeric globular form. When the ionic strength is raised, the monomers are polymerized into the fibrous structure (Tornberg, 2005)

1.2.2 Characterisation of proteins

Thermal denaturation, which involves the unfolding of proteins, is attributed to the rupture of intermolecular hydrogen bonds and is accompanied by heat uptake, which is seen as an endothermic peak in a differential scanning calorimetry (DSC) curve. During DSC measurements, the denaturation temperature serves as a measure of the thermal stability of a protein, and the enthalpy value, calculated from the endothermic peak, correlates with the net content of the ordered secondary structure of the protein. The heat input and transition temperature (T_{max}) characterise different types of proteins, corresponding to collagen, myosin, sarcoplasmic proteins and actin. The number of denaturation peaks described in the literature for cod proteins range between 3 and 8 when scanning from 0 C to 110 °C ((Hastings et al., 1985; Angsupanich, Edde, and Ledward, 1999; Thorarinsdottir et al., 2002; Skipnes et al., 2008; Bjørnstad, Meling, and Shamirian, 2018). Data from previous studies has indicated that the first peak corresponds to collagen and myosin, the peaks in between correspond to sarcoplasmic proteins and the last peak corresponds to actin. The heating rate is important, as a lower heating rate shifts the (T_{max}) to lower temperatures (Widmann, 2009, p. 77).

1.2.3 Water in the muscle

Water can have different forms in the muscle. The protein-associated water bind water tightly due to charged hydrophilic groups on muscle proteins. This water has very reduced mobility and does not move to other rooms and remains tightly bound even during the application of severe mechanical or physical force such as freezing and heating. This water will be continuously replaced with surrounding water molecules (Pearce et al., 2011).

The free water in the muscle is held only by capillary forces. The free water, about 85-90% of the total water, is located in the space between myosin and actin (Murray, 1995, p. 86). The forces that immobilise 'free water' within the muscle are generated by surface tension. More specifically, the water is held trapped within the muscle by capillary forces (Trout, 1988). The capillary forces are caused by the pores located between myosin and actin, which under normal conditions are approximately 10 nm (Bechtel, 1986, p. 28). 15% of the water is located outside the myofibrillary network (extra myofibrillar). Measuring water holding capacity (WHC) involves the use of force to measure the "free" water in the muscle structure. The contribution of the physically entrapped water to water holding capacity is much larger than those of the bound water (Damodaran, 1996, p. 370). "Protein-associated" water in the muscle is measured through an oven-drying process and the resulting values reflect "total water content" in the muscle (Warner, 2017).

1.3 Heat induced denaturation, shrinkage and water expulsion

The impact of heating cod is the denaturation and unfolding of myosin (Ofstad et al., 1993), which causes loss of structure within the thick and thin myofibril filaments. As myosin denature, the muscle proteins aggregate due to hydrophobic interactions, and the myofibrils shrink (Provost et al., 2016, p. 303). Because these long protein fibers are connected to the muscle fiber wall, the muscle fiber and fascicle shrink laterally and longitudinally (Offer and Cousins, 1992). In addition to heat, lateral shrinkage of the filament lattice can also be brought about by rigor contraction and a pH-fall closer to the isoelectric point (Offer and Knight, 1988). The shrinkage lead to altered distances between muscles fibers as well as the occurrence of pressure gradients(Tornberg, 2005). This squeezes water out of the muscle fiber. The water that is left behind accumulates first around the perimysial network and later around the endomysial network, giving rise to two extracellular water compartments (Offer et al., 1989). Heat also lead to the denaturation, shrinkage and the solubilisation of the connective tissue (Tornberg, 2005). Due to the shape of the long intertwined strands of collagen protein, denaturation causes collagen to shrink. The combination of myosin denaturation and loss of water binding with the shrinkage of collagen wrapped around the muscle fiber results in a loss of juice and an overall shrinkage of the muscle tissue. If proteins are large, they are more likely to be retained in the structure (Provost et al., 2016, p. 300).

According to the (1988) study of Offer and Knight (cited in Tornberg (2005)), the transverse shrinkage to the fibre axis occurs mainly between 40 °C and 60 °C. This widens the gap between the fibres and their surrounding endomysium. Then, between 60 °C and 65 °C, the connective tissue network and the muscle fibres cooperatively shrink longitudinally, and the extent of shrinkage increase with temperature. This shrinkage causes the great water loss that is obtained on cooking. It is then presumed that water is expelled by the pressure exerted by the shrinking connective tissue on the aqueous solution in the extracellular void. As muscle fibers are known to shrink by different degrees at different temperatures (Tornberg, 2005), this indicates that not one, but several proteins are involved in the shrinkage and water expulsion observed during cooking.

Pressure induced denaturation of muscle proteins in cod has been studied by Ofstad et al. (1993) Severe shrinkage of the myofibers occurred at 45 °C corresponding with maximum water loss. The muscle cell shrunk transverse to the fiber axis and intracellular cavities appeared where intracellular material may have leaked. The observed shrinkage at 45 °C was attributed to the denaturation of myosin and the reduced water-holding capacity of the tightly bound myofibrillar proteins. The shrinkage caused the immobilised cellular water to move and flow out at low pressure. At 60 °C detachment of the myofibers from the sarcolemma and some disintegration of the myofilamenteous structure was observed, and coagulated sarcoplasmic proteins could be seen in the extracellular compartment. The amount increased with increasing temperature, which was in accordance with the transition of sarcoplasmic proteins occurring at 45, 57 and 67 °C. At a higher magnification of muscle heated at 70 °C they observed granulated sarcoplasmatic proteins both in the inter- and intracellular space. The water loss decreased concomitantly with the appearance of the interstitial granulated material. They attributed the reason for the reduced water loss to aggregates of sarcoplasmic proteins and collagen, which are able to hold water and/or plug the intercellular capillaries, thus preventing water from being released during centrifugation (Ofstad et al., 1993)

Previous findings indicate that there are other mechanisms, in addition to denaturation of proteins, which cause the cod muscle to release water upon heating. The kinetics of protein denaturation measured by DSC and water loss are different. Skipnes et al. (2008) showed that the denaturation of proteins in cod takes place at lower temperatures (35-66 °C) than the occurrence of larger cooking losses (above 80 °C). With higher temperatures, the ability to hold water (WHC) was also reduced. This indicates the involvement of various processes such as water being expelled due to protein denaturation and shrinkage of muscle fibers on the one hand and improved water binding induced by myosin gelation, sarcoplasmic proteins or collagen on the other hand (Zielbauer et al., 2015). Skipnes et al. (2011) hypothesised that explanations for the loss of water may be based on thermodynamic changes in capillary forces. The water in cod could be expected to be available for expulsion by a weak mechanical force. Such a mechanical force could be due to gradients of temperature, pressure and water concentration.

1.4 Protein solubility

At constant pH and ionic strength, the solubility of most proteins generally increases with temperature. But protein unfolding (denaturation), exposure of nonpolar groups, aggregation, and precipitation, that is, decreased solubility contradicts this (Damodaran, 1996, p. 373). The next step in the structural changes to occur on heating are the protein–protein interactions, resulting in the aggregation of proteins. As Ofstad et al. (1993) did, many researchers have found that most sarcoplasmic proteins aggregate between 40 and 60 °C (Damodaran, 1996, p. 359). Sarcoplasmic globular proteins have been shown to form low quality gel, have low water holding capacity, and to enhance thermal gelation of myofibrillar proteins (Provost et al., 2016, p. 280). The results from Hatae, Yoshimatsu and Matsumoto (1984; 1990) indicated that differences in cook losses between fish species when heated were due to the different amount of sarcoplasmic proteins coagulated in the interstitial spaces and/or differences in the fiber diameter.

Several environmental factors, such as pH, ionic strength, type of salts, temperature, and protein conformation, influence the water binding capacity of proteins. Proteins exhibit the least hydration at their isoelectric pH, where enhanced protein-protein interactions result in minimal interaction with water. The innate ability of fish proteins to form heat-induced gels is optimal near neutral pH (Foegeding, Lanier, and Hultin, 1996, p. 937). Above and below the isoelectric pH, because of the increase in the net charge and repulsive forces, proteins swell and bind more water (Damodaran, 1996, p. 370). If the three-dimensional association of the proteins occurs in such a way that the attractive and the repulsive forces are so well balanced that a three-dimensional network is formed, a gel will set. This gel binds the water in the former solution, mainly by capillary forces (Hermansson, 1986)

The denaturation of myofibrillar proteins in solution usually results in gel formation, because especially myosin is unique in the sense that it form gels at very low concentration of 0.5% by weight (Hermansson and Langton, 1988). For comparison sarcoplasmic proteins need about 3% by weight to gel. When purified myosin is heated, the firmness of the gel reaches its maximum at 45 °C at pH 5.5 or at 60 °C at pH 6 (Sharp and Offer, 1992). If actin is present in the solution, a firmer gel is obtained. At ionic strengths >0.3 and at neutral pH, the myosin molecules are dispersed as monomers, forming a coarse network with large pores. At lower ionic strength the myosin molecules are assembled in filaments, resembling the natural thick filaments in the muscle. During heating a firmer gel is formed, especially if the filaments are very long. Such a gel consists of a finer and more uniform network, with smaller pores (Sharp and Offer, 1992).

1.5 Rheology

Rheology study presents an indirect techniques to study aggregation, as rheological properties of colloidal suspensions depend on the state of aggregation

of the suspended particles. Rheology is the study of flow and deformation of materials. Deformation and flow are referred to as strain or strain rate, respectively, and indicate the distance over which a body moves under the influence of an external force, or stress. For this reason, rheology is also considered to be the study of stress-strain relationships in materials.

A rheometer is a precision instrument that contains the material of interest in a geometric configuration, controls the environment around it, and applies and measures wide ranges of stress, strain, and strain rate (TA instruments, 2017). Viscosity values can be collected either in Controlled rate, CR, or in Controlled stress, CS, mode. In CR mode a deformation rare (shear rate) is imposed and the shear stress is measured. In CS mode a force (shear stress) is applied and the resultant deformation rate is measured. In theory, for materials with no time dependent properties both CS and CR flow curves should yield identical results. CS instruments generally provide good control and measurements of very small deflections, compared to CR (Kealy, 2007).

1.5.1 Non-newtonian behaviour

Flow tests are used to measure a material's "resistance to flow" or viscosity profiles. Most materials are non-Newtonian, i.e. their viscosity depends on the rate of deformation (TA instruments, 2017). For these materials the viscosity is not a single point value, but is represented by a range of values or a curve that can vary many orders of magnitude over a wide range of shear rates. A high zero shear viscosity plateau is linked to a lower infinite shear viscosity plateau by a shear thinning power law region.

Apparent viscosity, η* (Pa·s), is the ratio of shear stress, σ (Pa) and shear strain rate, γ (1/s). By Steffe (1996, p. 15) it has been evaluated (in one dimension) as:

$$\eta = \frac{\sigma}{\gamma} \tag{1.1}$$

Flow curves, i.e., shear stress vs. shear strain rate can be fitted using the Herschel–Bulkley model (Steffe, 1996, p. 20):

$$\sigma = \sigma_0 + K\gamma^n \tag{1.2}$$

where σ_0 is the yield stress, K is the consistency index, and n is the flow behavior index.

For protein solutions the shear rate dependence of the viscosity data are usually fit to the Carreau model, as illustrated by Equation 1.3, chosen for its simplicity to describe Newtonian behavior at low shear rates and power-law shear thinning at high rates (Dharmaraj et al., 2016; Liu et al., 2011). This model contains four parameters: the zero shear viscosity, v_0 and v_∞ is the viscosity at infinite shear rate, K is a characteristic time and n is power-law behavior

index. The parameters within this equation can be modelled using a proper software.

$$\sigma = v_\infty + (v_0 - v_\infty)((1 + K + \gamma)^2)^{(n-1)/2} \tag{1.3}$$

1.5.2 Parameters affecting viscosity

Increasing temperature cause an increase in intermolecular distance and protein-protein hydrophobic interactions, and apparent viscosity usually decreases (Dharmaraj et al., 2016). Yield stress tend to decrease with increasing temperature so long as there is no thermally induced structural enhancement at elevated temperature (TA instruments, 2017). Partial denaturation and/or heat induced polymerization increase the hydrodynamic size of proteins and thus increase the viscosity (Damodaran, 1996). But other factors, like shear rate, protein concentration, and pH may also affect viscosity (Sarangapani et al., 2015).

1.5.3 Viscosity of protein solutions

Recent studies of globular protein solutions have uniformly adopted a colloidal view of proteins as particles. Aqueous suspensions of some proteins are strongly shear thinning and presents viscosity values several times greater than those expected for an equivalent suspension of hard spheres. In its native globular state, charge repulsion interactions within the protein chain can overcome the attractive hydrogen-bonding interactions. Conformational changes can therefore be expected to have great significance on the shear viscosity and other rheological properties of protein solutions (Berli, Deiber, and Añón, 1999).

Colloid particles that are not symmetrically charged produces an appreciable dipole moment (Dharmaraj et al., 2016). Such non-uniformities and other local hydrophobic and van der Waals interactions can cause the particle to have an attractive potential at short range. The significant net charge produces repulsive interaction at long range. For protein dispersions, short-ranged attraction (SA) drives clustering and long-ranged repulsion (LR) limits the local range of association. The interplay between these short and long range forces is common for some proteins and it governs clustering, phase behavior, and solution viscosity. These forces can lead to local concentrations of protein molecules clustered together, sometimes producing interconnected percolated filamentous networks (Godfrin et al., 2014), within intermediate range order (IRO) (Liu et al., 2010b). With such clustering, the solution often becomes more viscous (Yearley et al., 2014).

1.5.4 Viscoelastic properties

Gels are viscoelastic materials. Weak gels are structured fluids, so they flow almost as liquids at large deformations. Therefore, dynamic rheological tests to evaluate properties of gel systems are well suited for studying the gelation behavior. The Cox-Merz rule (Cox and Merz, 1958) states that the apparent viscosity at a specific shear rate γ is equal to the complex viscosity η^* at a specific oscillatory frequency f). When this rule is valid, the rheological food properties can be determined by either oscillatory or steady-state stress experiments, which are useful due to limitations in each kind of the experiment.

1.5.5 Time dependent viscosity

Thixotropy is a relative measure of the extent and speed of recovery of the internal structure during and after shear. It allows an estimate of the effects of eg. cooking for prolonged periods. The theory is that the flow behaviour is defined for a range of shear rates and shear stresses so that its reaction to different processing conditions (cooking etc.) can be predicted (Kealy, 2007). It is likely that the effect of cooking on the viscosity of the liquid is similar to that of draining under gravity, which usually corresponds to a shear rate between 0.1 and 1 $\frac{1}{s}$ (TA instruments, 2017).

The viscosity of thixotropic materials does not follow the same path on structure breakdown as pure shear thinning materials. The terms thixotropy and anti-thixotropy (or rheopexy) are applied to materials which exhibit respectively an increasing or decreasing flowrate with time under a constant stress, which are often interpreted in terms of fracture or formation of a network under shear. An ideal solid shows an elastic response, i.e. an instantaneous deformation which then totally stops. A liquid flows independently of time with a constant flow rate, which may be simply called flow. Viscoelastic materials can show an intermediate response, where the deformation is not independent of time (solids), nor is the shear rate independent of time (liquids) under constant stress. A response where the deformation depends on time less strongly than linearly is sometimes referred to as creep, and can be indicative of different changes in the material, either destructive or formative.

1.6 Flow in porous media

In-situ viscosity has been suggested to describe fluid flow behavior of polymer solutions in porous media. In-situ viscosity is a macroscopic parameter that can be calculated using Darcy's law for single-phase non- Newtonian fluids (Skauge et al., 2018):

$$\mu_{apparent} = \frac{KA}{Q}\frac{\Delta P}{L} \tag{1.4}$$

Most of the models that have been developed are based on analytical solutions of non-Newtonian flow through capillary bundles, which simplifies the complex geometry of porous media.

Analytical solution for a power-law fluid at a given flow rate through a capillary tube with an arbitrary radius (R) can be defined. By comparing this equation with Poiseuille's volumetric flow rate for Newtonian fluids in a tube, an apparent viscosity and shear rate can be obtained. The analytical equation in a single tube can be extended to account for real porous media by using capillary bundle approach. An equivalent radius of capillary bundle model for porous media with known porosity (ϕ), permeability (K) and tortuosity (ψ) can be obtained. By calculating Darcy velocity and substituting equivalent radius into the analytical equation, apparent shear rate as a function of Darcy velocity can be obtained by equation (Skauge et al., 2018):

$$\dot{y} = 4(\frac{3n+1}{4n})^{\frac{n}{n-1}} \frac{U}{\sqrt{8K\phi\psi}} \tag{1.5}$$

A universally accepted model does not yet exist. Insufficiency of these models to predict in-situ viscosity may be attributed to their lack of incorporating time dependence and their use of oversimplified porous media models (e.g., capillary bundle).

1.7 Modelling the cooking of cod

. Blikra (2019) developed a model for heat and mass transfer during cooking of cod. She found that after heat treatment of small samples in a convection oven (150 °C, low relative humidity) between 8 and 10 minutes, the mass fraction of water was predicted lower than the measured trend line, respectively. During this stage of heating, cooking losses were expelled from the sample surface in addition to evaporation. In the model, the boundary conditions for mass transfer contained evaporation, but not mass lost in liquid form as cooking loss. This deviation can be accounted for in the future by adding equations for cooking loss to the model. To be able to model the pressure-driving mass transfer resulting from the shrinkage, the physical properties that must be known are the amount of water and protein available for the transport and the permeability of the fish.

Chapter 2

Objectives

The objective of this master thesis project was to investigate the properties of expelled liquid from Atlantic cod during processing and provide data for the project (Optimal II) which this work was a part of. The data provided from the master thesis should therefore be usable for later improvement of models for cooking of fish and optimization of the cooking process. By providing detailed information on the effect of heat load on muscle proteins, processing can be tailored and optimised to maintain the best quality of the fish product.

Specific goals

- Quantification of cook loss.
- Quantification of water, protein, salt, ash and fat.
- Thermal analysis for determination of specific heat capacity and residual protein denaturation enthalpy.
- Determine the pH, viscosity and density.
- Analysis of individual proteins using SDS-PAGE and scanning electron microscopy.
- Determination of permeability of cod.
- Surface tensile strength measurements between the cook loss and cod.
- Characterize mass transfer mechanisms in cod during heating.

Chapter 3

Materials and Methods

3.1 Cod samples

3.1.1 The cod

Farmed Atlantic cod (*Gadhus morhua*) was used as the main raw material throughout this thesis. The fish was supplied from the aquaculture station Tromsø AS. The fish was starved for 9 days before slaughtering, and killed by a blow to the head. A selection of 139 individuals weighing 2-4 kg were slaughtered, filleted (pre-rigor) and packed December 2017. Post-rigor fillets were cut into loins of 100-150 g and frozen immediately at -60 °C using liquid nitrogen. The loins were subsequently vacuum packed (92.2%) and stored in a freezer set at -80°C up until analysis, done in the time span between August 2018 and May 2019. Detailed information of the raw material can be found in the report of Blikra (2018).

3.1.2 The samples

The liquid that is expelled from a cod fish sample during heat treatment is termed cook loss (CL). CL samples were the main subject for analysis in this thesis. For a quantitative CL characterization the corresponding raw and cooked fish from the same individual was analysed for support. For a qualitative protein characterization, the corresponding thaw loss (TL)[1,2] from the same individual was analysed for support. The TL is defined as the liquid that is expelled from a -30 °C frozen cod fish sample during tempering to 0 °C.

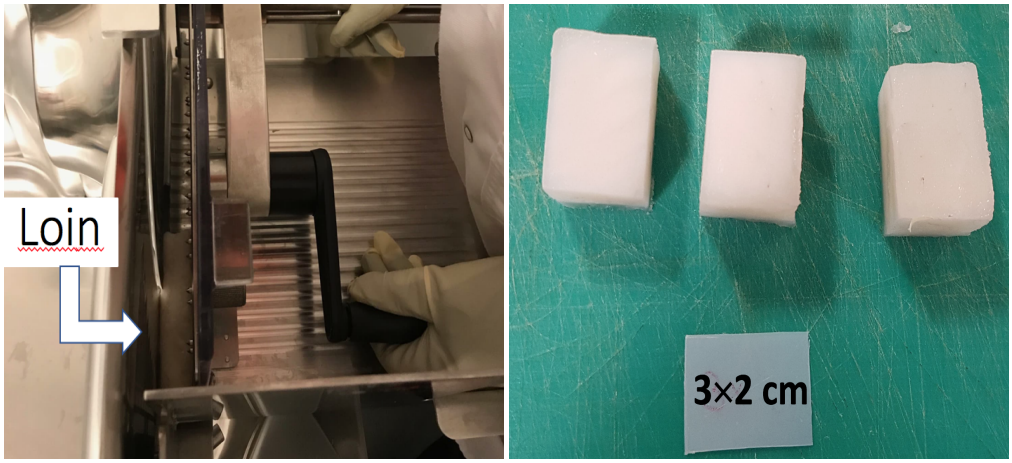
[1] From hereon referred to as TL or 0°C

[2] When describing the TL and CL simultaneously, they are referred to as liquid losses (LLs)

3.2 Sample preparation

3.2.1 Cutting

Before each experiment a selection of loins were tempered overnight in a freezer set at -30°C. The next day the loins were tempered furthermore for 30-60 minutes in a ventilated room set at 0-2 °C. In this room a slicing machine was used to cut away visible brown muscle, blood and gaping (see Figure 3.1 A). Each loin was subsequently cut with a sharp knife into three or four 3×2 cm (length×width) sample pieces (approx.10 g) with a height ranging from 10-17 mm (see Figure 3.1 B). The sample cutting was done quickly in room temperature (∼ 20 °C) using pre-cooled equipment. The samples were tempered furthermore overnight at 0-2 °C in zip lock pouches. The resulting TLs expelled from the samples were stored at 0-2 °C, and analysed within three days.



(A) Removal of brown muscle and gaping from a loin using a sclicing machine.

(B) Three 3×2 cm sample pieces cut from one loin.

FIGURE 3.1: (A) Removal of brown muscle, blood and gaping from a loin, using a slicing machine in a ventilated room set at 0-2 °C, following the 30-60 minutes tempering in the same room, and (B) three sample pieces cut from one loin with the use of a sharp knife and a 3×2 cm template.

3.2.2 Packing

A single cod piece sample was packaged in a 70 µ thick polyamide/polyethylene (20/50) pouch and vacuum-sealed (92.2%) using a vacuum-packing machine, as illustrated in Figure 3.2. Heating rate measurements using temperature loggers in relevant pouches (shrink, zip-lock, and PA/PE pouch) demonstrated the fastest heating rate of the pouch used (n=3, data not shown).

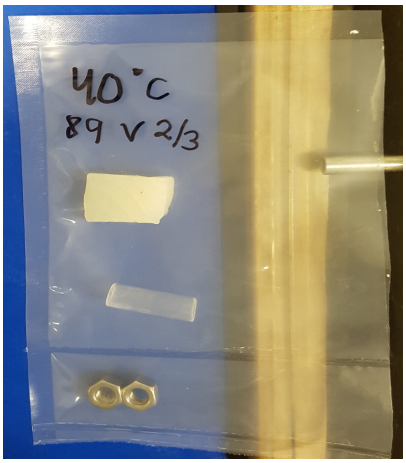


FIGURE 3.2: Vacuum sealing (92.2 %) a 3×2 cm cod loin sample from the side, together with a glass tube. Two metal nuts were sealed at the bottom of each pouch.

Sous vide packaging is designed to keep moisture within food during cooking, as was observed. For this reason, a 5 cm long glass tube (diameter×inner diameter: 1 cm×0.1 cm) was positioned vertically below the sample within the pouch. The glass tube was covered with BriteGuard® to minimize surface tension to the liquid loss. Two metal nuts were sealed at the bottom of each pouch in order to keep the pouches from floating during cooking. This also kept them upright, thus facilitating liquid loss sampling in the tube. The contents were vacuum sealed from the side in order to avoid incidents of the tube sticking to the cod piece sample.

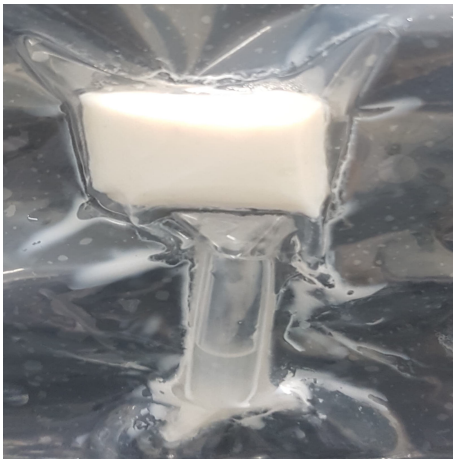
3.2.3 Heat treatment

The packaged samples were cooked in a circulating water bath (Grant, GD120, Cambridge, England), as illustrated in Figure 3.3 A. Up to seven bath temperatures (30, 40, 50, 60, 70, 80 and 90 °C)[3] and a cooking time of 10 minutes were analysed. The variations in bath temperature were measured using an ETS temperature sensor (Ellab, Hillerød, Denmark) for the ten first experiments, and evaluated using the Valsuite Pro Software. The temperature varied at most by 0.05 °C (n=30).

After cooking (see Figure 3.3 B), the samples were cooled immediately in an ice water bath for at least 10 minutes. The metal nuts and the glass tube was removed from the pouch, the pouch was sealed, and the CL was "homogenised" by means of a stomacher bag and by grinding the pouch towards the edge of a table. This was necessary for collecting the greater part of the CL in the pouch. The author is though aware of the uncertainties that follows this sample collection (as will be discussed in Section 4.1.3). The subsequent analyses, described in Section 5.1 to 5.4, were always done within three days after cooking.

[3]From hereon referred to by the common designation heat treatment parameters (HTP)

(A) Three samples were cooked simultaneously. The bath temperature was monitored by a temp. sensor. (±0.05 °C).



(B) The glass tube was designed to lead the expelled liquid away from the cod sample during cooking.

FIGURE 3.3: (A) Cooking of 92.2 % vacuum packed 30×20 mm cod loin samples at the HTPs, whilst measuring bath temperature using a temperature sensor and (B) collecting the CL using a glass tube.

3.2.4 General homogenisation procedure

Fish samples used for pH and quantitative protein analysis, and fish samples analysed by an external laboratory, which includes analysis of fat, total metals, and verification analysis of salt, were homogenised by grinding. The general procedure were as followed:

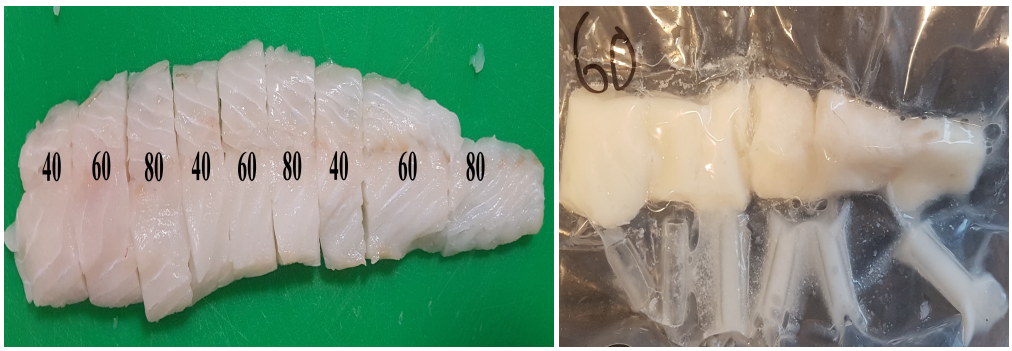
The fish was ground in a tube using an UltraTurrax homogeniser (IKA Werke GmbH, Staufen, Germany) at 1350 rpm/min^{-1} for 10 s. The inner walls of the tube, and the outer walls of the tube mill, were wiped using a spatula, and transferred to the body of the sample. Processing was continued for another 10 s, and wiped as before. The sequence was repeated for a third time.

3.2.5 Sample preparation: permeability

Visible brown muscle from a loin was cut away as before (see Figure 3.1 A). Afterwards, using the slicing machine, the loin was divided into three or four slices with a height of approx. 5 mm. Samples with a diameter of 27 mm were excised by a sharp-edged cylinder and the samples were tempered over night at 0-2 °C. These samples were used in measurements of intrinsic permeability and permeability upon centrifugation, as will be described in Section 3.6.

3.2.6 Sample and sample preparation: rheology

Wild Atlantic cod (*Gadhus morhua*) was used as the raw material for the rheological analyzes. Four skinless/boneless fresh cod loins, each weighing approx. 400 g, were purchased from a local grocery store. The fish was caught by longline fishing in the north-east atlantic by F-162 Båtsfjord, and exported by Nordic Group AS, located in Trondheim. It was filleted and put on ice immediately, until purchased four days later. At the same day and at room temperature, visible brown muscle was removed from the loins with a sharp knife. The loins were further cut into nine pieces, and these were distributed among the HTPs, namely 40, 60 and 80 °C, as illustrated in Figure 3.4 A. Three pieces were taken as one sample, and these were packed together with six glass tubes during cooking, as illustrated in Figure 3.4 B.



(A) Wild cod loin cut in nine pieces, and distributed among three cooking temp.

(B) Six glass tubes were used during cooking of the wild cod samples.

FIGURE 3.4: (A) Nine pieces, cut from one wild cod loin, were distributed among three cooking temperatures; 40, 60 and 80 °C (B) Three pieces were taken as one biological parallel, and cooked together with six glass tubes.

The CLs from the wild cod were analysed at their HTP temperature (eg. the 40 °C CL was analysed at 40 °C). For this reason, the heat treatment times were adjusted. This was done to avoid protein denaturation during the measurements, which could lead to results difficult to interpret. The times were as follows:

- 30-50 °C: 1 hour
- 60 °C: 30 min
- 70-90°C: 15 min

Using farmed Atlantic Cod (see Section 3.1.1), the water content of CLs collected after heat treating for longer times, at the HTPs 30, 50, 60, 70 and 90 °C, were tested. This was done as a basis for comparing the results with the results given for cod piece samples cooked for 10 min. Uncertainty is though connected to the fact that farmed Atlantic Cod was used for this comparative analysis.

3.3 Quantitative analyses

3.3.1 Liquid Losses

Mass changes due to thawing and cooking(g)[4] were calculated using Equation 3.1

$$Mass\ change = W_{before} - W_{after} \tag{3.1}$$

where W_{before} is the weight of the fish sample before thawing or cooking (g) and W_{after} is the weight of the fish sample after thawing or cooking (g). LLs were expressed as a percentage of the initial mass (w/w, wet basis), and evaluated using Equation 3.2

$$LL = \frac{Mass\ change}{W_{before}} \times 100 \tag{3.2}$$

3.3.2 Moisture by oven drying

Relative moisture content was determined gravimetrically following AOAC Official Method 950.46 "Moisture in Meat" (AOAC, 1991), and by utilizing the sand pan technique (ISO, 1999).

Cod piece samples were cooked at all the HTPs ranging from 30-90 °C. Fish samples of about 6-9 g, and total amount of TL and CL retrieved (0.2-1.5 g) from each HTP, were weighed before and after drying at 105 °C for 16-18 hours. A thin layer of pre-dried (105 °C, 1 hr) acid-washed sand (Pro Analysis, Merch KGaA, Darmstadt, Germany) was mixed with the LLs using a pre-dried glass rod. The glass rod was left in the tray during the gravimetric determination.

Moisture content was taken as equivalent to the water content.[5] The water content of the fish before cooking and the fish and LL after thawing or cooking (%) were evaluated using Equation 3.3

$$Water\ content(\%) = \frac{W_{wet} - W_{dried}}{W_{wet}} \times 100\% \tag{3.3}$$

where W_{wet} is the weight of the LL that was obtained for that sample (calculated using Equation 3.2), or the weight of the raw or cooked fish sample that was analysed for content of water(g), and W_{dried} is the weight after oven drying (g).

[4] From hereon used as a synonym for tempering and heat treating

[5] The mass removed also includes other volatile compounds, in the sample or formed during oven drying, as found for fish samples studied by Mota da Silva(2008). The results from the oven drying method is more correctly termed moisture content.

Water losses or water retained by a fish sample, after the LL had left the raw fish sample due to thawing or cooking, were expressed as a percentage of the initial water in the raw fish (w/w, wet basis), and evaluated using Equation 3.4 and Equation 3.5, respectively.

$$Water\ loss\ (g/100g\ raw) = \frac{Water\ content \times Mass\ change}{W_{before}} \tag{3.4}$$

$$Water\ retained\ (g/100g\ raw) = \frac{Water\ content \times W_{after}}{W_{before}} \tag{3.5}$$

in which mass change was calculated using Equation 3.1.

The analysis was carried out on three biological parallels (n=3) of fish and CL from all of the HTPs ranging from 30-90 °C. Fourteen samples of raw fish (n=14) and five samples of TL (n=5) were also analysed. Therefore, three biological parallels or more were used per temperature in calculation of average values and standard deviations(n≥3). In addition, three analytical parallels were used for the TL. The average values of these were used to average each biological parallel.

3.3.3 Protein by kjeldahl

Proximate protein content was measured indirectly, through measurement of the total nitrogen content in a sample, using the Kjeldahl method. Raw and cooked fish were prepared for analysis by homogenizing the 6-9 g samples, as outlined in Section 3.2.4. The fish samples and the corresponding CL samples were transferred to zip-lock pouches, stored in a freezer set at - 80°C, and analysed within 7 days. Before analysis the frozen samples were quickly thawed in a bucket of water and ice, under the drip of water.

Protein content was determined following the NMKL method no. 6 "Nitrogen. Determination in foods and feeds according to Kjeldahl." (Bøgh-Sørensen, 2003), with minor modifications. Approximately 1.0 g of CL and, using nitrogen-free weighing paper, 0.2 g of homogenised raw and cooked fish, was transferred to a digestion flask. 5 mL concentrated sulfuric acid (H_2SO_4) and a copper catalyst tablet was added and the flask was heated to 420 °C for 2 hr in a heat block ((Kjeltec system 2020 digestor, Tecator Inc., Herndon, VA, USA). The samples were allowed to cool at room temperature for 30 min, followed by addition of 75 mL of H_2O, neutralization and titration. Two flasks containing weighing paper were used as blind samples. The nitrogen content in % (w/w) were calculated based on the following:

$$Nitrogen\ content\ (\%) = 14.01 \times (V_a - V_b) \times \frac{M_{HCl}}{W} \times 100\% \tag{3.6}$$

where V_a is the amount of HCl used for titration (L), V_b is the blank value (L), M_{HCl} is the molarity of HCl (0.1M) and W is the weighed amount of sample (g). 14.01 is the molar weight of nitrogen (g/mol).

The amount of total nitrogen in the samples were multiplied with the traditional conversion factor of 6.25 [6], in order to determine total protein content (%) before or after cooking, as outlined below:

$$Protein\ content\ (\%) = Nitrogen\ content\ (\%) \times 6.25 \tag{3.7}$$

Protein losses or protein retained by a fish sample (w/w initial protein, wet basis) were expressed in an equivalent manner as for water, outlined in Equation 3.4 and Equation 3.5.

Kjeldahl analysis was carried out in triplicates (n=3) of fish and CL, respectively, from the heat treatment parameters ranging from 40-90 °C. The parameter 30 °C was omitted due to the low amount of cook loss that was retrievable. Two analytical parallels were used for each fish sample parameter. The average values of these were used as one biological parallel. Due to the lower amount of CL retrieved at 40 °C, two of the the biological parallels used consist of a mix of cook loss retrieved from two different individuals. This will be taken into account in later discussion.

3.3.4 NaCl by titration

Approx. 4 g of a well homogenised sample of raw and cooked fish (see Section 3.2.4 for procedure), and the total amount of LL retrieved (0.2-1.5 g), was diluted to about 50 ml with distilled water and allowed to stand for 1 hour at room temperature. Chloride was precipitated after acidification with nitric acid, HNO_3, and amount of chloride was determined by potentiometric titration with silver nitrate, $AgNO_3$, using a titrator (Mettler- Toledo T7, Zürich), in accordance to the principles outlined in the International Standard ISO 1841-2 "Meat and meat products - Determination of chloride content" (ISO, 1996). The cheese method, later discussed in Section 5.1.5, is based on this ISO standard.

The analysis was carried out on TL (n=2), and CL from the heat treatment parameters of 50 °C(n=5) and 70 °C (n=4). In addition, analysis of raw fish (n=5) and two biological parallels per cooked fish, from the heat treatment parameters between 30-70 °C, was carried out (n=2×5). Two analytical parallels were taken for each fish sample.

The result was calculated directly in % of sodium chloride, NaCl. As 1 molecule of titrant reacts with 1 molecule of Cl^-:

$$NaCl\ (\%) = \frac{V_{titr} \times C_{titr} \times 58.443 \times 100}{m_s \times 1000} \tag{3.8}$$

[6]The conversion factor of 6.25 is based on the assumption that the general total protein content in food is 16 %, and that all of the nitrogen is bound to protein. It has been proved that this conversion factor often overestimates the protein content of food, including cod fish (Mæhre et al., 2018, p.5), when compared to the protein content estimated by amino acid analysis. This will be taken into account during later discussion.

V_{titr} is the total volume (mL) of titrant needed to reach the inflection point, C_{titr} is titrant concentration (mol/L), and m_s is the sample amount (g) used during titration. It is possible to express a result in % of Cl- or KCl by replacing the atomic weight of the NaCl (58.443 g/mol) by the atomic weight of the Cl- (35.453) or KCl (74.551 g/mol) for the result calculation. The reported detection limit for this procedure is 0.25% NaCl.

Salt losses or salt retained by a fish sample (w/w initial salt, wet basis) were expressed in an equivalent manner as for water and protein, outlined in Equation 3.4 and Equation 3.5.

Due to the results being close to the reported detection limit, chemical analyses of salt of raw and cooked fish were performed in duplicates by an aaccredited laboratory (BioLab, Nofima, Norway). Approx. 5 g of three biological parallels (n=3) were homogenised into one sample and sent to the laboratory. Salt was determined according to the AOAC Official Method 937.09 "Salt (Chlorine as Sodium Chloride) in Seafood" (AOAC, 1937). Five analytical parallels were taken, and the results were given in %NaCl. This method differs from that used within this thesis. Here, Cl is precipitated as AgCl by $AgNO_3$, and boiled with HNO_3, before titration with Ammonium thiocyanate, H_4SCN.

3.3.5 Ash and Fat by external laboratories

Total amounts of metals in raw and cooked fish were analysed by the accredited laboratory ALS Laboratory Group Norway AS. Approximately 6 g of four raw or cooked fish samples were homogenised into one sample (see Section 3.2.4). The trace elements were digested in accordance to the NS-EN method 13805:2014 "Foodstuffs - Determination of trace elements - Pressure digestion" (Standard Norge, 2014), and analysed according to the EPA-method 200.8 "Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry" (U.S. EPA, 1994). Inductively coupled plasma sector field mass spectrometry (ICP-SFMS) was used as the principle of measurement. Results were given in mg/kg sample. The measurement uncertainty was indicated as an extended measurement uncertainty (JCGM, 2008) calculated with a coverage factor of 2, which gives a confidence interval of about 95%.

The results were converted into % metal content, and the metals with a prevalence higher than 0.1 % was summarised and taken as the total ash content (%). The combined standard uncertainty was found by squaring the uncertainties, adding them all together and then taking the square root of the total. Ash losses (g/100 g raw) were calculated as outlined in Equation 3.9.

$$Ash\ loss = Ash\ content_{\text{raw}} - (Ash\ content_{\text{cooked}} \times \frac{W_{\text{retained}}(average)}{100\%}) \quad (3.9)$$

where W_{retained} is the average weight of a cooked fish sample (g), considering 100 g raw sample, in which values were obtained by earlier experiments.

The fat content of raw fish was analysed in order to get a complete overview of the biochemical content of the fish. Two 5 g samples (n=2) of raw fish were homogenised into one sample (see Section 3.2.4), and sent to Nofima BioLab (Norway). The sample was analysed for content of fat according to the AOCS Official Method Ba 3-38 "Oil in Seed Meals and Cakes" by the principle of Soxhlet extraction (AOCS, 2017). Five analytical parallels were taken, and the results were given in %fat.

3.4 Qualitative analyses

3.4.1 Residual denaturation enthalpy by DSC

Differential scanning calorimetry (DSC) was used to determine the protein denaturation characteristics of the LLs. It was performed with a DSC1 (Mettler Toledo, Schwerzwnbach, Switzerland). LL samples (approx. 50 mg) were accurately weighed into a steel sample pan, medium pressure Ø7 mm with pin (Mettler Toledo, Switzerland). A reference pan was filled with water corresponding to the water content in the LLs (measured as described in Section 3.3). The sample pan and the reference pan was hermetically sealed and heated from 2 °C to 100 °C at 2.5 °C/min, in which a peak area of the resulting curve was defined as the integral of the heat flow into the sample within a specified temperature range.

The protein denaturation peak temperature (T_m, °C) and residual denaturation enthalphy (ΔH J/g) was determined using the STARe Software version 14.00 (Mettler Toledo, Schwerzwnbach, Switzerland), in which four peaks were considered as the point of denaturation belonging to four separate protein groups. The baseline was determined after a repeat measurement of each sample, and used as a guideline as to integrate the individual peaks using a spline baseline, whilst using a line baseline when quantifying the total area ΔH (J/g) of overlapping peaks.

The analysis was conducted on samples of TL and CL from all of the HTPs between 30-70 °C. Results are reported as averages of three biological parallels (n=3). [7] The DSC heating curves are represented with endothermic denaturation peaks which points downwards.

3.4.2 Specific heat capacity by DSC

It is known that the specific heat capacity c_p of a sample is represented by the area under a DSC curve. c_p can be determined directly by differentiating the heat flow with respect to temperature, as in Section 3.4.1, but this procedure is

[7] During the measurements a selection of reference pan weights were written incorrectly. By comparing these integration values to the values of analytical parallels, with a correctly written weight, it was observed that the results were not affected. This observation is supported by Widmann (2009, p. 103), who states that the mass of the reference curve is not important. The important parameters for T_m (°C) are sample mass and heating rate.

inaccurate. In "c_p by sapphire" the heat flow through a sample is determined by comparison to the heat flow in a sapphire reference sample, with a known specific heat, measured under the same conditions.

A custom made nine-step DSC procedure was followed to measure specific heat capacity (Widmann, 2009, p. 126). Additionally, testing procedures adhered to protocols set forth in the ASTM E1269 - 11(2018) "Standard Test Method for Determining Specific Heat Capacity by Differential Scanning Calorimetry" (ASTM, 2018). The nine-step DSC procedure was designed with a heating regime that treated each 10th temperature equally. Ten isothermal segments of 3 min were separated by nine dynamic segments of 2 min, employed with a heating rate of 5 °C/min, covering the temperature range between 10-100 °C.

The first measurement was taken with two empty sample pans, namely standard aluminium crucibles with lid and pin (Mettler Toledo, Switzerland), loaded into the DSC. During this measurement, the baseline heat flux was obtained. The indicated bias in the machine was accounted for in the measurements that follows by blank subtraction. A second measurement was of a pan containing 45 mg synthetic sapphire, replacing one of the empty pans. The heat flux into the reference sample was recorded throughout the identical heating regime. The third measurement was made using approx. 40 mg LL samples, replacing the reference sample. The heat flux into the sample was recorded during an identical heating regime as the previous two measurements. The heat flux curves from the three measurements were used to comparatively determine the specific heat capacity of the LL, using the "c_p by Sapphire" DSC function of the STARe software.

The analysis was conducted using samples of TL and CL from a selection of HTPs, namely 30-60 and 90 °C. c_p values for analysis were calculated at every 10th °C. The c_p curves of three biological parallels or more (n$\geq$3) were plotted against temperature. A simple linear regression model was employed for each curve, and the average and standard deviation was evaluated for the slope, a, and the intercept, b. In order to indicate the extent to which the two variables (c_p and temperature) were linearly related, the Pearson correlation number [8] of the total regression curves, retrieved for each HTP, is represented in the results part.

3.4.3 Protein molecular weights by SDS-PAGE

LL was subjected to SDS-PAGE (Sodium dodecyl sulphate - polyacrylamide gel electrophoresis) analysis as to complement the DSC analyses. In SDS-PAGE, proteins are broken down to their denatured form and separated according to their size.

LL proteins were resolved by SDS-PAGE according to the procedure given by BioRad (10026447), which is based on the Laemmli method (Laemmli, 1970). Electrophoresis was performed in a Mini-PROTEAN apparatus (BioRad), using a vertical mini-slab gel (Mini-PROTEAN® TGX™; BioRad) composed of 4 to 20 % acrylamide and run at constant voltage of 200 volts for 30-40 min.

[8] The Pearson´s R is a number between -1 and 1. More than 0.45 indicates strong correlation.

Amounts of 10 μL of protein solutions were loaded into each well of the gel. The same volume of Mark^{12} protein standard (Invitrogen, Thermo Fisher Scientific) was added in a separate well alongside the samples. After electrophoretic separation, the gel was stained with Coomassie blue solution (0.25% Coomassie brilliant blue R250; BioRad, 50% (v/v) methanol and 10% (v/v) acetic acid) for 1 hour at room temperature and subsequently destained in the destaining solution (5% (v/v) methanol and 7.5% (v/v) acetic acid) for 1 h. The gel image was captured and analysed using an Odyssey CLx scanner (Li-COR Biosciences, Lincoln, USA). The molecular weights of the protein subunits were determined by comparison of their relative mobilities of migration with those of the high range protein molecular weight standard.

The first SDS- trial revealed that the CLes were to concentrated to be separated. After this, CL protein solutions of 2.5 %(w/v) were prepared by diluting the samples with water at a ratio of 0.5:9.5 (w/w) and mixing with loading buffer (5% 3-mercaptoethanol; BioRad) at a ratio of 1:1 (sample solution:loading buffer, (w/w)). CL proteins were solubilized and reduced by heating at 90°C for 2 min before loading unto gels. TL and CL comprising all of the heat treatment parameters 30- 90 °C were loaded unto one gel. Every second lane in the gel represent LL from the same individual, but retrieved at a different HTP temperature. Two gels were made (n=2). The experiment was repeated twice, one time as a 5% dilution and one time as a 2.5% dilution of the LL protein solutions.

3.4.4 Protein sizes by SEM

Scanning Electron Microscopy (SEM; Zeiss Supra 35 VP) was used to visualise the microstructure (resolution $\geq$ 50 nm) of the cook loss proteins, and the attached x-ray emission spectrometer was used for proximate elemental analysis. One loin was cut into four cod sample pieces, and heat treated at four temperatures; 30, 50, 70 and 90 °C (n=1). Due to high vacuum conditions, CL protein solutions were freeze dried using liquid nitrogen. A small sample was sputter coated with a layer of palladium atoms and scanned.

The SEM produces images of a sample by scanning the surface with a focused beam of electrons. The incoming (primary) electrons supplies energy to the inner-shell electrons that are present in the sample, which can then be released as secondary electrons. The secondary electrons produce SEM images and yield characteristic X-rays that can be used to identify and measure the abundance of elements in the sample.

3.4.5 pH measurements

pH was measured in samples of raw and cooked fish and LL from the HTPs 0, 40, 60 and 90 °C. 5 g fish and 0.2-1.5 g of LL was mixed 1:1 (w/w) with 0.1 M KCl, and the fish was homogenised as outlined in Section 3.2.4. pH measurements were made by using a Toledo Five Easy Plus FEP20 pH meter (Mettler

Toledo, Zürich, Switzerland) with an electrode (LE438 Mettler Toledo, Zürich, Switzerland) that was calibrated with buffer standard solutions of known pH.

The pH values are reported as the average and the standard deviation of six biological parallels (n=6). Two analytical parallels per sample was retrieved by changing the position of the electrode in the solution.

3.4.6 Density measurements

The apparent density of CL, from the HTPs between 40- 90 °C (n = 2-5), was measured by reading the volume (0.4-1.5 mL) within a 2 mL graduated pipette, followed by weighing the pipette containing the sample and subtracting the weight of the pipette without sample. Density ρ_{CL} was taken as the mass of sample per unit volume (g/mL) , and the results were expressed with two decimals. The reason for using the 2 mL graduated pipette is based on the following: the accuracy of density values obtained using two different pipettes were tested, using water. The density values for a 1 mL pipette were consistently overestimated by at least 0.05 g/mL, whereas the 2 mL pipette always produced values within 0.02 g/mL from the literature value.

Apparent density of myotomes from raw fish (n=2) and fish cooked at 50 °C (n=2) and 60 °C (n=3) was measured using a pycnometric procedure. A symmetric ring was drawn midway up the neck of a 50 mL Erlenmeyer flask. The mass of the flask was recorded (m_0), along with the mass of the myotome (m_1), and the flask filled with water to the mark with myotome (m_2) and without myotome (m_3). The temperature of the water used was measured to the nearest decimal in order to make use of the literature density value of water ρ_w for calculation.

The density of a myotome ρ_m was calculated as outlined in Equation 3.10

$$\rho_m = \frac{(m_1 - m_0) \times \rho_w}{m_2 - m_3} \tag{3.10}$$

3.5 Rheological characterisations

Rheological properties of CL was measured using a rotational Discovery Hybrid Rheometer, HR-2 model (TA instrument, UK) equipped with a hatched parallel plate geometry (plate diameter = 40 mm; gap width = 0.7 mm) and a Peltier Plate temperature control system. The measurements were performed at the temperature equivalent to the HTP temperature the CL was retrieved from, which were 40, 60 and 80 °C. A solvent trap cover was used to reduce any solvent loss during the rheological experiments and to improve temperature uniformity.

CL samples were prepared from fresh cod as outlined in 3.2.6, and, after tempering, the CL samples were allowed to equilibrate at room temperature for

30 min before analysis. Average values were calculated using three or four biological (n = 3-4) -and two analytical parallels per temperature.

Rheological properties were measured using four different procedures, as described by the following list:

1. Dynamic oscillation temperature ramp (1.59 Hz, 1 Pa), covering temperatures from 20- 80 °C with a ramp rate of 2.5 °C/min, to investigate effect of thermal treatment on gelation.

2. Dynamic oscillation strain (%) sweep (0.1 Hz), covering 0-2000% strain within 240 s, conducted at 20 °C and at HTP temperatures, respectively. The frequency of 0.1 Hz was utilized to ensure that the flow curves were within the linear viscoelastic regime. The storage modulus (G´), loss modulus (G´´) and complex viscosity η^* were thus obtained, as a function of strain (%) at that frequency.

3. Shear stress (Pa) controlled ramp covering 0.01 - 100 Pa within 240 s, performed at HTP temperature. In the flow mode, the rheometer applies a wide range of shear stresses (or rates) to the sample in a stepped or continuous fashion, and the resultant shear rate (or stress) is measured. The calculated apparent viscosity is plotted as a function of the control variable and this curve is referred to as a flow curve.

4. Peak hold at a constant shear rate of 0.02, 1, and 250 1/s, respectively, as a function of a 600 s time period. Also performed at HTP temperature. The constant shear technique involves imposing a single, constant shear rate (of shear stress) on the material and monitoring the response. The thixotropy of different materials can be compared by comparing the times taken to reach an equilibrium value (Kealy, 2007).

Two biological parallels of CL retrieved from the standard pre-frozen cod (see 3.1.1) were additionally analysed using method 1 and 2, as a basis for comparison. Significant differences were not calculated for the viscosity measurements. This was largely due to a lack of time. The following results and discussion will be based on explaining the flow behaviours, and looking at trends.

3.6 Permeability

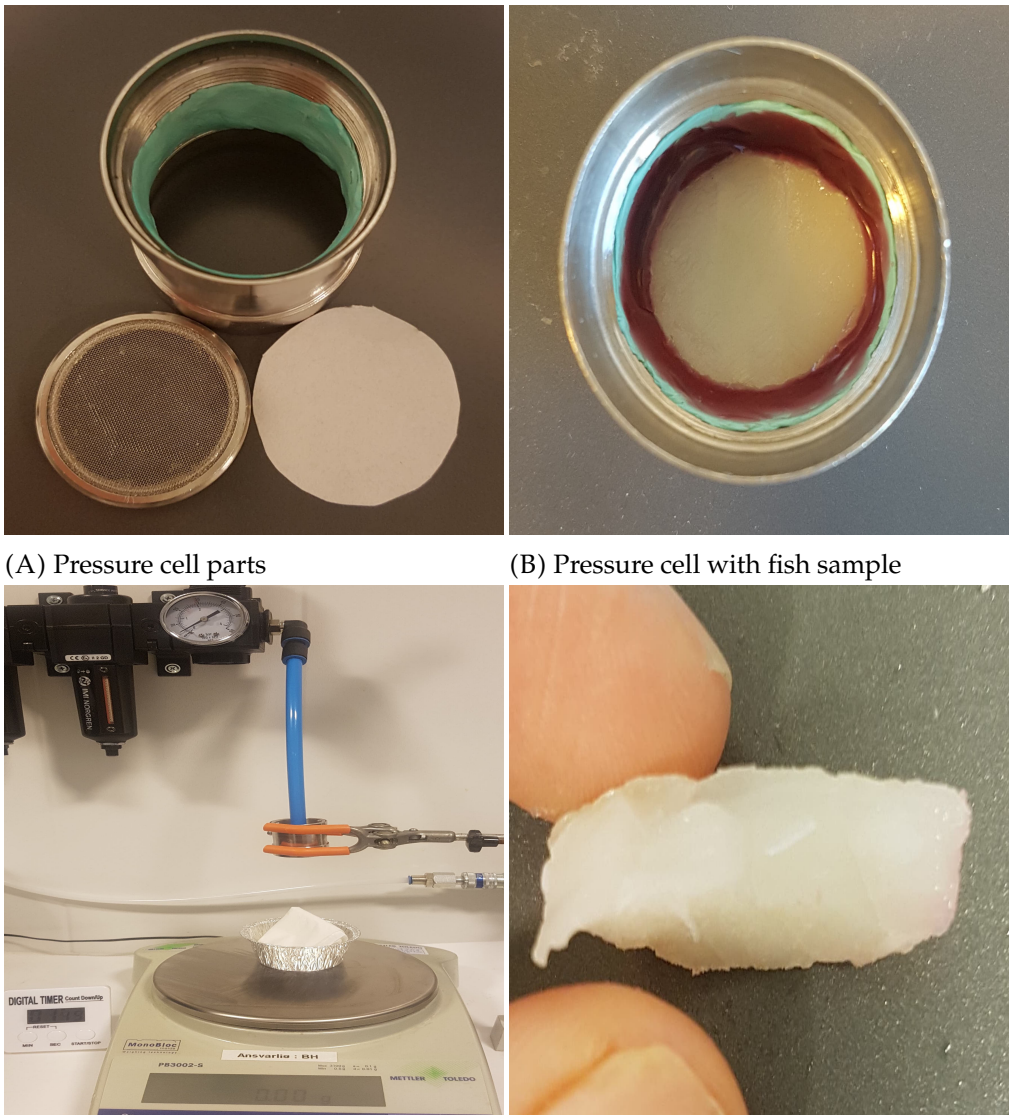
The general idea in permeability measurements is to apply a pressure on a liquid that is situated above a material of which permeability is of interest. The amount of liquid collected over a certain time is equal to the flow velocity, which can be used in Darcy´s law, together with known pressure and thickness, to get the permeability.

3.6.1 Intrinsic permeability

Part of the goal with this study was to develop an experimental set up using air pressure controlled flow of water through a cod piece sample, to measure the intrinsic permeability, as was done for raw whole meat by Datta (2006) and for fried hamburgers by Oroszvári et.al. (2006). The experimental set up for this procedure is illustrated in figure 3.5.

Cylindrical shaped samples (prepared as outlined in Section 3.2.5) were placed in a custom made pressure cell, based on a prototype sample cup (Hettich, Tuttlingen, Germany) described by Skipnes, Lund Østby, and Hendrickx (2007). The basic parts of the pressure cell are shown in 3.5 A. Modelling clay was used to tighten the continuous threads within the sample cup. The filter was mounted at the bottom and covered by a filter paper. The circumference of a fish sample was made to react with superglue. This was done in order to seal the porous entrances of the fish meat, before using epoxy glue to seal the fish sample within the pressure cell, as shown in Figure 3.5 B. Superglue cures within seconds when in contact with water. The epoxy glue was mixed with food coloring in order to monitor any contamination of the fish sample. Approximately 3 mL of water were put on top of the fish sample. To complete the pressure cell, a lid with a hole was mounted on top, and an air pressure hose was used to connect the pressure cell to a manometer, as shown in Figure 3.5 C. Air pressure of 0.3 bar were allowed to flow through the pressure cell, and the time and the amount of water collected were noted every 2 min for 30 min. As can be seen in Figure 3.5 D, the epoxy glue did not penetrate the fish sample.

The intrinsic permeability of whole raw fish was determined using two biological parallels (n=2), as this procedure, and the method development (see Section 4.5), was time consuming.



(A) Pressure cell parts

(B) Pressure cell with fish sample

(C) Set up for water flow measurement

(D) Fish sample after measurement

FIGURE 3.5: Measurement of intrinsic permeability using a custom made pressure cell.

3.6.2 Permeability upon centrifugation

Oroszvári et.al. (2006) also measured the permeability upon centrifugation. In this case, water was not mounted above the hamburger samples. Instead, they made use of the fact that there was already water present within the hamburgers. They assumed that the water would not be a limiting factor at short centrifugation times (5 min). In fact, they found that the permeability upon centrifugation was in accordance with the intrinsic permeability measured with the air pressure cell.

Minced hamburger meat could obey different flow regimes compared to whole meat, and compared to fish meat. Several measurements of the permeability upon centrifugation, at different applied pressures, were made for the fish

samples. The results pointed towards one fact; the permeability was decreasing with time, and reached a stabilized value after about 15 min. This trend was in agreement to the permeability value obtained, plotted against time, using the pressure cell. The reason for the decreasing permeability was attributed to the fact that the duration of time was a limiting factor in calculations. The same amount of water passed through the sample in the pressure cell at short times, compared to at longer times, but when using short times in the calculation, the values became biased and overestimated. An agreement between the intrinsic permeability and the permeability upon centrifugation were observed after stabiliaation (as will be presented in section **??**).

Due to the outlined reasons, the centrifuge method was used for raw fish and cooked fish. Cylindrical shaped samples (see 3.2.5 were placed in updated versions of the sample cups, in a similar fashion as was done by Skipnes (2007). The cups were centrifuged (Rotina 420R, Hettich, Tuttlingen, Germany) at 550 and 1770 RPM (corresponding to approx. 50 and 500 g, calculated using Equation 3.14), in which 500 g corresponds to approx. 0.3 bar)), respectively, for 15 minutes at a temperature of 20 °C. This was done for raw fish. Due to an agreement of the permeability obtained at 50 g and 500 g, the permeability of water through cooked fish samples were determined by the centrifuge method at 50 g. A selection of samples were heat treated at 40 °C (n=6), and at 60 and 90 °C (n=3) in a water bath (Grant, GD120, Cambridge, England) for 10 min and subsequently cooled in a ice water bath for at least 10 min. The cups were centrifuged as described for raw fish.

3.6.3 Equations for permeability calculations

Similarly to that done by Oroszvári et al. (2006) and Datta (2006), the permeability (K) was calculated using Equation 3.11

$$K = \frac{\bar{u} \times \mu \times h_{fishsample}}{\Delta P} \tag{3.11}$$

where $\bar{u}$ is the flux of water (m s^{-1}), μ is the viscosity of water at ∼20 °C (1.00 $\times 10^{-3}$ kg m^{-1} s^{-1}), $h_{\text{fish sample}}$ is the height of the fish sample (5.0 $\times 10^{-3}$ m) and ΔP is the pressure gradient (Pa). For the permeability upon centrifugation a number of pre-calculations were made. The flux of water (m s^{-1}) was calculated by:

$$\bar{u} = \frac{V}{A \times t} \tag{3.12}$$

where V is the volume of water that passes through the fish sample (m^3), A is the area of the fish sample (m^2), and t is the duration of time (s). Density of water, ρ at ∼20°C of 998 kg m^{-1} is used to convert the weight of collected

fluid into volume. The pressure (Pa) during centrifugation was calculated in a similiar fashion as that done by Oroszvári et al. (2006):

$$P = \frac{F}{A} = \frac{RCF \times g \times W_{fish\,sample}}{r^2 \times \pi} \tag{3.13}$$

where F is the force (N), g is the acceleration of gravity (9.81 m/s^2), $W_{fish\ sample}$ is the weight of the fish sample, r is the radius of the fish sample during centrifugation (0.013 m) and RCF is the relative centrifugal force which, as directed by the centrifuge manual, was calculated using the following equation:

$$RCF = \frac{4 \times \pi^2}{g \times 60^2} \times r_c \times RPM^2 \tag{3.14}$$

where r_c is radius from center of rotor to center of fish (m), and RPM is revolutions per minute.

3.7 Statistical analysis

The results were analysed for significant differences using IBM SPSS Statistics (version 25). The tests utilized were one way ANOVA and the Tukey posthoc test with a 95 % confidence interval. The same confidence interval was used for examining possible outliers and for the linear regression curve fitting done for the specific heat capacity results (section 5.3.2).

Each cod piece sample of raw and cooked fish, and the corresponding LL, was always analyzed individually, representing one biological parallel. The average were taken of all analytical parallels before statistical analyses, thus meaning that the presented standard deviations only represent biological parallels. The selection of these parallels, derived from the same individual were, always in a consistent and systematic manner, varied among the HTP temperatures used.

Fish samples analysed by an external laboratory, which includes ash, fat and salt analysis, consisted of biological parallels that were ground into one sample. These results are presented with the uncertainties given by the laboratory.

Chapter 4

Method development

4.1 Sample preparation

4.1.1 Vacuum used in pouches

The effect of vacuum used in the pouches were tested. One fish loin was cut into four sample pieces, packed with 90.2, 92.2, 94.2, and 96.2 % vacuum, respectively, followed by cooking at 50 °C for 10 min. It was observed that the amount of CL increased with approx. 1.5 g for each increment in %vacuum, mainly due to water being expelled. It was observed that the increase in CL was somewhat lower the higher %vacuum applied. The increase in CL could be due to the fish getting more squeezed with higher % vacuum. This observation is supported by Garieby (1986), who observed that the negative pressure applied during vacuum packing of rabbit meat resulted in extraction of liquid from the meat, causing increased LL in the pouch. Lower vacuum is compromised by a slower rate of heat absorption, due to presence of more oxygen. With this in mind, 92.2% vacuum was rendered a good choice. This vacuum was also practical in the sense that it could be used while still having the possibility of moving the glass tube within the pouch after sealing.

4.1.2 Temperature conditions for sample cutting

Brown muscle was cut from a loin using a slicing machine, and afterwards a knife was used to cut the loin into sample pieces, as described in Section 3.2.1. As it was very cold in the 0 °C ventilated room, alternative locations for this sample preparation were examined.

The effect of performing the meat slicing (MS) with the machine and afterwards, using the knife for sample cutting (SC), were tested at three different temperature conditions, as illustrated in figure 4.1. The MS and SC was done as quickly as possible at all temperature regimes, and fish samples were cooked at 50 and 70 °C for 10 min. When the MS and the SC was done at 0 °C (n=4), similar CL amounts per 100 g raw fish was obtained, as to when the subsequent SC was done at 20 °C (n=3).

The same was not true for when the meat cutting is done at 20 °C (n=4), as an apparently increase in CL is observed, especially when the fish sample is cooked at 50 °C. Although figure 4.1 illustrates a trend of increase in CL following sample preparation at different temperature regimes, the differences are not significant ($p<0.05$). Considering these results, it was rendered safe to perform the sample cutting at 20 °C, which was done throughout this thesis.

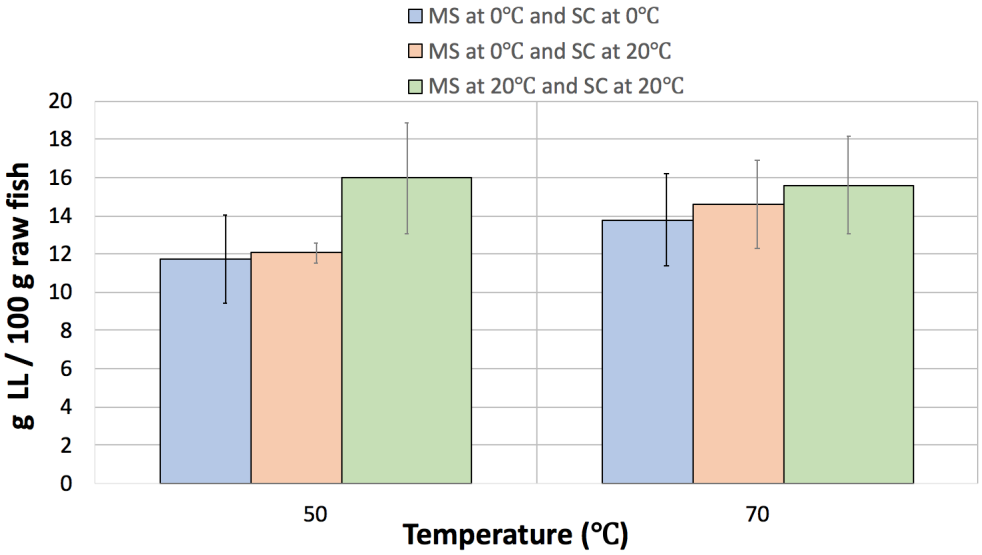


FIGURE 4.1: LL of samples sliced and cut at different temperatures treated for 10 min at 50 and 70°C

4.1.3 Sample collection

The performance of the sample collection used throughout this thesis was indirectly tested. An additional measurement of the dry matter content of CL from the HTPs 50, 70, and 90 °C was carried out (n=4). In this case the contents in the pouches were wiped up using a filter paper. As filter papers are hygroscopic by nature, they were dried (for six hours) in order to determine their intrinsic water contents. After equilibrating the filter papers at room temperature, they were used to wipe the inside of each CL, and subsequently dried as in section 3.3.2. The intrinsic water contents of the filter papers were subtracted from the measured water loss of the CL samples. Sample collection using filter paper reduced the measured water content of the CLs by 0.5-1.5 %, probably due to the collection of more proteins that were stuck within the pouch. Significant differences were actually observed ($p<0.05$) between the water content measured at 50 and 70 °C, compared to the contents measured using the standard sample collection. This is probably due to more proteins being expelled at these HTP, relative to water. The author considers this sample collection more accurate, but in practical terms, it was not possible to utilise it in most of the subsequent analyses. Therefore, results are presented using the standard sample collection, whilst always keeping in mind this uncertainty that follows.

4.2 Moisture by oven drying

The sand pan technique, described in the method "ISO6496: Animal feeding stuff- determination of moisture and other volatile matter content", was utilized when analyzing the cook loss. This technique can help to spread the liquid sample and prevent the formation of a surface crust, in such that evaporation is less hindered (Bradley Jr., 2010, p. 90). The technique was tested on a cook loss (50 °C, 40 minutes) from a whole loin. Dry matter content with and without sand (n=3) were analysed, and a significant difference was observed ($p<0.001$). With sand the water content was 90.718 $\pm$ 0.005 %, without sand it was 90.47 $\pm$ 0.01%.

4.3 NaCl by titration

Amount of salt was determined by titration with silver nitrate, $AgNO_3$, after sample dissolution in water. The procedure was explained in detail in section 5.1.5.

The extraction process of the originally tested ISO method was intended for cheese products. In this procedure, salt is extracted from the sample for one hour at 55 °C. Dyer (1943, p. 2) found that complete extraction of salt from a minced raw, fresh cod sample was obtained by boiling for 2 to 5 minutes or by extracting for approximately 3 hours at room temperature.

The cheese extraction method was tested and compared to extraction at other temperatures. The following extraction temperatures were tested:

- Room temperature, approx. 20 °C
- 55 °C
- 80 °C

Samples of 1.5 g from one (Trial 1) and three (Trial 2) ground raw cod loin were extracted for 1 hour at each of the three temperatures. In trial two, half of the ground cod was analyzed by Nofima Biolab, an accredited laboratory.

TABLE 4.1: Measured amount of salt (%) after extraction for 1 hour at three different temperatures. Three and five analytical parallels were taken in trial 1 and 2, respectively.

% NaCl in two trials		
Extraction temp.	Trial 1 (n=1)	Trial 2 (n=3)
20 °C	0.18 ±0.01	0.18 ±0.00
55 °C	0.14±0.02	0.14±0.00
80 °C	0.14±0.01	- - -
100 °C (Nofima Biolab)		0.16±0.05

The following observations were made:

1. A significant difference is observed between the % NaCl in the samples extracted at 20 and 80°C ($p < 0.05$) in trial 1.

2. A significant difference is observed between the % NaCl in the samples extracted at 20 and 55°C ($p < 0.001$) in trial 2.

3. No significant differences are observed in comparing the % NaCl extracted at the different temperatures and the result at Nofima BioLab °C ($p < 0.05$)

There was an additional problem when measuring the amount of salt in the LLs. Often, the instrument did not manage to calculate a number, and the result was NaN (not a number). The problem was more prominent for the resultant LLs from HTPs at higher temperatures. This observation could indicate presence of a decreasing amount of salt with higher temperatures, which in turn could be accredited to a low amount of salt in general, possibly below the detection limit. The problem was though solved by lowering the treshold value of the electrode potential per volume, dE/dV (mV/mL), meaning that the instrument used more time at low electrode potential values.

4.4 Rheological characterisations

At first a 40 mm 1° cone and plate geometry was used due to its higher precision at low viscosity values, which was evident at high shear rates (measured using an S3 calibration oil). Later on it was revealed that by using this geometry the viscosity was overestimated by 10 folds at low shear rates (as compared to values obtained using the 40 mm hatched parallel plate, and also a 40 mm smooth parallel plate). The conclusion made was that the particles in the CL were too large, and subsequently they were trapped underneath the small truncation gap (49 um) during low velocity conditions (eg. low shear rates), resulting in falsely large viscosity values.

For rheological analyses, a sample size of at least 0.5 ml was necessary. This relatively high amount of CL was often not retrieved from the small cod fish pieces cooked at temperatures below 70 °C. Adding in to the equation the time consuming sample preparation and large number of failed experiments, the procedure quickly developed into a costly affair. For this reason, it was decided that fresh cod was to be purchased from a store and that larger samples were to be used, as described in Section 3.2.6.

Surface slip was occasionally observed at high velocities. All these measurements were omitted. It was also observed that analysis, done within a short time span after cooking, yielded results that reminded of the viscosity of water. All these results were discarded. The explanation behind this trend was addressed to the lack of homogenisation. The CL was analysed "as is", and within a short time span after cooking large visible aggregates were present to various degrees. These aggregates were most likely formed after the CL was expelled from the fish, while in this present work, the state at which the CL is flowing within the fish was considered more important. This aggregation

phenomena was considered to have a large impact on the measurement results, as large particles do not contribute much to flow. As a consequence, it is the viscosity of flowing water that is measured and not that of the protein solutions. After the samples had been rested for some time (around 1 hr) the aggregates seemed more dissolved within the solution, and consequently the viscosity values increased and became more reproducible.

4.5 Permeability measurements

For the permeability upon centrifugation measurements, samples of about 10 mm height were used in the beginning. This height lead to visible deterioration, or grounding, of the fish samples when cooked in the sample cup at 90 and 60 °C, in which this was most prominent for the latter. This was probably due to high pressure, maybe in combination with swelling, during cooking. A seemingly increasing permeability of the fish samples cooked at higher temperatures were observed. These results were disproved after repeating the measurements with 5 mm samples, in which minimal deterioration was observed. These results will be presented in Section 5.4.

Intrinsic permeability using the custom made pressure cell rendered many obstacles during its design. The most difficult task was to seal the fish within the cell. This was solved by using superglue and epoxy glue. Two successful measurements were made, using the method described in Section 3.6.1. The values obtained were in close agreement to the values measured using the centrifugation method, as will be presented in Section 5.4.

When trying to perform additional measurements, suddenly no water passed through the sample, even under high pressures and several hours of duration. The hypothesis made explaining this new issue was that the new hose was leaking. Another possible explanation could be that the modelling clay started to get worn down, as it seemed after repeated measurements, thereby contaminating the water and sealing the pores of the fish sample. Due to the lack of time of investigating the underlying issues, only two biological parallels were obtained.

4.6 Mass transfer coefficient

Surface tensile strength of expelled liquid was attempted measured by measuring the contact angle between CL and fish samples. This was supposed to be used for determination of the mass transfer coefficient. Contact angle measurements were made using the CL on myotomes from fish cooked at various temperatures from 30- 90 °C. In all cases, the contact angle was 0 °. From this it was concluded that the surface of the fish may be considered as fully wetted and therefore not interesting to investigate further.

After a thorough literature review, it was found that finding the mass transfer coefficient proved to be more time consuming, demanding and difficult for the extent of this thesis.

Chapter 5

Results

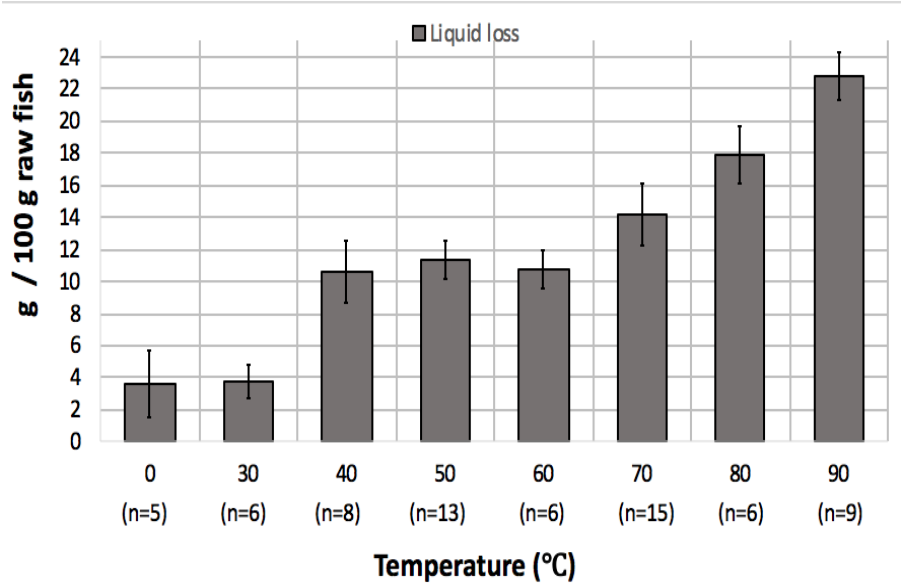
5.1 Liquid losses

Samples of raw and cooked fish, and TL and CL, were characterized with regards to amount of LLs, and content of water, protein and salt. A selection of raw and cooked fish were additionally analysed for content of ash, whilst raw fish, in addition, was analysed for content of fat.

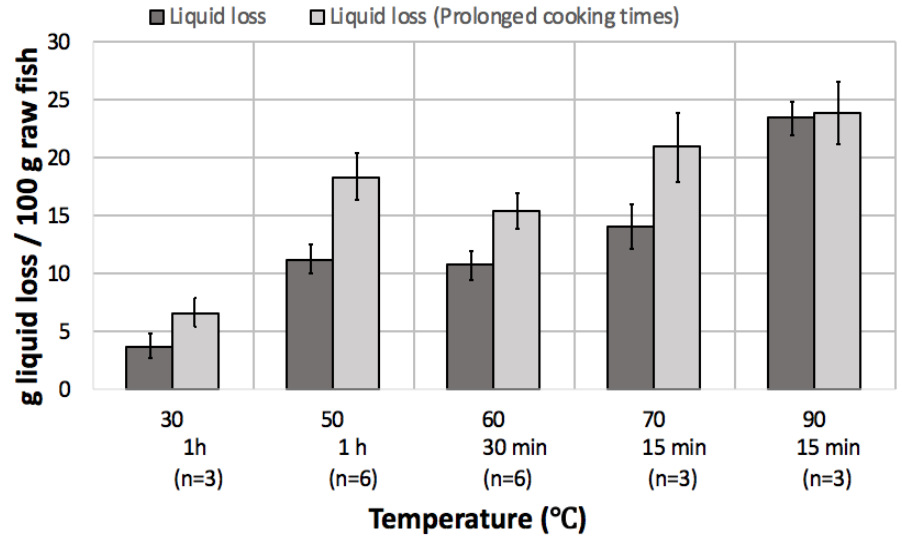
5.1.1 Amount of liquid losses

The LLs ocurring at different temperatures are shown in Figure 5.1 A. A significant increase in the LLs are observed from 30 to 40 °C, from 60 to 70 °C, from 70 to 80 °C and from 80 to 90 °C ($p<0.05$). No significant differences was found from 40, 50 and 60 °C.

Measurements of the LLs used for rheological analysis, as described in section 3.2.6, are shown in figure 5.1 B. The LLs are consistently larger at all temperatures, and significantly larger at 50, 60 and 70 °C, in comparison to the samples cooked for 10 min.



(A) Liquid losses that occurs in response to thawing (0 °C) and cooking 3×2 cm cod fish samples in vacuum pouches for 10 min



(B) Comparison between the amount of liquid expelled at two different cooking times (10 min (black) and >15 min (grey).

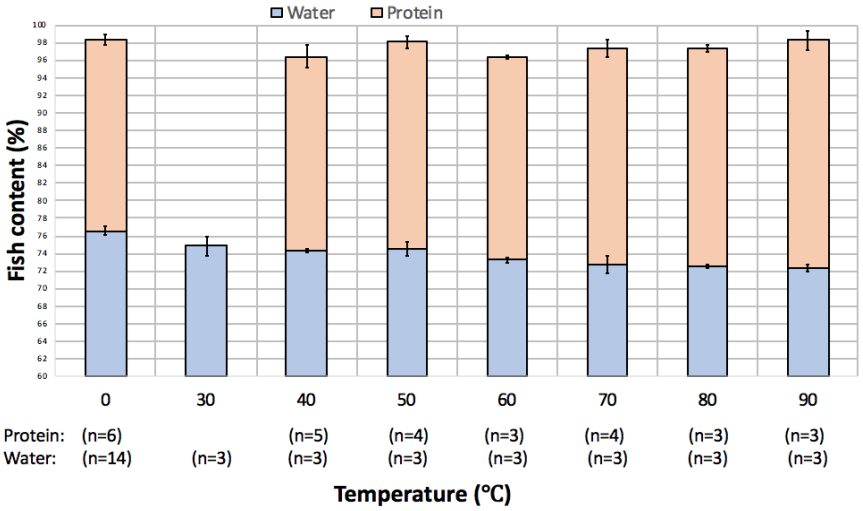
FIGURE 5.1: Liquid losses((g/100 g raw fish); the number of biological parallels per temperature are labeled in the figure) as a result of thawing from -30 °C to 0 °C and cooking at temperatures from 30-90 °C for 10 min. The amount of liquid that is expelled (B) during cooking at longer times (grey bars, >15 min; labelled for each temperature at 30, 50, 60, 70 and 90 °C. The values are compared to the liquid that is expelled during 10 min cooking. Mean values are shown with standard deviation.

5.1.2 Water and protein contents

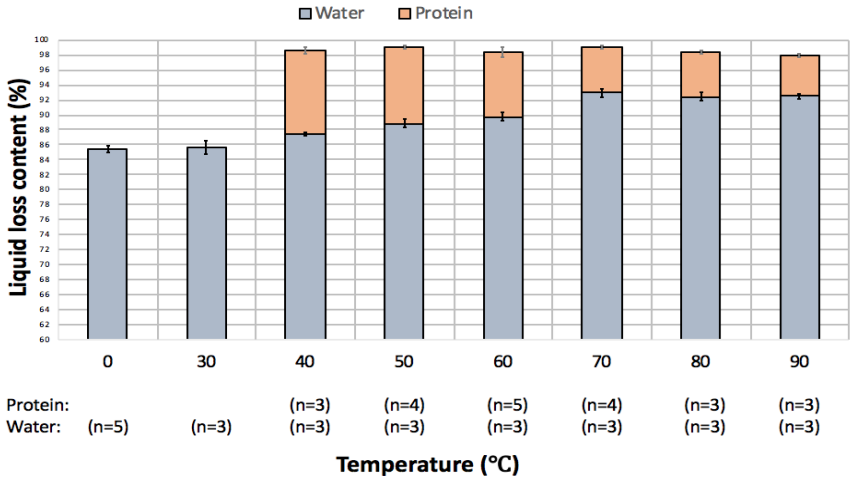
The water and protein contents relative to the (cooked) fishes (%) are shown in figure 5.2 A. A significant decrease in % water for cooked fishes above 70°C is observed, compared to when the fish is cooked below 50 °C. An increasing trend in % protein is observed for the cooked fishes, but the increase is not significant until cooking at 70°C. It seems like the fish cooked at 40 and 60 °C has a higher content of ash and/or fat, when inspecting the total %content.

The water and protein contents relative to the LLs after thawing or cooking (%), respectively, are shown in figure 5.2 B. The water content of the CL at 50 °C is significantly larger in comparison to the TL and CL at 30 °C ($p<0.001$). No significant differences are though observed between the water content of 40, 50 and 60 °C CL. Furthermore, the water content increases significantly from 60 to 70 °C, but does not increase at the remaining higher temperatures. Significant decreases in the % protein content of the CLes are observed between 40/50 °C and 60 °C, and from 60 °C to 70/80/90 °C. Changes in the %contents of water nor protein are observed between 70-90 °C .

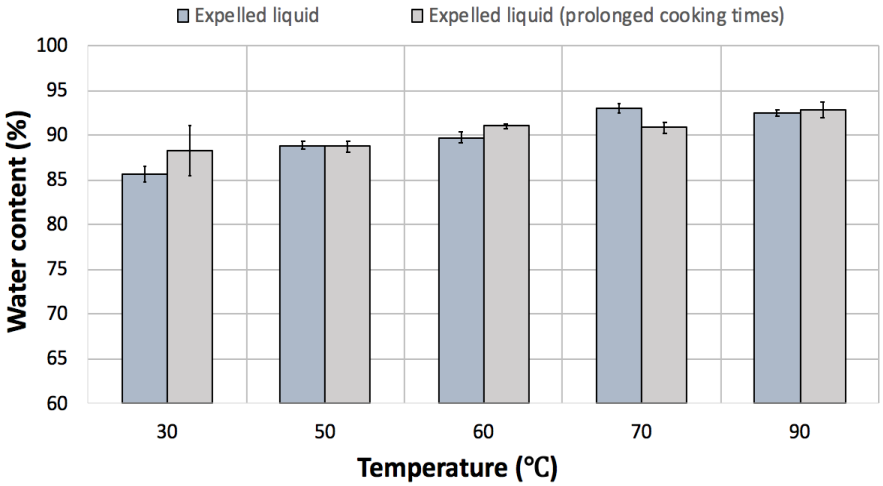
Measurements of the water content of samples used for rheological analysis, as described in section 3.2.6, are shown in figure 5.2 C. A significantly higher water content is observed after 30 °C cooking for the liquid expelled after one hour in comparison to 10 min. Except for this, no significant differences are observed between the water content at different cooking times.



(A) Water and protein content (%) of raw and cooked fish



(B) Water and protein content (%) of liquid losses boiled for 10 min



(C) Water content (%) of liquid losses after longer cooking times

FIGURE 5.2: (A) Water and protein content (%) of fish and (B) for liquid losses, as a result of thawing from -30 °C to 0 °C and cooking at temperatures from 30-90 °C for 10 min. The number of biological parallels (n) per temperature are labeled in the figures. (C) % water content during cooking at longer times (grey bars, >15 min; n= 3). The values are compared to the liquid that is expelled during 10 min cooking. Mean values are shown with standard deviation.

5.1.3 Water and protein losses

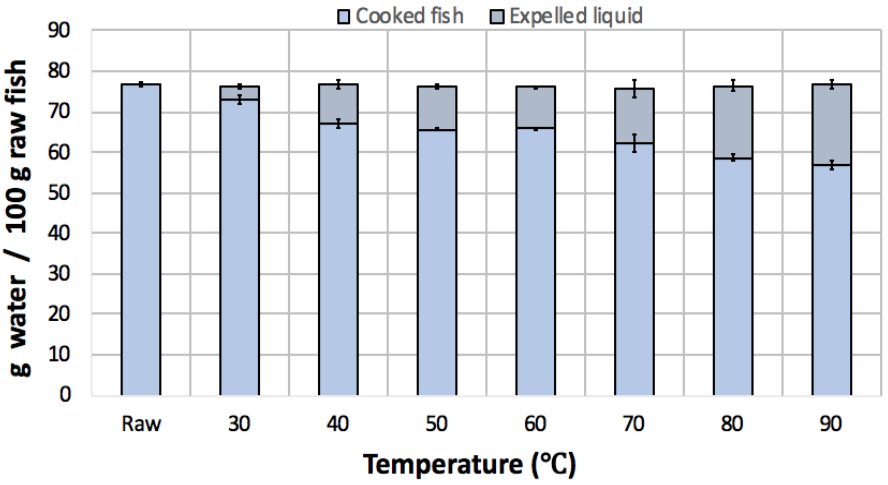
It is difficult to observe if the relative water and protein contents increase and decrease simultaneously, or if the observed decrease in protein contents is due to an increase in the water content. Therefore, it can be more feasible to look at the contents relative to original content in the raw fish.

The Figures 5.3 A and 5.3 B illustrates the total amounts of water and protein (g/100 g raw fish), respectively, retained in the cooked fish and lost as LL. The total amounts do add up, to a good degree, to the amounts present in the raw fish. This indicates that mass conservation is attained. Uncertainties associated with sample collection (see Section 4.1.3), weighing, and/or leaking out of the pouches during cooking should therefore be low.

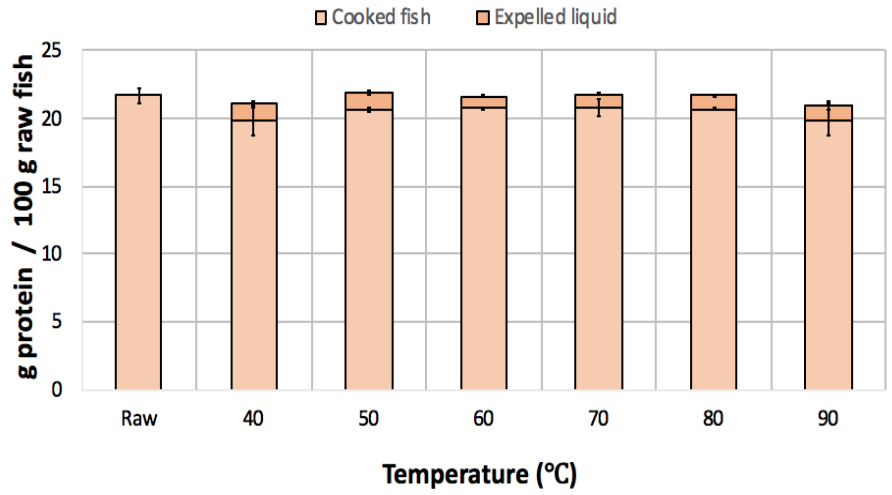
About 6 g more water is significantly expelled when cooking at 40 °C, compared to cooking at 30 °C. The increase in cook loss at 40 °C (see figure 5.1.1), compared to at 30 °C, seems to be due to a greater amount of water being expelled. About 4 g more water is expelled for each temperature increase from 60-90 °C, with a significant increase between 60 and 70°C, and between 70 and 80 °C. The reason for the increases in CL observed at these temperatures also seems to be due to water being expelled. The same, but opposite, trend can be observed for the water retained in the fish.

Approx. 1.3 g of protein is expelled at 40 and 50 °C, but after this temperature the amount of proteins expelled drops down to 0.9 g. A significant difference is in fact observed ($p<0.05$). After 60 °C the proteins expelled rises steeply, and attains a significantly higher value at 90 °C. Proteins are possibly being hindered from being expelled in the 60 °C fish sample matrix.

As only around 1 g of proteins are expelled during cooking of a 100 g fish sample, this could be the reason for the lack of observed significant differences between the around 20 g of proteins that is present in 100 g of cooked fishes.



(A) Water regained and water lost during cooking



(B) Protein retained and protein lost during cooking

FIGURE 5.3: (A)Water losses and water retained (g/100 g raw), as a result of thawing from -30 °C to 0 °C and cooking at temperatures from 30-90 °C for 10 min. (B) Protein losses and protein retained (g/100 g raw) in response to cooking at 30(40)- 90 °C. The number of biological parallels are the same as for the % content as illustrated in figure 5.2 B and 5.2 A

5.1.4 Ash and fat contents and losses

Metals that were found present in a relatively high amount (%) in raw and cooked (50 and 90 °C) fish samples are summarized in table 5.1. The most pronounced metals present were potassium, sulfur and phosphorus (between 0.3 and 0.5 %). This result was, in fact, also indicated by the scanning electron microscopy analysis of cook losses, which will be presented in 5.2.1. The presence of sodium, magnesium and calcium were rather low (between 0.01 and

0.04 %). The total amount of the metals were taken as the total ash content. As can be seen in the table, there is a minor decrease in the contents of ash when fish is cooked, compared to when it is raw.

TABLE 5.1: Metals that make up 0.01 % or more of raw and cooked (50 and 90 °C) cod loins, and the sum taken as total ash. Results are shown with extended standard uncertainty, and combined standard uncertainty, respectively.

High content metals (% content)			
Metal	Raw	50 °C	90 °C
K (Potassium)	0.53 ±0.11	0.46 ±0.10	0.47 ±0.11
S (Sulfur)	0.37 ±0.18	0.29 ±0.06	0.33 ±0.07
P (Phosphorus)	0.33 ±0.16	0.23 ±0.05	0.24 ±0.05
Na (Sodium)	0.043 ±0.021	0.030 ±0.006	0.031 ±0.007
Mg (Magnesium)	0.032 ±0.007	0.029 ±0.006	0.031 ±0.007
Ca (Calcium)	0.010 ±0.002	0.009 ±0.002	0.010 ±0.002
Total ash	1.32 ±0.27	1.04 ±0.13	1.10 ±0.14

The relative contents of ash (%) are illustrated, together with the total water and protein content in Figure 5.4. Additionally, the figure presents the measured % content of fat, along with its standard deviation of five analytical measurements (n=3) in raw fish (0.30 ±0.03). As can be observed, the amount of water, protein, ash and fat practically adds up to 100 % in total for the raw fish. Although this observation strengthens the validity of all the quantitative analysis done, the total % contents measured could be several percent higher or lower than 100 % taking into account the standard deviations/uncertainties, also that of water and protein as illustrated in 5.2 A.

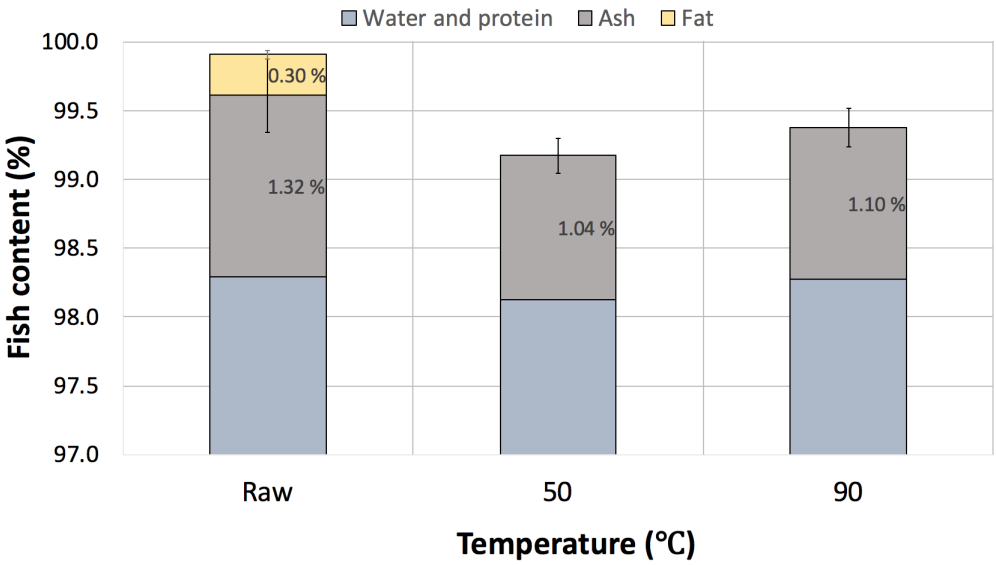


FIGURE 5.4: Ash and fat content (%) in raw fish and ash content of fish cooked at 50 and 90 °C

The relative ash content values (%) were used to calculate the amount of ash

lost during cooking, as illustrated in Table 5.2. For a 100 g raw loin sample approximately 0.4 and 0.5 g of ash are lost during cooking at 50 and 90 °C, respectively.

TABLE 5.2: Calculated losses of ash

Sample	Fish (g/100 g raw)	Ash loss (g/100 g raw)
Raw fish (orig. present)	100 g	1.32 ±0.27
Cooked fish, 50 °C	88.66 ±1.16[a]	0.39 g
Cooked fish, 90 °C	77.14 ±1.47[a]	0.47 g

[a] Value and uncertainty (figure 5.1 A) used in calculation of ash loss.

5.1.5 Salt contents and losses

The measured contents of chloride (%) and the corresponding values of sodium chloride (% and g/100 g raw fish) are illustrated in Table 5.3. No significant differences were observed for the % content nor the salt retained (g/100 g raw) during cooking, between that in the raw fish and the fishes cooked at temperatures between 30-70 °C. Therefore, the average and standard deviation of the salt content was taken as the total of that in the raw fish and that of the cooked fishes, respectively. The average of the relative salt content (%) of the raw fish was 0.17±0.02 (n= 5). Similarly, the relative salt content measured for raw fish at Nofima BioLab in Bergen was 0.16 ±0.05 % (n= 5), which is practically the same result. Even though all the salt content values given in Table 5.3 lie close to the detection limit of the instrument, which undermines the validity of the values given by chloride titration, the agreement with the Nofima Biolab result strengthens the validity.

The average of the salt retained (g/100 g raw fish) in the cooked fishes were 0.18 ±0.02 g (n= 10), which is not significantly different to the salt content in raw fish. A significant difference was, on the contrary, observed between the relative salt content %) and salt losses (g/100g raw fish) between that in the TL and CLs analysed. The salt losses (g/100 g raw) was 0.01 ±0.00 for the TL (n=2), and 0.02 ±0.00 for the cook loss at 50 °C (n=5) and at 70 °C (n=4). This could indicate that salt is more expelled during cooking, compared to at tempering. These values and the difference is though very small, and as mentioned earlier, the values can not be fully trusted. The detection limit for reported for the procedure used was 0.25% NaCl.

What can be said is that the amount of salt expelled confidently can be asserted to be below 0.02 g, when cooking 100 g of raw sample at the two temperatures tested. This small amount could explain the lack of significant differences observed between the about 0.17-0.18 g of salt that is present in 100 g raw and cooked fish. A variation of 0.02 g is less than the amount of salt present in biological different fishes,as is illustrated by the standard deviation of 0.02 g for the raw fish, and as was observed for the cooked fishes as well.

An interesting observation is made when focusing on the amount of Cl^- instead of NaCl. Approx. 0.10 % Cl^- is measured to be present in raw fish, using

this titration method. But the results from the metal analysis, illustrated in Table 5.1, showed that only 0.04 % sodium was present in raw fish. On the contrary, potassium was present in a relatively high amount, namely 0.5 %. With this in mind, it can be indicated that at least half of the Cl^- is bound to potassium, instead of sodium. Therefore, the conversion of Cl^- directly into NaCl is debatable.

TABLE 5.3: Contents of Cl^- (%) and salt as NaCl (%) in raw and cooked fish, and LLs. And NaCl retained by cooked fishes and lost in the LLs (g/100 g raw). Different superscript symbols for the g NaCl /100 g raw represent significant differences.

Sample	Cl^- (%)	NaCl (%)	NaCl (g/100 g raw fish)
Raw fish (n=5)	0.10 ±0.01	0.17 ±0.02	0.17 ±0.02^{+}
Cooked fish, 30-70°C (n=10, 2 per temp.)	0.12 ±0.02	0.20 ±0.03	0.18 ±0.02^{+}
Thaw loss, 0 °C (n=2)	0.13 ±0.00	0.22 ±0.00	0.01 ±0.00°
Cook loss, 50 °C (n=5)	0.10 ±0.02	0.17 ±0.00	0.02 ±0.00*
Cook loss 70 °C (n=4)	0.07 ±0.01	0.12 ±0.02	0.02 ±0.00*

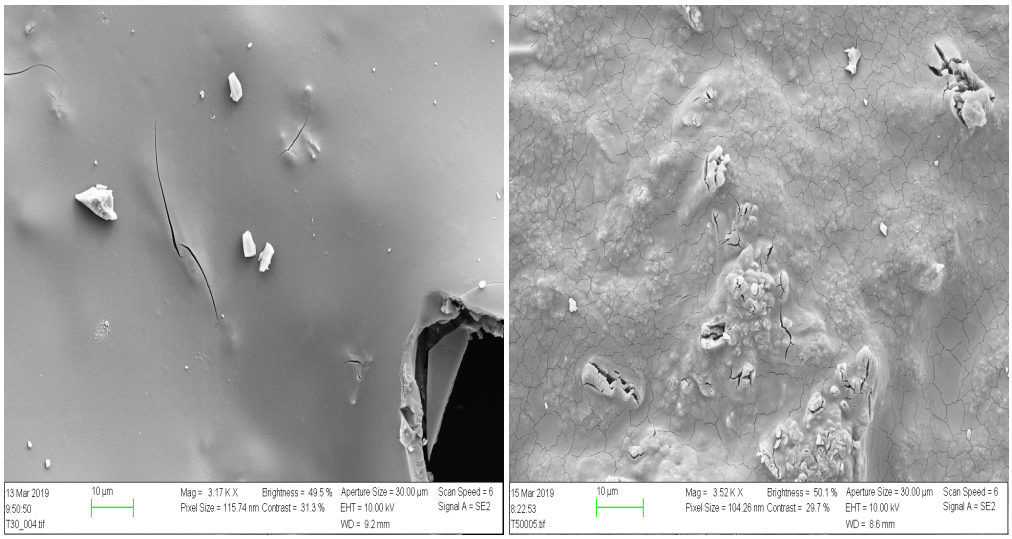
5.2 Proteins in liquid losses

5.2.1 Scanning electron microscopy

The results from the SEM analysis indicated that carbon, oxygen and nitrogen were the elements that were present in largest amount in all of the CLs analysed. With the exception of these, potassium, sulfur, phosphorus, and chloride, in that order, were consistently quantified in a large amount in the CLs, already at 30 °C. These results are in agreement with the % metal contents measured by BioLab, as previously illustrated in table 5.1.

Figure 5.5 presents SEM images of CLes retrieved after cooking at (A) 30 °C, (B) 50 °C, (C) 70 °C and (D) 90 °C. The resolution is 10 um. The most noticeable observation is that the proteins get bigger in size for larger temperature CLs, larger than 10 um in the 70 and 90 °C CLs.

The surface of the 30 °C CL exhibits a large compact and uniform sheetlike structure. The CL samples from higher temperatures seem to contain more globular proteins. However, the surfaces appears to be more fragmentary and folded for each temperature increase. Fibrous proteins seem to be apparent in all CLs, with increasing size according to increasing HTP.



(A) Cook loss 30 °C (B) Cook loss 50 °C

(C) Cook loss 70 °C (D) Cook loss 90 °C

FIGURE 5.5: SEM images of freeze dried liquid expelled at (A) 30 °C, (B) 50 °C, (C) 70 °C, and (D) 90 °C. The green scale line represent 10 µm.

5.2.2 Residual denaturation enthalpy

Figure 5.6 A illustrates the average thermograms (n=3) obtained for TL and CLs from the HTPs 30-70 °C, previously heated in a water bath for 10 min. The DSC analyses gave a thermogram with four clearly defined peaks with minima at about 42-44, 58, 70-73 and 80°C. With increasing temperatures up to 70 °C, the four endothermic peaks diminish gradually and according to the denaturation temperature (with the exception of peak 4), until no peak can be detected.[1]Peak 1 disappears when heating at 40 °C. Peak 2 and 3 are strongly reduced for each step increase in HTP temperature between 30 and 60 °C. Peak 2 disappears at 60 °C, whilst peak 3 disappears at 70 °C. At 40 °C a 4th peak emerge. This peak can be slightly observed at 60 °C, but it was too small to be integrated. On rescanning the samples no peaks were seen indicating that the new or residual structure could be destroyed by heat. The integrated peaks of residual denaturation enthalpy, RDE (ΔH), as a function of maximum denaturation temperature (T_{max}) are plotted in Figure 5.6 B. The following observations were made for each peak:

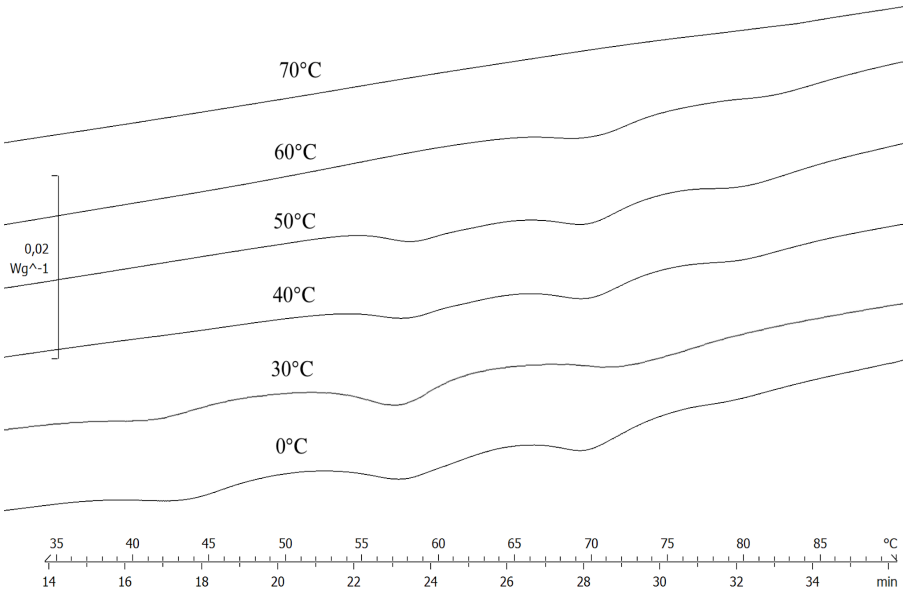
1. A significantly ($p<0.05$) lower T_{max} value is observed for the 30 °C CL compared to the TL. Does not exist in any other CLs.

2. ΔH decreases significantly ($p<0.05$) for the CL between 30 and 40 °C.

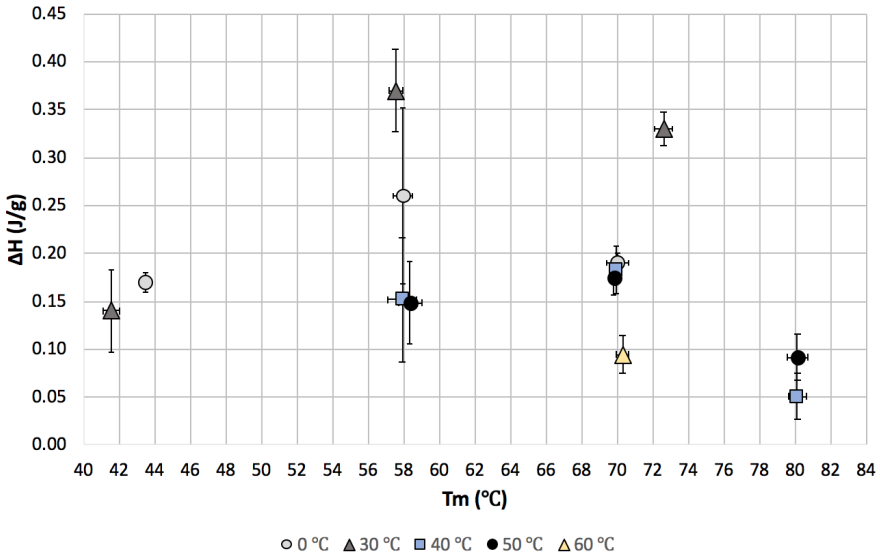
3. Has a significantly ($p<0.05$) higher T_{max} value for the 30 °C CL compared to all other CLs, which could be influenced by presence of peak 4. Peak 3 ΔH decreases significantly ($p<0.05$) for the CL between 30 and 40 °C, and between 50 and 60 °C.

4. Start to prevail in the CL at 40 °C, the ΔH is not significantly different from the CL at 50 °C ($p>0.1$), and the peak disappears for the CL at 60/70 °C.

[1]However, further unfolding of proteins cannot be precluded if additional heat load is applied.



(A) DSC thermograms; curves have been shifted along the y-axis for comparability reasons.



(B) Integrated DSC enthalpy peaks

FIGURE 5.6: (A) Average DSC thermograms (n=3) for TL (0°C) and CL (30-70°C) and (B) the ΔH peaks as a function of maximum denaturation temperature (T_{max}). The heating rate was 2.5 °C/min. Mean values are shown with standard deviation (n=3).

The integration value of the complete thermograms, which includes overlapping peaks, for each sample parameter is illustrated in table 5.4 (n=3). A significant decrease in total ΔH is observed going from 30 to 40 °C, and from 50 to 60 °C.

TABLE 5.4: Total denaturation enthalpy of the peaks. Values with different footnotes represent significant differences ($p<0.05$). NO: not observable.

Sample	ΔH_{tot} (J/g)
0 °C	$1.19 \pm 0.18^{+}$
30 °C	$0.98 \pm 0.06^{+}$
40 °C	$0.62 \pm 0.05^{*}$
50 °C	$0.66 \pm 0.01^{*}$
60 °C	0.09 ± 0.02^{o}
70 °C	NO

5.2.3 SDS-PAGE

Approximate molecular weights of proteins expelled in the LLs are illustrated by the SDS-PAGE image in Figure 5.7 [2]. The quantitative amount of proteins seem to decrease consistently with higher HTP temperatures, especially between 60 and 70 °C, which is in agreement with the % content findings that were presented in Figure 5.2 B. A distinct simultaneous increase in %water and decrease in %protein was observed for the LLs between 60 and 70 °C. In addition, new distinct proteins of large molecular weights ∼200 kDa are expelled at 70-90 °C.

In order to characterize these compounds, further research is required, including analysis using peptide mapping or mass spectroscopy among other techniques. Nevertheless, their origin can be presupposed. The spots were tentatively identified mainly based on the SP found in the drip loss of cod (Vang, 2007) and two of these SP proteins were identified based on two proteins that are present in a high amount in cod (G3P and TPI) (Kjærsgård, Nørrelykke, and Jessen, 2006), identified by mass spectrometry. The molecular weight of collagen is based on the findings of Aminudin et al.(2015), who extracted collagen from three different freshwater fishes. They found that collagen forms high molecular weight bands between 60-200 kDa. Collagen is most likely present in the TL and CL at low temperatures, as elucidated from the SEM image in Figure 5.5 and DSC analysis in Figure 5.6.

The SDS-PAGE indicates that large molecular weight MF proteins are expelled at higher temperatures. Therefore, other MF proteins could be present in the LLs, and these are represented in parenthesis in Figure 5.7. The MF protein bands are identified based on weakly discerned bands observed in the drip loss of cod (Vang, 2007) and after the findings of Rosas-Romero et al. (2010), who analysed the CL from squid and found only MF proteins. In addition, the identification is based on MF proteins present in high amount in cod (Kjærsgård, Nørrelykke, and Jessen, 2006). The MF bands are supported by findings made by Hashimoto et al. (2004) and Abdollahi and Undeland (2018), who extracted salt-soluble proteins from cod.

[2]The two gels produced yielded similar band patterns, thus indicating that heating of fish promoted an analogous protein and/or peptide denaturation and lixiviation regardless of the age, size or physiological condition of the individual.

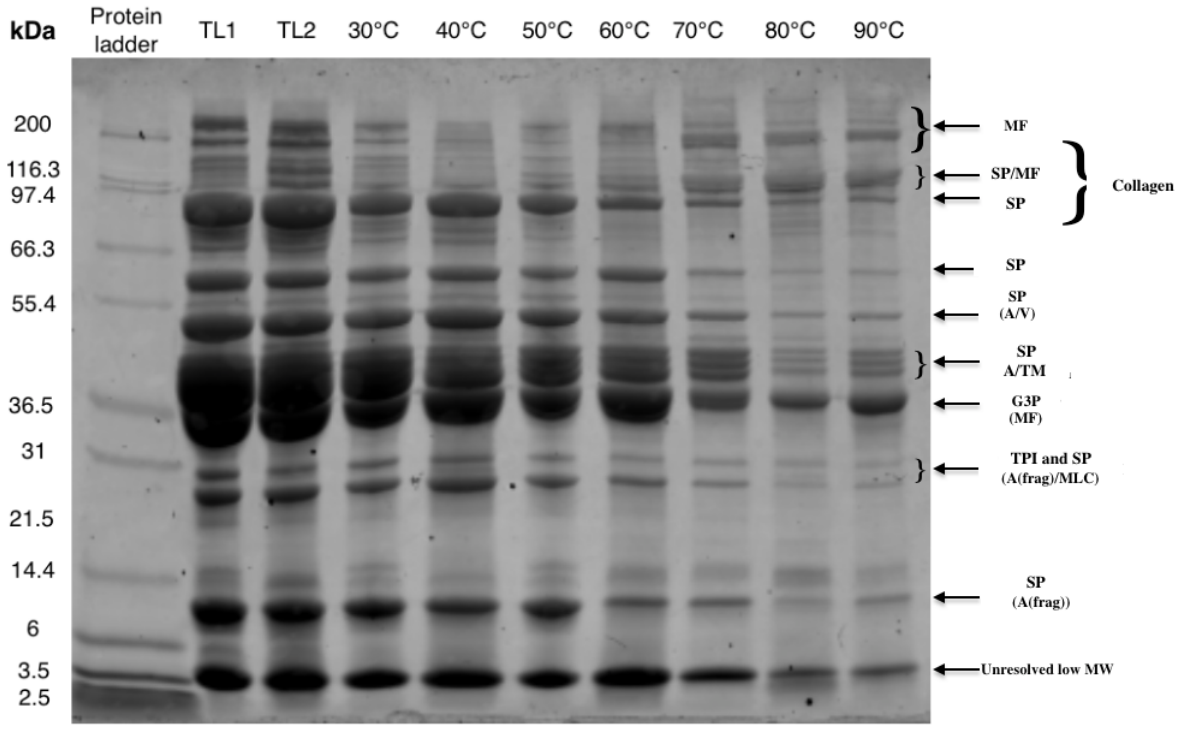


FIGURE 5.7: SDS-PAGE of 2,5 % diluted TL and CL (30-90°C). Every second lane represent TL and CL from the same loin. MF: myofibrillar, SP:sarcoplasmic, A: actin, V:vemitin TM: tropomyosin, G3P: glyceraldehyde-3-phosphate dehydrogenase, TPI: triose-phosphate isomerase, A (frag.): actin fragment, MLC: myosin light chain. Based on findings of (Vang, 2007; Kjærsgård, Nørrelykke, and Jessen, 2006; Aminudin et al., 2015; Rosas-Romero et al., 2010)

5.2.4 pH

5.8 illustrates the change in pH for cod fish and liquid losses in response to heat treatment. The pH of the cook loss increases significantly ($p<0.05$) from about 6.2 up to about 6.35 between 40 and 60 °C. A similar trend can be observed for the fish, but the increase in pH is not significant. The pH of the fish increases significantly ($p<0.05$) from 6.3 up to about 6.5 between 40 and 90 °C. It seems like the expelled liquid consistently has a slightly lower pH than the fish, and a significant difference is in fact observed between the fish and CL at 90 °C.

These results indicate that the same change in protein configurations occur in both fish and CL at 60 °C, but a significantly different change in configuration occurs at 90 °C. These results can be interpreted in light of the residual denaturation results obtained by DSC. Between 40/50 and 60 °C sarcoplasmic proteins with a Tm of 58 is completely denatured. Between 60 and 70 °C sarcoplasmic proteins with a Tm of 70 °C is completely denatured, and myofibril proteins with a Tm of 80 °C are almost completely denatured.

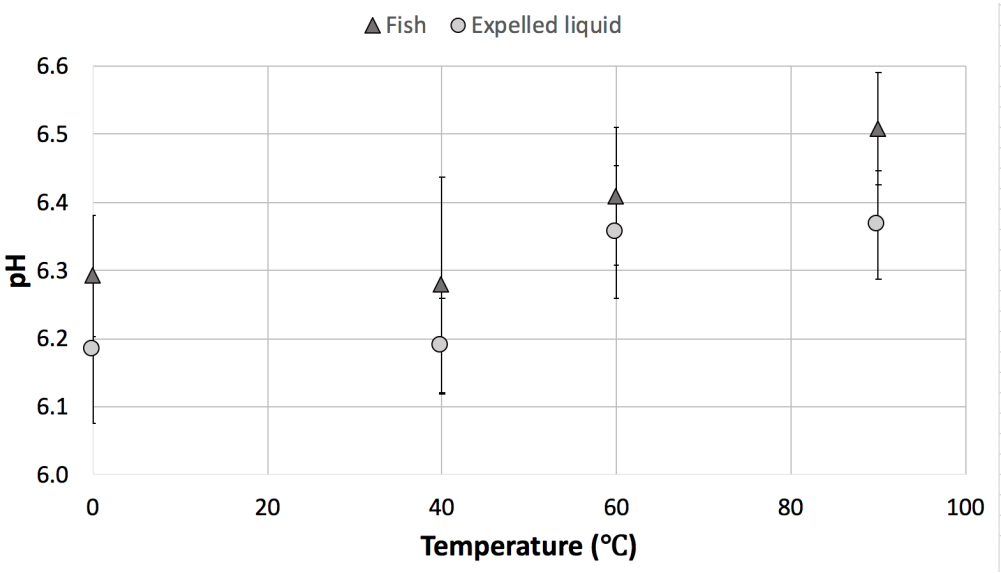


FIGURE 5.8: pH of raw fish and thaw loss (0°C) and cooked fish and cook loss (40, 60 and 90°C). Mean values are shown with standard deviation (n=6).

5.3 Chemical and physical properties

5.3.1 Density

The density of myotomes from fish samples cooked at 50 and 60 ° was measured to be 0.93 ±0.01 g/mL (n=2 and n=3, respectively). These represent a slightly lower density, although not significant, than that for raw fish, which were measured to be 0.95±0.01 g/mL(n=2).

Figure 5.6 illustrate the measured values of density for CL. In addition, the density of thaw loss was measured to be 1.05 ±0.01 g/mL (n=3). An apparent decrease in density is observed with increasing temperature, thereafter the density starts to increase again for the liquid expelled at 80 °C. The observed differences are though not significant ($p<0.05$)

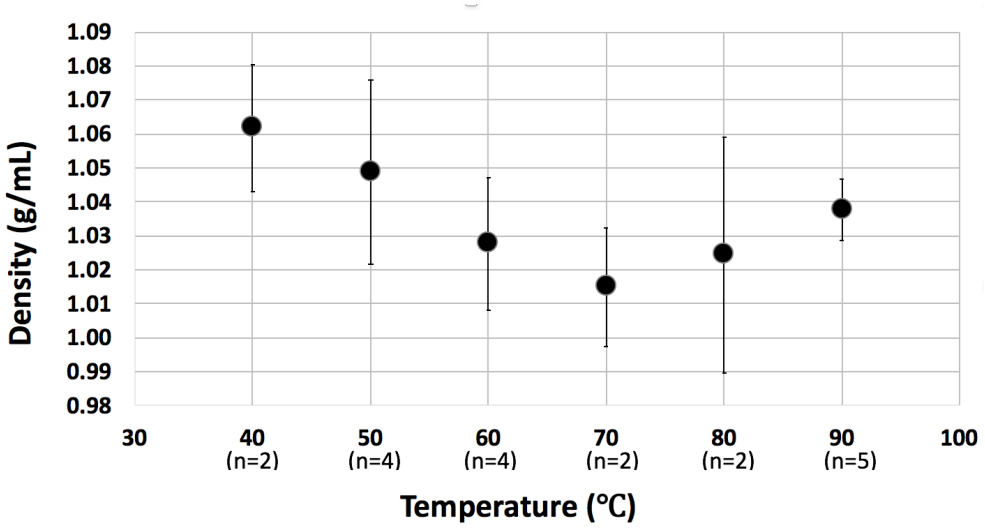


FIGURE 5.9: Density of cook loss expelled at 40-90°C.

5.3.2 Specific Heat Capacity

Figure 5.10 represent the result from the Cp measurements. The R^2 values were always higher than 0.96, with the exception of one curve (60(2)), which had an R^2 value of 0.86. The mean coefficients obtained by simple linear regression, and their standard deviations, are summarized in 5.5. After a small decrease in the Cp (intersection value) from 3.2 to 3.0 between 40 and 50 °C, the Cp increases significantly and attains a value of 3.4 at 60 °C.

The Cp for the CL at 30 and 40 °C increases linearly with increasing temperature, as indicated by the high Pearson´s R (<0.9). At 50 °C (R=5) the Cp increases more as an S-curve with increasing temperature, but linearity is higher for the Cp of CLs at higher temperature.

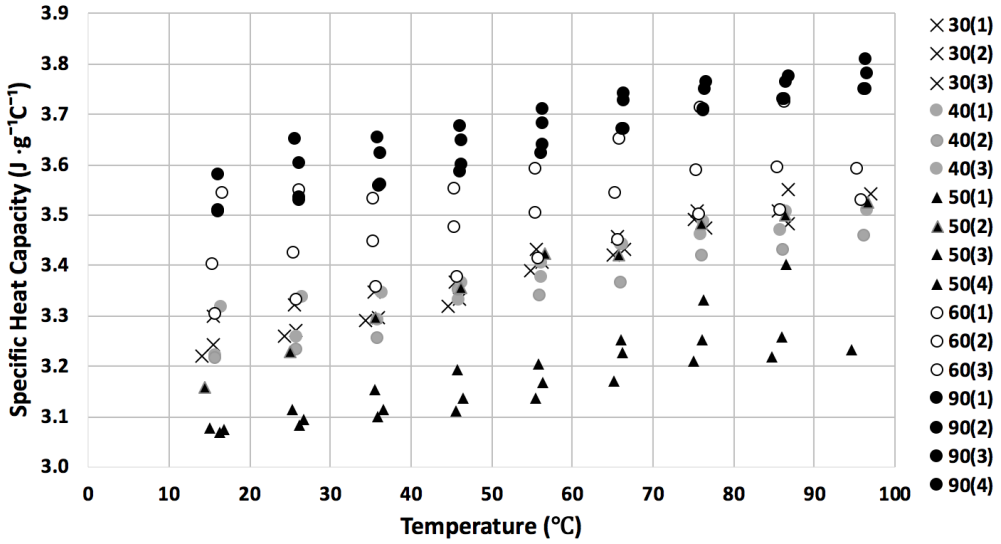


FIGURE 5.10: Specific heat capacity as a function of temperature from 10 to 100 °C for the expelled liquid resulting from cooking at 30, 40, 50, 60 and 90 °C. The numbers in parenthesis represent biological parallels

TABLE 5.5: Mean coefficients a and b of the simple linear regression line of specific heat capacity as a function of temperature for liquid expelled at 30, 40, 50, 60 and 90 °C. Values with different footnotes represent significant differences ($p<0.05$). The Pearson correlation (a number between -1 and 1) indicates the extent to which the two variables are linearly related (more than 0.45 indicates strong correlation)

Linear regression of specific heat capacity points			
Cook loss	a_{mean}	b_{mean}	Pearson´s R
30 °C (n=3)	0.004±0.001	3.19±0.03*	0.97
40 °C (n=3)	0.003±0.001	3.20±0.04*	0.91
50 °C (n=4)	0.003±0.000	3.04±0.03*	0.51
60 °C (n=3)	0.003±0.000	3.42±0.09^{+}	0.62
90 °C (n=4)	0.003±0.000	3.50±0.03^{+}	0.74

5.3.3 Viscosity

5.3.4 Complex viscosity

Figure 5.11 presents the changes in complex viscosity corresponding to the change in temperature over the range 20 - 80 °C at a heating rate of 2.5 °C/min. As can be observed, the complex viscosity increases above their cooking temperature, in correspondence to their denaturation transition temperatures measured by DSC (see 5.6).

A prominent observation is the markedly larger increase in the complex viscosity for the 60 °C CL expelled from the farmed, pre-frozen fish, compared to the 60 °C CL expelled from fresh, wild fish.

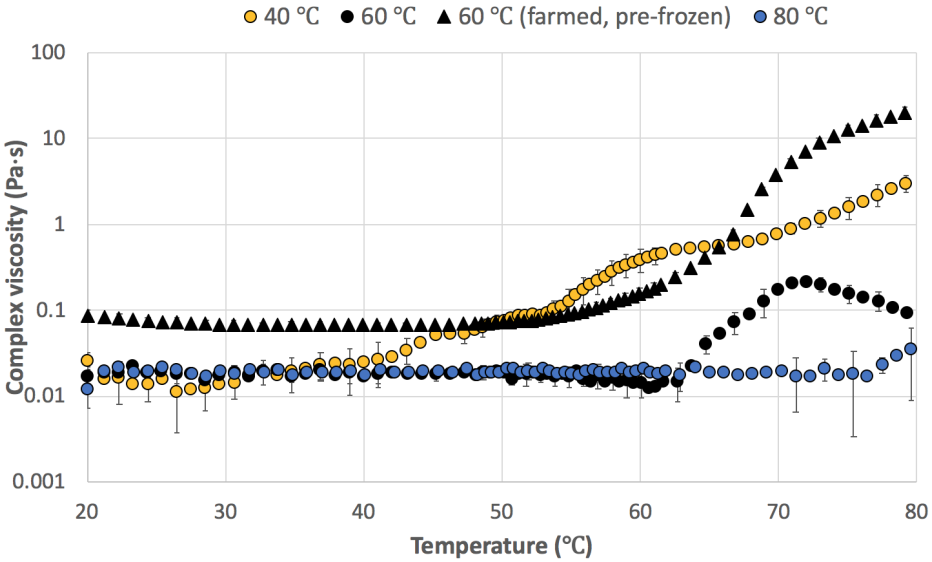
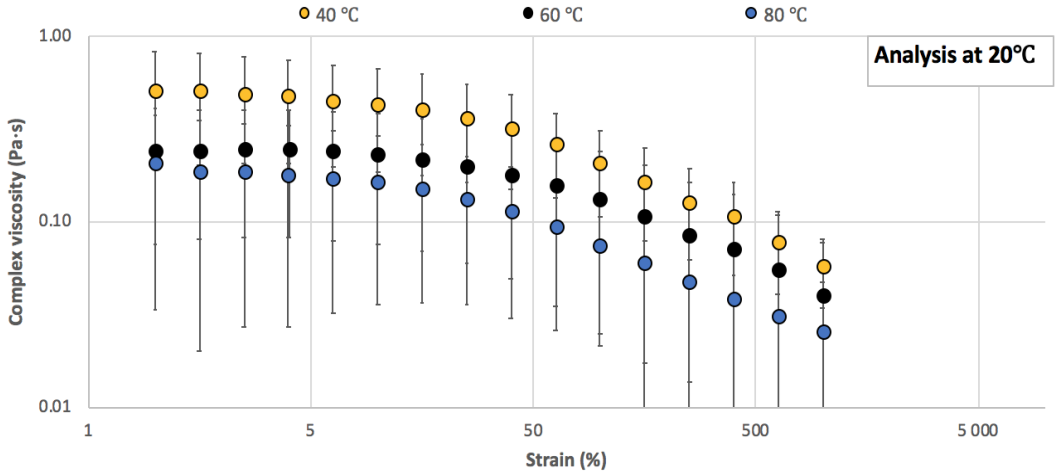
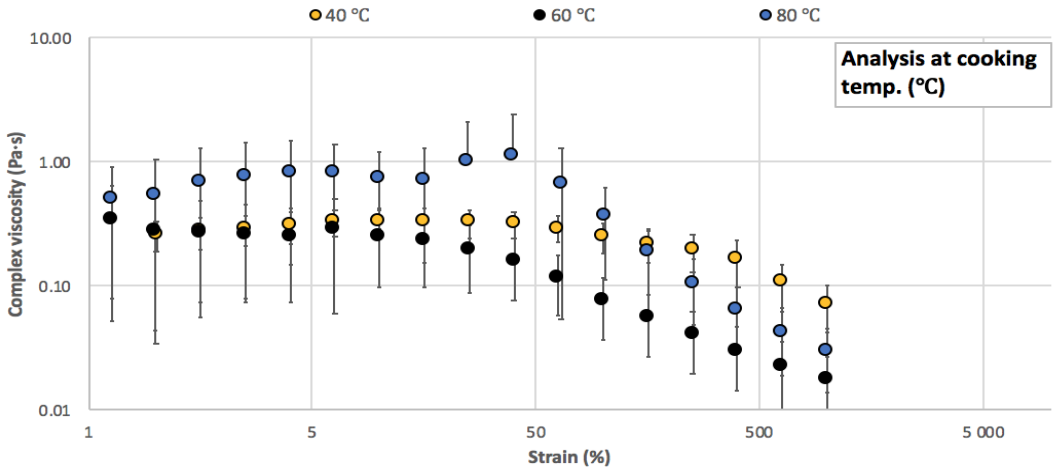


FIGURE 5.11: Temperature ramp (ramp rate 2.5 °C/min, 1.59 Hz, 1 Pa) from 20-80°C of liquid expelled from fresh cod cooked at 40, 60 and 80 °C, and from pre-frozen cod cooked at 60 °C.

Figure 5.12 B presents the complex viscosity of the CLs in their linear viscoelastic region, and the changes that occur when the measurement is performed at room temperature compared to at their respective cooking temperature. The complex viscosity of the 80 °C CL increases slightly due to an observed simultaneously increase in complex storage and loss modulus (not shown). Concurrently, the yield stress against flow, increases slightly from about 0.2 to 1. This observation is just indicative. Relatively large tan δ (= G“/G‘ > 0.1) values, typical of so-called weak gels (not shown), were observed for all samples.



(A) Dynamic oscillation amplitude sweep performed at 20 °C



(B) Oscillation amplitude sweeps at cooking temperatures

FIGURE 5.12: Dynamic oscillation amplitude sweep at 0.01 Hz, and from 0.01-1000 % strain, performed at (A) room temperature (20 °C) and (B) the equivalent HTP temperature of 40, 60, and 80 °C. Mean values are shown with standard deviation (n=4).

5.3.5 Time independent viscosity

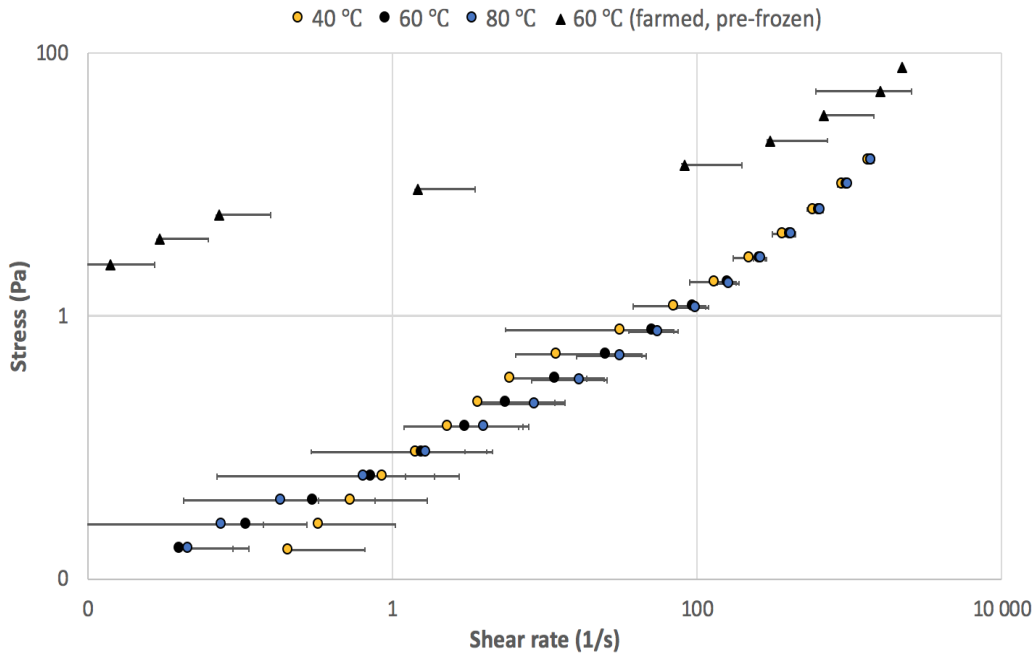
Figure 5.13 illustrates the shear rate that resulted as a function of the applied stress (A) and the corresponding viscosity curve (B) for fresh, wild cod CL from the HTPs 40, 60 and 80 °C, analysed at their respective HTP temperature. The Figure also illustrate the values obtained when analysing the 60 °C CL from pre-frozen, farmed cod. All the curves exhibit non-Newtonian shear-thinning behavior, which is a curvature downwards on the viscosity axis.

Figure 5.13 A reveals a complex flow behaviour that was not possible to model using only one model. The lower part could be modelled using a power law

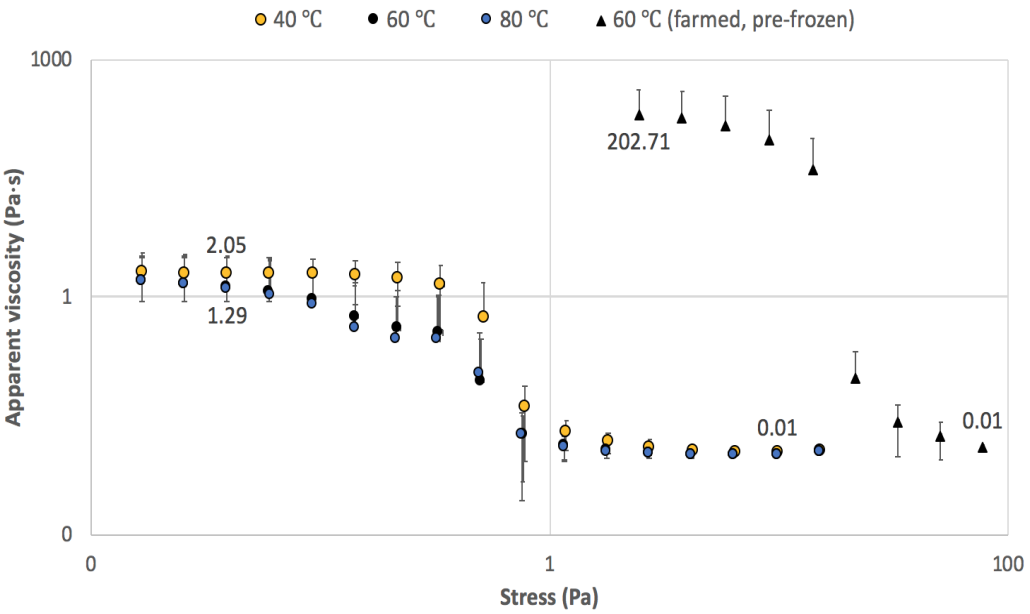
model, while the upper part could be modelled using the modified power law model, namely the Herschley- Buckley model. Using only the latter model would indicate an apparent yield stress, but it can be observed that the CL actually flows beneath this yield stress.

Figure 5.13 B shows that, at relatively low stress, corresponding to a low shear rate, all solutions had a constant viscosity which could be averaged to determine the zero shear rate viscosity. The cross model proved to be a good fit (data not shown), as it usually is for food systems. By inspection of the plateau, the zero-shear viscosity seem to be around 1-2 Pa· s for CL from wild, fresh cod. For pre-frozen, farmed cod the viscosity is about 202 Pa·s (more than 4 SD), which is markedly larger.

The shear-thinning behaviour is similar for all CLs, but the onset and extent depend slightly on temperature, and largely on the type of CL (eg. fresh, wild or pre-frozen, farmed). A slightly larger stress is needed for the 40 °C CL to yield, whilst a stress above 1 Pa is needed for the farmed, pre-frozen CL to yield. The infinite rate viscosity's seems to reach a stabilised value of about 0.01 Pa·s, for all CLs.



(A) Logarithmic plot of stress (Pa) against shear rate (1/s), or flow curves, of CL at 0, 60, and 80 °C. Vertical bars represent the standard deviation in each value.

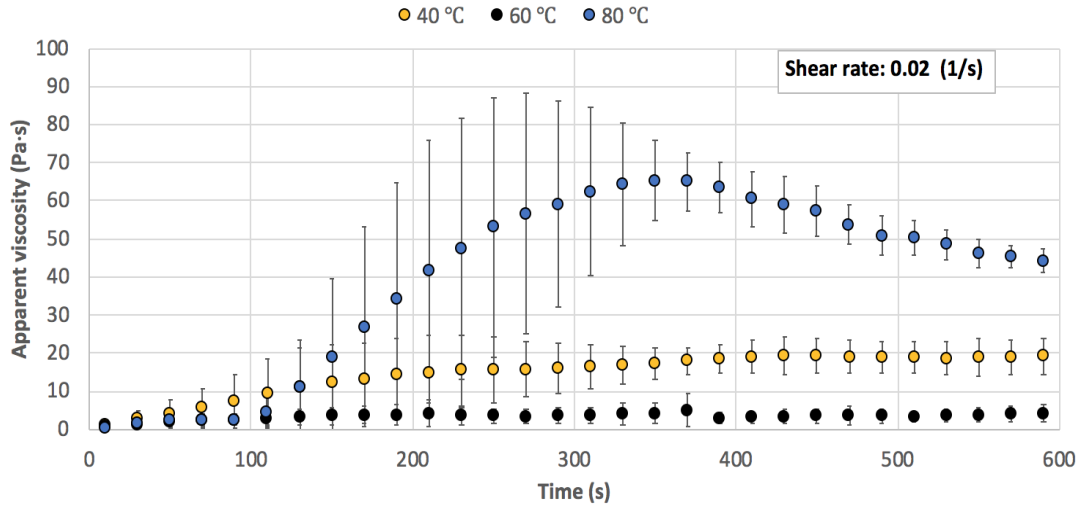


(B) Logarithmic plot of apparent viscosity (Pa•s) as a function of stress (Pa)

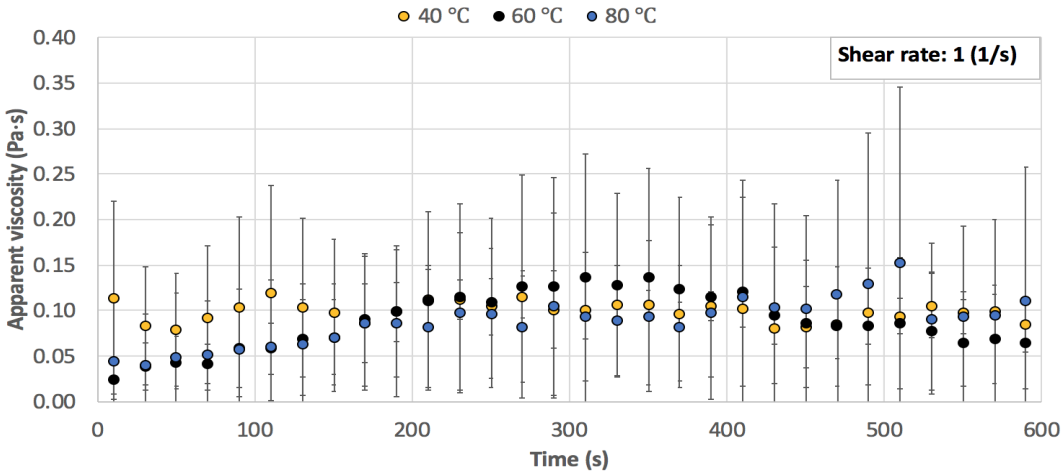
FIGURE 5.13: Stress ramps from 0.01-100 Pa of liquid expelled from fresh cod cooked at 40, 60 and 80 °C, and stress ramp from 0.1-100 Pa of liquid expelled from pre-frozen cod cooked at 60 °C, performed within 240 s at HTP temperatures. The stress ramps are illustrated as (A) the resulting shear rate (1/s), written on the x-axis as is standard, against stress (Pa), and (B) the calculated viscosity (Pa•s) against stress (Pa). Mean values are shown with standard deviation (n=3).

5.3.6 Time dependent viscosity

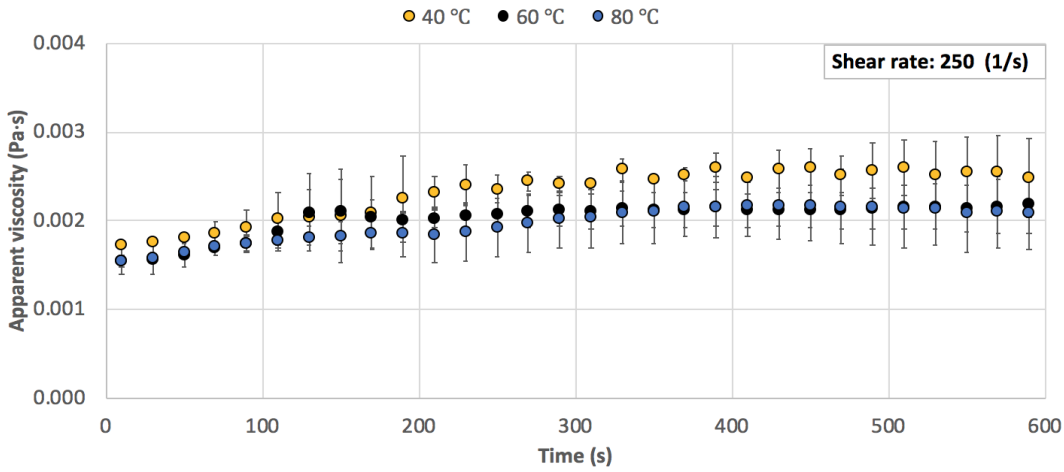
The time-dependent apparent viscosity of the CLs at constant shear rates of 0.2, 1 and 250 1/s are illustrated in Figure 5.14. It can be observed that the magnitudes of the time-dependent viscosity are reduced with higher shear rates, which was expected. This reduction is most prominent for the 80 °C CL, which shows the most time-dependent increase in viscosity at low shear rates. This was not expected.



(A) Constant shear rate: 0.02 1/s



(B) Constant shear rate: 1 1/s



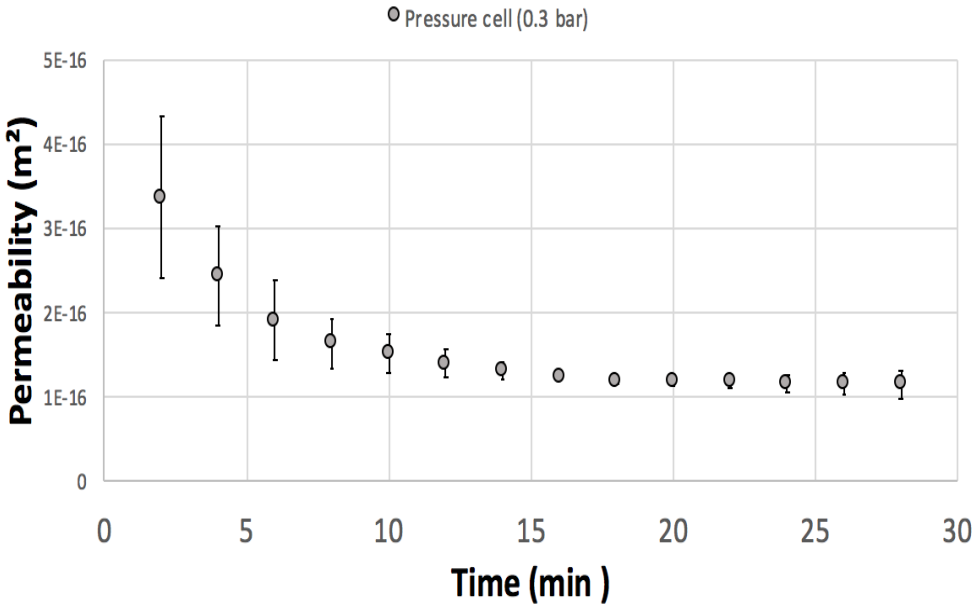
(C) Constant shear rate: 250 1/s

FIGURE 5.14: Apparent viscosity as a function of time at constant shear rates of (A) 0.02 1/s, (B) 1 1/s, and (C) 250 1/s, performed at HTP temperatures. Cook losses tested were from wild, fresh cod cooked at 40, 60 and 80 °C. Mean values are shown with standard deviation (n=3).

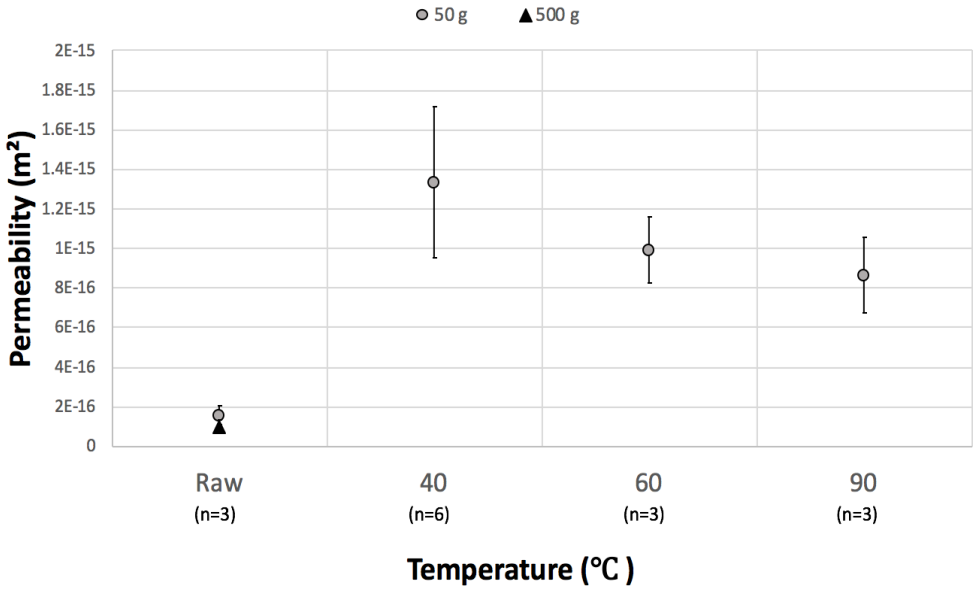
5.4 Permeability of cod fish

The results from the permeability measurements are illustrated in Figure 5.15. The permeability of raw fish upon centrifugation was independent of the two pressures tested ($p<0.05$), and the value was similar to the permeability measured using a pressure cell.

Significant differences are observed between the permeability of raw fish and all the cooked fishes ($p<0.05$). The data in Figure 5.15 B indicates a decrease in permeability of fish cooked at 40 °C compared to that cooked at 60 °C ($p=0.2$) and 90 °C ($p=0.09$).



(A) Permeability of raw fish vs time measured using a pressure cell.



(B) Permeability of raw and cooked fish upon centrifugation

FIGURE 5.15: (A) Permeability of raw fish plotted against time and measured with a pressure cell. Mean values are shown with standard deviation (n=2). (B) Permeability upon 15 min centrifugation of raw (50g and 500 g) and cooked (50g) fish. Mean values are shown with number of parallels and standard deviation

Chapter 6

Discussion

6.1 Liquid losses

The amount of TL obtained in the present study was about 4%, which is similar to the CL obtained after heating at 30 °C. The difference is the high standard deviation of the TL compared to the CL. The amount of TL is in agreement with previous measurements of drip loss from farmed Atlantic cod (1-3 %,Vang2007) and TL from vacuum packed pork fillets (Zielbauer et al., 2015) (4-14 %). After storage at 24 °C, the latter authors reported a LL of 5 %, which was reduced to 2% when not using vacuum. It seems like some of the LL at 30 °C, obtained in present thesis, could be helped to be expel by the vacuum used in the pouches, and such indications could also be observed.

The amount of CL increased markedly with temperature from 30 to 90 °C (see Figure 5.1.1), which is also the trend seen in earlier studies of whole and ground cod muscle (Hodnefjell, 2019; Skipnes, Østby, and Hendrickx, 2007; Skipnes et al., 2011; Ofstad et al., 1993), and of salmon (Kong et al., 2007a; Ofstad et al., 1993). In present study, the CL increased significantly from 4% at 30 °C to ∼11% at 40, 50 and 60 °C. The CL values were almost constant when heating in the range 40 to 60 °C, which is in agreement with the literature. A small increase at 50 °C was observed, as was also reported by Hodnefjell (2019). The CL decreased slightly from 50 to 60 °C, but not to the same extent as observed by Hodnefjell (2019), who cooked minced cod muscle in an oven with 100% humidity for 10 min. At 60 °C, she observed the lowest CL amount (∼5%).

The amount of CL measured in previous studies are generally lower than the amounts observed in the present study. Sous-vide cooked tuna only had a CL of 5 % when cooked for 13 min at 60 °C (Llave et al., 2017). Skipnes, Østby, and Hendrickx (2007) and Skipnes et al. (2011) cooked ground and whole cod muscle (myotoma) for 10 min in a water bath, in a stainless steel sample cup with a filter of 0.213 mm. The CL values were of ∼4 % in the temperature range of 30-60 °C. In present study, glass tubes were used in the vacuum pouches. A reasonable explanation for the discrepancy could be that the pressure from the vacuum squeeze extra liquid out from the samples (as was indicated, see Section 4.1.1. However, Llave et al. (2017) found that directional shrinkage and CL of tuna under vacuum were smaller than those observed with conventional heating processes. It could be that the glass tube used within the present study

did prevented the build up of any possible concentration gradient around the sample, and at the same time it did not interfere with the heating rate, and consequently liquid was easier and faster expelled from the fish, compared to without the glass tube. The CL obtained within the present study is lower than the centrifugation loss (210 g, 15 min) from cod, as measured by Ofstad et al. (1993), which was ∼20 % between 5 and 30 °C. This could be due to methodological differences, since Ofstad et al. (1993) used centrifugation at 210 g, which could suffice to separate more of the liquid within the muscle. Centrifugation in the range of 200-800 g has also proved to change the microstructure of a sample (Trout, 1988).

Ofstad et al. (1993) attributed the centrifugation loss to structural changes appearing in the connective tissue at low temperatures (5-40 °C), due to denaturation and melting of collagen. They observed, after an initial delay, that the centrifugation loss increased rapidly as a function of temperature, reaching a maximum at 45 °C, thereafter it decreased slightly until reaching a temperature of 60 °C. This trend is in accordance with the CL in the present study. According to Ofstad et al. (1993), the maximum centrifugation loss was attained when the muscle cell shrank due to denaturation of myosin. The reduced centrifugation loss at higher temperatures (50-70 °C) was probably caused by aggregates of SP proteins stabilizing the aqueous phase. However, centrifugation and cooking are different. In the present study, the CL increased significantly and steadily with approximately the same amount (3.5 %) for each 10th temperature increase from 60 - 80 °C, and the increase was somewhat higher (5 %) between 80 and 90 °C. The rapid increase in CL is in agreement to previous literature regarding cooking of cod, although the start temperature for this major increase varies (Hodnefjell, 2019; Skipnes, Østby, and Hendrickx, 2007; Skipnes et al., 2011). The underlying mechanisms causing this CL are still being investigated. In the plastic pouch used within present study, the water vapour should be saturated, which should be a hindrance for water vapour to escape by diffusion mechanisms.

Important findings

- Maximum CL is attained at 40-60 °C, and increases up to 90 °C, in accordance to literature.
- The glass tube in the pouch probably increase the amount of CL, compared to literature.

6.2 Water and protein contents

For raw Atlantic cod, the measured contents of water (77 %), protein (22%), ash (1.3 %, of which 0.17 % is NaCl) and fat (0.3 %) are in close agreement with previous literature (Bjørnstad, Meling, and Shamirian, 2018; Ofstad et al., 1993; Skipnes et al., 2008; Vang, 2007; Dyer, 1943; Ghaedian, Decker, and McClements, 1997). The reported values of water and protein contents are only

slightly higher and lower, respectively, than the values obtained in present study.

Skipnes et al.(2011) found that the water content (∼78%) of ground farmed cod muscle did not decrease significantly during heating, even if a CL was observed. They concluded that the content of dissolved proteins in the CL could be comparable to the level of proteins in the fish muscle. They separated the CL while the fish was still hot, to avoid coagulation of dissolved proteins on the fish surface. This was contradicted by the results of Kong et al. (2007a). When heat treating salmon (>100°C), they found that the dry matter content of the CL was <15 %. In their study, the CL was separated from the fish after cooling, and this could lead to proteins coagulating on the fish surface. In present study, the protein content of the CL obtained at 90 °C was even lower, only about 7%. The CL was separated after cooling, but was continuously collected in the glass tube during heating. Coagulation effects should therefore be limited.

In the present study, the water content of the raw cod fish (∼77%) decreases significantly at 70°C (∼73%), accompanied by a similar increase in the protein content (22 to 25 %) (see Figure 5.2 A). The trends observed for the fish is amplified when looking at the CL. The water content in the CL is significantly larger at 50 °C (∼89%), compared to the TL and CL at 30 °C(∼86%), probably due to a large expulsion of water resulting from denaturation of myosin. The CL obtained from heating at 50 °C contains 10 % protein, a slightly lower content than the CL obtained from heating at 40 °C (∼11 %). Obviously, a large amount of proteins are expelled already at this temperature.

As the protein content was not measured for the CL obtained by heating at 30 °C CL, a large amount of proteins could also be expelled at this point, as has been observed for drip losses by previous authors. Drip loss from raw fillets of farmed Atlantic cod contained 4% to 14% protein (Kristoffersen et al., 2007). Savage, Warriss and Jolley (1990) revealed an average of 11% protein in drip loss from pork, and the protein concentration of drip loss from broiler breast meat was about 15 % (Bowker and Zhuang, 2013).

Going back to the present study; from 50 to 60 °C, the water content in the CL again increases significantly (to ∼90%), followed by a simultaneous decrease in protein content (to ∼9%). The slight increase in water content could be due to a delayed expulsion of water after myosin has been denatured, concurring simultaneously with a stabilisation of the aqueous phase, as deduced by Ofstad et al. (1993). Furthermore, the water content increases significantly from 60 to 70 °C (to ∼93%), accompanied by the same decrease in protein content (to ∼6%). After 70 °C, no significant changes occur, even though the amount of CL continue to increase. This can indicate that water and protein are expelled in comparable amounts.

Important findings

- Contents of dissolved proteins in the CL are not comparable to that in the fish muscle; the CL obtained from heating at 40 °C CL contain 10 %

protein.

- The water contents of the CL continuously increase with increasing temperature, followed by a decrease in protein content.
- The water contents of the CL increase significantly at 50 °C and 70 °C.
- Water and protein are expelled in comparable amounts from 70-90 °C.

6.2.1 Validity of the measured protein contents

As noted in Section 3.3.3, using a conversion factor of 6.25 could overestimate the protein content. But looking back at the total % content measured for the raw fish in Figure 5.4, the contents do add up to 100 %. Therefore, it seems that the conversion factor is adequate. Mæhre et al. (2018) analysed cod with brown muscle included, and this could contribute to a lower conversion factor. Even if the protein contents are overestimated, the relative changes in protein contents during cooking, observed in present work, should still hold true, as long as the sample collection are performed in the same manner (discussed in Section 4.1.3, but approved in Section 5.1.3).

6.3 Water and protein losses

Taking into account the original amount of water present in 100 g raw fish (∼77 g), about 3 g is expelled at 30 °C. 9 g of water is significantly expelled already when cooking at 40 °C, accompanied by the expulsion of 1.2 g of protein of the ∼22 g present in raw fish. This is the temperature in which myosin and collagen denatures, and it seems like the expulsion of water and protein occurs quickly in the process of the denaturation.

Further cooking at 50 °C leads to the expulsion of an additional 1 g of water, but no additional protein. SP proteins start to denature around 45 °C, and this could lead to the expulsion of some water. However, it seems more likely that the small LL is due to a delayed expulsion occurring due to denaturation of myosin, as no additional proteins are expelled. Another reason could be that denaturation of SP proteins cause expulsion of water, but at the same time aggregates of SP proteins are formed, stabilising the aqueous phase and contradicting the expulsion of water, and/or proteins. This latter hypothesis is strengthen by the fact that the expulsion of water increases just very slightly (∼0.4 g) when the temperature is raised to 60 °C, at the same stage in which the second group of SP proteins denatures (∼57°C). At this point the amount of proteins expelled are significantly lower, about 0.9 g. A slight increase in the expulsion of water, combined with a decrease in expulsion of protein, is most likely due to aggregates of SP proteins, in which proteins are more retained than water.

From 60 °C the proteins expelled increases to some extent, and attains a significantly higher value at 90 °C (∼ 1.1 g), but at the same time the water expelled

rises steeply as about 3-4 g water is expelled for each temperature increase from 60-90 °C. This could correspond to some denaturation of myosin and the third group of SP proteins (∼69°C), and/or actin (∼76°C).

The total amount of water soluble proteins has been measured to be ∼2.4% of the wet weight of farmed Atlantic cod muscle with a water content of ∼83% (Hultmann and Rustad, 2007). This would mean that, when cooking 100 g cod, 2.4 g of proteins can be expelled. In the present study, this would mean that half of these soluble proteins are expelled (∼1 g) in the CL. In the literature, regarding drip loss, the proteins expelled are smaller. 0.42 g/100 g protein was lost in the drip of farmed Atlantic cod (Vang, 2007). Only 0.03 g protein/100 g of muscle was expelled in the drip from broiler breast meat (Bowker and Zhuang, 2013). It has been hypothesised that relatively large contents of protein in drip losses would mean that the protein content of CLs depends on the heat load and especially denaturation of SP proteins. Obviously more than twice the amount of proteins are expelled in the CLs in the present study, compared to drip losses. But the main loss occurred already at 40 °C, before the main denaturation of SP proteins. Additional heat load did not cause any additional losses before cooking at 90 °C. Similar to these findings, Murphy and Marks (2000) found that the total soluble proteins in ground chicken breast patties, relative to the raw patties, did not decrease before reaching meat temperatures of 80 °C (oven, 95% humidity). Aggregate formation at 60 °C could hinder expulsion of proteins, until eg. the pressure is large enough to expel additional proteins trapped within the fish matrix.

Important findings

- Maximum protein loss occur already at 40 °C. A large amount of water is expelled.
- A significantly smaller amount of proteins is expelled at 60 °C, compared to at 40 °C, maybe due to aggregates of SP proteins.
- A significantly larger amount of proteins is expelled at 90 °C, compared to at 60 °C. Water expelled rises steeply.

6.4 Ash losses

Figure 5.4 indicated that the amount of water, protein, fat and ash added up to 100% for the raw fish. For the contents of the cooked fishes to add up to 100%, the remaining % could be presence of fat. If so, it seems likely that the amount of fat increases when fish is cooked, compared to when it is raw. This could be due to water, protein and some ash being expelled during cooking, in favour of fat.

Variations of NaCl would strongly influence CL and physical properties (Vaka, 2018), but such variations were not observed in present study (see table 5.3). This indicates that NaCl does not play a significant role in influencing the

amount of LLs occurring during cooking of unsalted cod muscle. The high amounts of potassium ions in cod, as found in the LLs by SEM and from the metal analysis (see Section 5.1.4), could though have an impact on the amount of CLs occurring during heat treatment. As most of the ash was already lost at 50 °C (see Table 5.2), potassium (for example) could play a role at a point up to this temperature. Potassium can destabilise many proteins which have a closed native conformation and stabilise those which have an open conformation (Puolanne and Halonen, 2010).

For comparison, oyster contains similar amount of water as cod, but a large amount of NaCl (8.5% ash, in which 7.6% was NaCl)(Kim et al., 2000). Thus, NaCl probably plays a larger role in the CLs from oyster.

Important finding

- Potassium ions could play a role in influencing the amount of CLs occurring at temperatures <50 °C, whereas NaCl most probably has no influential effect.

6.5 Scanning electron microscopy

The SEM image of the CL obtained from heating at 30 °C revealed a thin and sheet-like collagen structure (see Figure 5.5), and this collagen probably stems from inter-cellular compartments. This conclusion is made based on similar appearance to SEM images of inter-cellular collagen from sponges, taken at the same magnification of 10 μm (Tziveleka et al., 2017). The same authors also snapped images of insoluble collagen, which appeared to have a more thread-like structure. The collagen from fish usually has a porous structure. Aminudin et al.(2015) extracted collagen from freshwater fishes, and observed a porous structure under 15000X magnification with SEM. But when fish has been subjected to frozen storage, this porous nature of the cell surface disappears and the collagen fibrils thickens, as has been observed for collagen in Atlantic cod muscle (Gill, Walton, and Odense, 1991).

Globular proteins could be present at 30 °C, disguised by the large collagen structure, but they are mainly observed in the CLs from above 50 °C HTPs, which probably corresponds to SP proteins. They appear more clearly in higher HTP CLs, probably due to the expulsion of more SP proteins with higher temperatures. The globular appearance could also stem from myosin. When Hermansson and Langton (1988) studied fine-stranded gels of myosin from bovine muscle, fringes of globular material surrounding the filament backbone was seen. Sharp and Offer (1992) studied purified myosin in a KCl solution using an electron microscope, and found that at temperatures around 50 °C, large globular aggregates formed. At temperatures above 50 °C structural changes of the myosin tail occurred and hydrophobic interactions lead to network formation. Aggregation could also be affected by presence of NaCl, as has been seen for soy protein gels using SEM (Hermansson and Buchheim, 1981). From

the present work it was found that most of the ash was expelled at 50 °C, mostly comprising potassium ions. Therefore, it does not seem like these ions contribute to the more aggregated structure for higher HTP CLs.

Large fibrous proteins was clearly apparent in the 70 and 90 °C CLs. This indicates that heat treating cod muscle, in a vacuum pouch, could lead to fragmentation of the muscle structure. This could maybe be linked to the shrinkage of fibre bundles concurring with denaturation.

Rocha et al. (2017) extracted soluble and myofibrillar proteins from croaker fish. Using SEM, they observed that the surface of the soluble proteins exhibited a fragmentary structure with the particles forming multilayered sheets. However, the surface of the myofibrillar proteins appeared to be more homogeneous, although folded and wrinkled. The soluble and myofibrillar proteins did not seem to differ much in their structure when freeze dried and pictured using SEM. Also, the SEM images of the CLs presents a combination of collagen, SP proteins and fibrous proteins, and therefore it is difficult to state any conclusions.

Important findings

- Collagen is expelled at 30 °C.
- More SP and/or myosin proteins are expelled at higher temperatures
- Fibrous proteins are expelled at higher temperatures.

6.6 Residual denaturation enthalpy

As found by DSC, the total ΔH decreased between 30 and 40 °C and between 50 and 60 °C. This result suggests that the amount of native proteins expelled in the CL decrease largely around these two temperatures. Less folded, native structures indicates fewer non-covalently bound water molecules (Offer and Knight (1988) as cited by Tornberg (2005)). The lower ability to bind water could mean that they are more prone to aggregation. The proportion of native protein in a sample; could be equivalent to the extent of ordered structure.

In the present study the TL and CL at 30 °C exhibited two significantly different peaks, namely at 42 and 44 °C, respectively. Hastings et al. (1985) isolated connective tissue from the myocomatta of a cod as a source of collagen, and two transition peaks were found at ∼32 °C and ∼40 °C, respectively. Skipnes et al. (2008) found that the collagen transition occurred at 38 °C within cod muscle. As deduced from the SEM images in Section 5.5, collagen is most likely the main protein present in CL obtained from heating at 30 °C CL, and therefore probably in the TL as well. The difference in T_{max} can be attributed to many factors. Previous DSC analyses of meat have shown that T_{max} can be

affected by sample mass, pH, the size of the fish, and the composition of proteins and content of connective tissue (Stabursvik and Martens, 1980; Guzmán-Meza et al., 2017). The T_{max} and ΔH data represents thermal stability of the protein and proportion of native protein in a sample. The pH of the TL and the 30°C CL is probably similar (see Figure 5.8), but the nature and content of collagen and SP proteins could be different, and the amount of native collagen could be lower in the CL obtained from heating at 30°C.

In the present study, two peaks are observed with a T_{max} of 58°C and 70°C. These probably correspond to SP proteins, in accordance with literature. The expelled SP fraction from cooking of hake showed three T_{max} at 45, 59 and 76°C (Beas et al., 2006). The transition of isolated SP proteins from cod occurred at 45, 57 and 67 °C (Hastings et al., 1985). In the study of Skipnes et al. (2008), SP proteins in cod muscle were attributed to T_{max} values at 57 and 69 °C. Zielbauer et al. (2015) found, by DSC, that the centrifugal drip contained the same SP proteins as the whole raw meat of pork fillets, with T_{max} values close to each other. The peaks (53, 63, and 68 °C) were more distinct in the centrifugal drip, due to the lesser amount of connective tissue.

The native SP proteins are present in a larger amount, compared to native collagen, in the CL obtained from heating at 30 °C. These SP proteins could not be observed in the SEM-image (see Figure 5.5). This can mean that the collagen that is expelled has been more denatured in the CL obtained from heating at 30 °C. It could also mean that the expelled SP proteins are small, and/or that the collagen sheet is covering their appearance.

Native SP proteins are present in the largest amount in the CL obtained from heating at 30 °C, compared to CLs obtained from heating at higher temperatures. The first SP group have a ΔH value that is larger at 30 °C, compared to at 40 °C. The second group of SP proteins in the CL obtained from heating at 30 °C has a T_{max} that is slightly shifted towards the higher range of values. The ΔH is also larger. The SP proteins could be less denatured at higher temperatures. According to Thorarinsdottir et al. (2002), a lower content of water can also be the cause. The %water content of the CL obtained from heating at 30 °C is significantly lower than CLs from higher temperatures, as was observed in Figure 5.2 B. Hägerdal and Martens (1976) explained that proteins then become more thermostable due to the formation of inter- and intramolecular electrostatic interactions and the formation of hydrogen bonds in moieties that could be occupied by water. At 60 °C, the second SP group attains an even lower ΔH value. As it has been found earlier, the same amount of protein is expelled at this temperature; as at 40 °C. Therefore, it is logical to assume that the SP protein group is more denatured at this temperature.

A peak with a T_{max} of about 80°C was observed for the CLs obtained after heating at 40 and 50 °C CLs. Actin in whole muscle was found to have a T_{max} of 75-76°C (Bjørnstad, Meling, and Shamirian, 2018; Hastings et al., 1985; Skipnes et al., 2008), which is lower than when isolated. Actin, isolated from an acetone powder, have shown a peak at 81 °C (Hastings et al., 1985). Hastings et al. (1985) also observed that a SP protein sometimes were present in the isolated SP fraction at this temperature. Nevertheless, this protein was not found to be present in the CL obtained from heating at 60 °C CL.

Important findings

- Fewer native protein structures are expelled at 40 and 60 °C. Aggregation is more likely.
- Collagen in the CL obtained from heating at 30 °C is less thermostable than collagen in TL.
- More native and/or thermostable SP proteins are present in the CL obtained after heating at 30°C.
- Actin or a SP protein is expelled at 40 and 50°C, but not at 60 °C. Is probably trapped within the fish matrix.

6.7 SDS-PAGE

The overall trend in Figure 5.7 is that the amount of proteins seem to decrease with higher water content in the CLs obtained from heating at higher temperatures. This is seen as a decrease in some medium/low molecular weight proteins, mostly comprising SP proteins, but could also comprise MF proteins like actin. High molecular weight proteins seem to increase with higher HTP temperatures at 70 °C and above. At 30 °C these proteins are probably collagen, whilst at higher temperatures, they probably correspond to MF proteins, such as MHC.

The background for the tentative identifications made is now explained. Vang (2007) analysed the drip loss from farmed Atlantic cod during iced storage at 4 °C. She observed minor differences between the SP muscle proteins extracted from cod fillets and proteins in the drip loss. The molecular weight of the SP proteins were in the same range as the ones observed by Tadpitchayangkoon, Park and Yongsawatdigul (2010), who studied SP proteins from striped catfish using SDS-PAGE. The molecular weights ranged from 11 to 97 kDa, but most had a molecular weight of 43 kDa. Bands in Figure 5.7 with similar molecular weight has been attributed to these SP proteins. Identification of triosephosphate isomerase (TPI) and glyceraldehyde-3-phosphate dehydrogenase (G3P) was based on the findings of Kjærsgård, Nørrelykke and Jessen (2006), who studied the change in protein composition of cod fish during frozen storage. They found, after separation of proteins using 2-DE SDS-PAGE and identification using MS/MS, that SP proteins involved in glycolysis were present in a large amount (but changed in concentration during frozen storage), and TPI and G3P were two of them. These molecular weights corresponds to two SP proteins found in the drip of cod (Vang, 2007). Two α-actin fragments (A (frag.)) increased during frozen storage, thereby implying an α-actin breakdown, which was expected to result in a softer structure of the thin filaments of muscle sarcomers. As argued by the authors, this would lead to an increased exposure of enzymes like TPI and G3P, located in this region, in respect to both proteolysis and mechanical stress. These SP proteins could consequently also be prone to be expelled during thawing and cooking.

Glycogen phosphoryalse (G)P expelled in the CL from broiler meat was found to have a molecular weight of ∼90 kDa (Bowker and Zhuang, 2013), and could be a SP protein present in Figure 5.7 with the same molecular weight. In fact, the authors who identified this SP protein, also prescribed TPI and G3P to the exact same molecular weights, as was done for these two enzymes in cod (Kjærsgård, Nørrelykke, and Jessen, 2006). GP in CL from broiler meat seemed to decrease with higher temperatures, as it seems to do within the present work. Greaser and Sosnicki (1997) demonstrated that denaturation of GP decreases the solubility of the normally water-soluble protein and causes it to adhere to the myofibrils during subcellular fractionation procedures. Thus, increased protein denaturation and diminished water solubility could have caused the decreased abundance of the apparent GP band in the LLs analysed within the present study.

High molecular weight proteins seem to be present in the TL bands in Figure 5.7, and also for the CL bands from the HTPs 70-90 °C. Vang (2007) observed weakly discernable bands of MF proteins in the drip loss of cod, mostly comprising high molecular weight proteins of approximately 220 kDa. Similarly, past studies have reported that high molecular weight bands of MF origin accumulate with aging in the centrifugal drip fluid of intact pork (Kolczak et al., 2003) and in SP extracts from ground beef (Xiong and Anglemier (1989). Lin, Park, and Morrissey (1995) assumed that washing of minced fresh surimi with water would remove sarcoplasmic proteins which in turn concentrates myofibrillar proteins. However, high losses of myofibrillar proteins during washing and dewatering were observed. The solubility of myofibrillar proteins increased as number of washing cycles increased. Murphy and Marks (2000) found that the myofibrillar protein subunits of molecular weight greater than 43 kDa decreased in chicken breast patties, with increasing temperature from 23°C to 80 °C as analysed via SDS-PAGE. A small amount has been observed in the drip of broiler meat, in which only ∼50 % of the soluble proteins were SP proteins (Bowker and Zhuang, 2013). In present study, some of the the high molecular weight bands are not observed in the CLs from the HTPs 40-60 °C. This could mean that high molecular weight proteins are hindered from being expelled from the muscle matrix, which results from DSC-analysis also indicate.

Vang attributed a band with a molecular weight of 202 kDa to MHC. This conclusion is supported by Abdollahi and Undeland (2018), who made salmon and cod protein powders using a pH shift method, and found that the MHC, the most abundant polypeptide, had a molecular weight of ∼205 kDa. MF protein fractions, from freeze dried cod muscle, showed two major bands as MHC and actin at 200 kDa and 50 kDa, respectively (Hashimoto et al., 2004). It is hard to discriminate which of the high molecular weight bands in Figure 5.7 are MHC, but the band with increased intensity (∼200 kDa) in the lanes between 70-90 °C could likely be MHC. The intensity increase at the same time as the water content increase, which indicates that the amount of this protein increase to a large extent above 70 °C.

Abdollahi and Undeland (2018) found that after MHC, actin (A)was the most abundant protein in cod, having a molecular weight of ∼42 kDa. The MF fraction studied by Vang (2007) gave rise to strong bands attributed to actin and

tropomyosin (TM), but the SP fraction did likewise exhibit strong bands indicating similar molecular weights. Her drip loss samples exhibited similiar strong bands, but whether these resulted from presence of actin or tropomyosin, or SP proteins, or a combination, is hard to evaluate. Similar observations can be made for the TL and CL samples in Figure 5.7, and the same argument can be made; the presence or absence of actin and tropomyosin can not be stated with confidence. Gebriel et al. (2010) found that a number of proteins were repeatedly found in more than one gel sample when analysing SDS-bands from cod using MS. They suggested that proteins either were partially hydrolysed and/or that different protein isoforms were present. Due to these observations, the bands in Figure 5.7 was attributed to not only SP proteins, but also various MF proteins

Michalczyk and Surówka (2007) extracted salt soluble myofibrillar proteins from rainbow trout and analysed them using SDS-PAGE. They attributed a band of 215 kDa to MHC, and two bands of 102 and 107 kDa to α-actinin. The latter protein could be present in the gel in Figure 5.7, but a lot of SP and other MF proteins have molecular weights in this region. There are several proteins having molecular weights in the range between approx. 25 and 60 kDa, as can be deduced from Figure 5.7. Of MF proteins in rainbow trout, these included actin ($\sim$43), β-tropomyosin($\sim$40), α-tropomyosin($\sim$36). Some of the proteins were assigned to the myosin light chain (17.6–35 kDa). Sets of band appearing at molecular weight $\sim$14 kDa were assigned to non-separated low-molecular proteinaceous substances. Thorarinsdottir et al. (2002) found that the MHC was cleaved into smaller sub fragments in the salting process of cod, with the two heavy meromyosin fractions and the light meromyosin fraction being the most abundant. Actin was less affected than myosin. These findings could have analogous mechanisms as to heating of cod, since both processes lead to protein denaturation.

The presence of lower molecular weight MF proteins in the TL and CL is supported by the findings of Rosas-Romero et al. (2010). They analysed the cooking effluent from squid after 25 min cooking at 95 °C. The main components of the soluble proteins and/or peptides in the effluent were attributed to structural proteins, namely the X-protein (X), vimentin (V), and a myofibrillar regulatory protein fragment (MF, 37 kDa). They stated that the latter could be troponin T, γ-actin, α or β chain of tropomyosin, or a hydrolysis product of MHC. Murphy, Marks and Marcy (2000) found that increasing cooking temperature resulted in fragmentation of muscle proteins with high molecular weight (> 40 kDa), when cooking chicken breast patties.

There were far less bands in the drip loss from cod (Vang, 2007) compared to the bands observed in Figure 5.7. This indicates that a larger selection of proteins are expelled from the LLs within present thesis study. This observation could be attributed to the fact that samples studied in present work was TL and CL from pre-frozen cod. Vang studied drip loss from fresh farmed cod in her study. Kjærsgård, Nørrelykke and Jessen (2006) concluded that the biggest alterations in cod fillet protein composition could be subscribed to the frozen storage time, and that distinct changes appear between 6 and 12 months of frozen storage (-30 °C). The fish used within this thesis has been stored at -80 °C for more than 12 months. On the contrary, other authors have not observed

any effect of frozen storage on protein content of TL from beef or drip loss from pork (Diamante, 2016; Ngapo et al., 1999), or any evidence of denaturation of the meat proteins.

Important findings

- Relative amount of protein decrease corresponding to an increase in water content at higher HTP CLs.
- High molecular weight proteins are possibly hindered from expulsion in the cooked fish matrix at 40-60 °C.
- MHC is most likely expelled at 70 °C, and the amount increase to a large extent with higher temperatures.
- When comparing date from results from literature, frozen storage could alter protein composition in cod.

6.8 pH

The pH of raw fish after filleting, rigor mortis, freezing and storage was 6.3, which is similar to the pH measured for pre-rigor slaughtered farmed Atlantic cod in the literature, whether pre-frozen or fresh (Vang, 2007; Ofstad et al., 1993; Skipnes et al., 2008) or treated with 0 % and 1.5 % brine (Vaka, 2018). These relatively low pH values are due to high concentrations of glycogen in the farmed fish muscles at the time of slaughter, as pointed out by Ofstad et al. (1993). Glycolysis supplies ATP; however, under post-mortem anaerobic conditions the muscle cannot maintain normal ATP levels, which leads to a decrease in pH due to accumulation of lactic acid (Gebriel et al., 2010). Similar pH values has been measured for squid (Rosas-Romero et al., 2010), and for its LL after cooking, in which minimal variations were found between the two samples. Vang (2007) found that the pH in drip from cod was approximately 0.2-0.3 units higher than in the muscles, and attributed this to differences in buffering capacity or soluble amines. Within present study, the pH of the LLs were similar to the fish, but always somewhat lower.

When cod was treated with 4.5 % brine the pH was significantly reduced (Vaka, 2018). In present study, it was found that heating temperature influenced the pH of the fish, which increased from 6.3 to 6.5 between 40 and 90 °C. This indicates changes in protein configuration, probably in accordance with denaturation of myosin, SP proteins and/or actin. From the literature it can found that heating meat usually increases the pH. For example, Kauffmann et al. (1964) found that pork muscle usually increased about 0.35 units in pH upon heating. Hultmann and Rustad (2007) also observed an increased muscle pH for temperature abused Atlantic cod fillets during storage. Hamm and Deatherage (1960) found an increase of about 0.4 pH units when heating beef. The latter authors showed that the major decreases in WHC of beef muscle with temperature were paralleled by a decrease in the number of acidic

groups on the protein chains, and by an increase in pH. They attributed the decreases in WHC to the decrease in electrostatic repulsion between the protein chains due to the disappearance of acidic groups. The decrease in repulsion permits closer packing of the protein chains, with less room for immobilised water. It has been pointed out that a pH closer to the isoelectric point (pI) of the MF proteins will increase the protein-protein attractions (Hermansson, 1986). Increased pH is consistent with the large water loss above 70 °C, as was seen in Figure 5.3 A.

In the present study, the pH of the CL increased from 6.2 to 6.45 between 40 and 60 °C. Consequently, when the pH of the CL is closer to the pI of proteins, they have less charge, and the lack of charge may cause molecules to be randomly aggregated. The increased pH observed in the present study would make myosin more soluble (Korzeniowska, Cheung, and Li-Chan, 2013). The myosin tail has an increasing negative charge from pH 5.5 to 6.5 (Offer and Knight (1988), as cited by Tornberg (2005)). pI of MLC and actin (fragments) in Atlantic cod is in the range of 3.6 to 5.4, whilst pI of enzymes are in the range of 7.1-8.7 (Kjærsgård, Nørrelykke, and Jessen, 2006). The pI of catfish SP proteins has been determined to be pH 5 (Tadpitchayangkoon, Park, and Yongsawatdigul, 2010). The observed increase in pH could therefore make MF and SP proteins slightly more soluble, but enzymes slightly less soluble. Many polymers (eg. myosin) have the ability to gel, and the transition from one to another can occur by slight changes in pH or ionic strength (Hermansson and Langton, 1988). Swelling and water holding of proteins in general has a maximum at pH 3.0, a minimum at pH 5.0 and from there a constant increase within the physiological pH range of 6.4–7.2 (Hamm (1972) as cited by Tornberg (2005)). Increased disulfide formation can also occur with pH increases above 6 (Tadpitchayangkoon, Park, and Yongsawatdigul, 2010).

The pH of the fish and CL at 90 °C differ. This could mean that more protons are expelled in the LL, which could be in accordance to a large LL expulsion.

Important findings

- pH of the fish increase between 40 and 90 °C, wih correlation to the large water loss above 70 °C.
- pH of the CL increase between 40, 60 and 90 °C, which could increase gel formation.

6.9 Thermophysical properties

Densities

Density and C_p was measured for future modelling purposes of transfer phenomena in cod fish. The values obtained for density within the present study was about 1-04-1.05 g/mL for the CL obtained after heating between 40 and

90°C. This density is slightly higher than for pure water, as is traditionally used for modelling of mass transport in food. Higher density can mean that the water is less structured, due to a lack of a stabilization effect eg. ions have on the proteins. (Puolanne and Halonen, 2010).

The density values of the CLs as a function of temperature was compared to values which were calculated, based on the contents of protein and water (see Figure 5.2 B), according to the equation of Choi and Okos (2001, p. 15). The density of the CL obtained from heating at 50 and 90 °C, using this calculation, was 1.03 g/mL at 25 °C, which is close to the values obtained within the present study. When taking into account the thermal expansion of water, the calculated densities became 1.02 and 0.99 g/mL. If the ash and fat contents (∼2%, see Figure 5.4) had been taken into account in these calculations the density would probably be slightly higher.

The density of raw and cooked myotomes (50/60 °C) at 20 °C, measured in present study, was 0.93- 0.95 g/mL, which is lower than the density of whole cod muscle at 0 °C, which was found to be 1.05 g/mL (Skipnes, Østby, and Hendrickx, 2007). Chopped cod at 19 °C was found to have an even higher density of 1.16 g/mL (Hodnefjell, 2019).

Specific heat capacity

At their respective cooking temperature, the C_p values were 3.2 and 3.8 $g^{-1}C^{-1}$, for the CLs obtained from heating at 50 and 90 °C, respectively. From interpretation of the DSC scans, it was observed that the proteins were not completely denatured after 10 min cooking at 50 °C (the C_p curve was shaped like a S), and this could be the reason for the low C_p value measured. In the start of the cooking process, in which the proteins denature, the C_p would probably have a smaller value. If the liquid is expelled before the proteins denature completely, then the measured value in present work would be more close to the true value. The C_p values have also been calculated according to Choi and Okos (2001, p. 886). The C_p of CL obtained after heating at 50 and 90 °C using this calculation was 3.9 and 4.0 J $g^{-1}C^{-1}$, respectively at both 25 °C and at their respective cooking temperature. This is close to the C_p of pure water ($\sim$4.2$g^{-1}C^{-1}$). These values differ to a large extent to the values obtained within present study.

C_p values normally increase with amount of water. This was usually the case within present study, except for the CL obtained after heating at 50 °C, which exhibited the lowest C_p. Murphy, Marks and Marcy (1998) found that the C_p of SP proteins and MF proteins were strongly influenced by temperature; however, the C_p of stromal proteins were nearly constant across the temperature range considered (10 to 100 °C). Some variations of the C_p values measure within this thesis could be due to the different contents of SP and MF proteins. At last, the C_p values measured for the CLs are lower than that for raw farmed cod muscle (Skipnes, Østby, and Hendrickx, 2007), which is 3.65 $g^{-1}C^{-1}$, even if the CL has a lower content of protein.

Important findings

- The density of the CL was slightly higher than that of pure water.
- The C_p of the CL, with its constituents proteins still in the process of denaturing, was markedly lower than that of pure water, especially for CLs obtained from lower HTPs.

6.10 Viscosity

6.10.1 Complex viscosity

The complex viscosity (CVi) of CLs obtained from heating wild cod samples at 40, 60 and 80 ° was measured in the range 20-80 °. The CVi of the CLs increased rapidly after reaching their respective HTP temperature (see Figure 5.11). The CVi of the CL obtained from heating at 40 °C started to increase at 40 °C, and then the CVi peaks at two additional temperatures, namely at 55 °C, and at about 70 °C. This is in accordance with the DSC peaks observed for this CL (see Figure 5.6), namely denaturation of collagen, two groups of SP proteins, and one actin/SP protein group. The CVi of the CL obtained from heating at 60 ° peak at about 70 °C, corresponding to denaturation of the second group of SP proteins. Meat juice analysed by Shibata-Ishiwatari, Fukuoka and Sakai (2015) exhibited two endothermic peaks as measured by DSC, corresponding to two groups of SP proteins. The CVi consequently increased within this temperature range, starting at 60 °C.

Shibata-Ishiwatari, Fukuoka and Sakai (2015) performed dynamic viscoelasticity measurements of meat juice containing 79 mg/mL of protein, using the same parameters as within the present study (1 Pa, 1.59 Hz). The CVi was observed to decrease continuously with the increase in temperature, up to 49 °C. However, the complex viscosity showed a drastic increase when the temperature exceeded 60 °C, with the maximum value being observed at 70 °C. At this temperature the complex viscosity was larger than 1000 Pa•s. In present study, the CVi did not attain such a high value. The highest value obtained was about 20 (Pa•s). This CL was obtained from heating farmed, pre-frozen cod at 60 °C. It contained a markedly lower amount of protein (∼9%), compared to the meat juice, which could be a contributing factor to the lower CVi.

It can be observed, from the temperature ramp in Figure 5.11, that the CVi, before it increased, at first decreased with higher temperature. This can be observed for all CLs from the fresh, wild cod. It seems like this is due to some changes in protein configurations occurring just before the proteins start to denature. The CL from the farmed, pre-frozen cod decreased over a higher temperature range before it increased, just as for the meat juice analysed by Shibata-Ishiwatari, Fukuoka and Sakai (2015). The complex viscosity of this CL also increases to a larger extent compared to the complex viscosity of all the other CLs from the fresh, wild cod. The CL from the fresh, wild cod could contain more water, as was indicated by visual inspection, which could result in

the markedly lower CVi. This could be due to the fact that brown muscle was cut from the wild, fresh cod loin at room temperature. This was performed at about -0 °C for the pre-frozen, farmed cod loin. Indeed, in Section 4.1.2 it was observed that cutting the cod loin in room temperature could lead to a higher water content in the CL. The fresh, wild cod was also stored at 0 °C for four days before it was purchased and heat treated. It has been shown that protein from fresh cod may be spoiled within three days, while frozen proteins may be more stable (Shaviklo, Thorkelsson, and Arason, 2012). Another explanation for the observed difference in CVi between these two loins, could be based on the fact that pro-longed frozen storage can alter the protein contents of the loin, as discussed in Section 5.7. Finally, it would also be reasonable to think that wild and farmed cod could naturally have a different biochemical content, as have been found in other studies (Rustad, 1992).

Important findings

- The CVi increased markedly corresponding to denaturation of the CLs constitute native proteins.
- The increase in the CVi of the CLs was markedly smaller than for meat juice, most likely due to less protein in the CLs.
- The increase in CVi of CLs from fresh, wild cod was markedly smaller than for the CLs from frozen, farmed cod.

6.10.2 Viscoelastic properties

A solid-like mechanical response to oscillating small strains was observed for all CLs. This response was expected to decrease with higher temperature, and lower protein concentration. But the dynamic strain dependence measurements (see Figure 5.12 and 5.12 B) indicated that in the linear viscoelastic region, the gel properties of the CL obtained after heating at 80 °C increased when it´s complex viscosity was analysed at 80 °C, compared to at room temperature. No changes were observed for the CLs obtained after heating at 40 and 60 °C. This could be due to the presence of MHC in the CL obtained after heating at 80 °C, and consequently gel formation could be more feasible. Strain sweeps of soy protein isolates was performed by Liu et al. (2011). They found that after heating a suspension gel of soy proteins, the storage modulus increased 10 fold. Storage modulus of heat induced gels can increase with increase in protein concentration and pH, as has been shown for whey protein dispersions (Dissanayake, Ramchandran, and Vasiljevic, 2013), and the pH of the 80 °C CL was slightly larger, as was shown in Figure 5.8. It has also been shown that the gel structure of blood plasma decreased with increased protein concentration, due to an increasing degree of random aggregation of the protein gel network (Hermansson and Buchheim, 1981).

The SDS-PAGE (see Figure 5.7) indicated that the CL obtained after heating at 80 ° generally contained larger proteins, including the MHC. A high breaking

force of thermally induced MHC cod gels was observed by Abdollahi et al. (2019). Liu et al. (Liu et al., 2010a) found that, above the isoelectric point, fish myosin swelled and bound a large volume of water. They attributed this to the presence of many charged groups and repulsive forces. At pH values closer to the isoelectric point, the proteins tended to coagulate due to increased protein–protein interactions. During heating at 1 °C/min, myosin formed gels in the pH range 5.5–7.5 (Liu et al., 2010b). The gel properties of fish myosin was also temperature dependent. It has also been demonstrated that MHC in solution forms disulfide bonds when heated at 50 °C and higher (Angsupanich, Edde, and Ledward, 1999).

Important findings

- The CLs behaved viscoelastically at the given experimental conditions.
- The storage modulus seem to increase for the CL obtained at 80 °C, when measured at 80°C.

6.10.3 Time independent viscosity

The stress ramp in Figure 5.3.5 illustrated a shear thinning behaviour of the CLs, which is often observed for colloid dispersions (Dissanayake, Ramchandran, and Vasiljevic, 2013; Dharmaraj et al., 2016; Brenner, 2009). The stress ramp was performed in a controlled stress mode, and this was done as to control relaxation behaviour that often occur for colloid dispersions in controlled shear mode. In the stress ramp, the CL obtained after heating at 40 ° exhibited a slightly larger zero-shear apparent viscosity compared to the two other CLs, and the onset of shear-thinning occured after a slightly larger applied stress. The corx merx rule did not seem to apply for the CLs. The CVi in the linear viscoelastic region, measured at oscillating small strains (discussed in Section 6.10.2), were slightly lower than the zero-shear viscosities, and resulted in different dynamics (eg. the zero shear viscosity was largest for the CL obtained after heating at 40 °C, while the oscillating CVi was largest for the CL obtained after heating at 80 °C). It should be pointed out that the differences are relatively small, and that significant differences has not been investigated. It should also be pointed out that the oscillation sweep was done in controlled strain mode, which is equivalent to shear, and this could affect the viscosity.

The zero- shear apparent viscosity of the CL from the farmed, pre-frozen cod at 60 ° was markedly larger than for any of the fresh, wild cod CLs, analogous to the temperature dependent CVi, as discussed in Section 6.10.1. This indicates that it is not only denaturation that cause a larger viscosity for this CL, but also its physical nature. A larger zero-shear plateau usually correspond to stronger interactions between particles, and consequently a larger stress must be applied in order to make the solution flow. This indicates that more or different proteins are present in the wild, fresh cod solution.

For all CLs the apparent viscosity reduced to a similar value at large stresses,

about 0.01 Pa·s, which is above that of pure water. It is likely that the effect of cooking on the viscosity of the liquid is similar to that of draining under gravity, which usually corresponds to a shear rate between 0.1 and 1 $\frac{1}{s}$ (TA instruments, 2017). This shear rate correspond to a viscosity in the zero shear and/or shear thinning region of the flow curve measured.

Important finding

- The CLs are shear thinning.
- The zero-shear viscosity is markedly larger for the farmed, pre-frozen CL.
- The infinite rate viscosity is similar for all CLs.
- The viscosities of the CLs were always above the viscosity of pure water.

6.10.4 Time dependent viscosity

As was seen for the CL obtained after heating at 80 °, in Figure 5.14 A, the apparent viscosity increased substantially with time when sheared at a low shear rate (which corresponds to the zero-shear viscosity plateau), illustrating "rheopectic" behaviour, or time-dependent increase in strain, known as viscoelastic creep. Thereafter the apparent viscosity decreased slightly, showing thixotropic behaviour, before it stabilised into a constant value. The apparent viscosity of the CL obtained after heating at 60 °C was barely affected by the shear rate and time. The viscosity of the CL obtained after heating at 40 °C was moderately affected. The substantial "rheopectic" behaviour shown for the CL obtained after heating at 80 °C has been observed for preheated protein isolates from cod gels at pH >9 (Brenner, 2009). An elastic response was built up very quickly, followed by creep, and above a critical strain the gels fractured and began to flow.

The exact mechanisms causing this behaviour are probably very complex, but could be attributed to the amount of charge the particles in the solution have, as has been shown for charged globular proteins (Dharmaraj et al., 2016). When the particles are charged, dynamics of short and long ranged attractions are present. This causes competition of attraction and repulsion at intermediate range length scales. If the dispersion have a sufficiently long relaxation time, the structure will rebuild during shearing. This would cause an apparent increase in viscosity, but at a certain point the shear will be sufficient as to disrupt the structure completely, and then the particles can flow. This behaviour has been demonstrated for non-aggregating polysaccharide dispersions (Ikeda and Nishinari, 2001).

This creep behaviour seem to be less present when the applied shear is larger, as was illustrated in Figure 5.14 B and 5.14 C. The increased deformation rate probably gets more and more sufficient as to disrupt the structure at an earlier

time. As could be observed, the apparent viscosities of the CLs reduced to a similar value, and the viscosities became time independent.

Important findings

- The apparent viscosity of the CL obtained after heating at 80 ° increased markedly with duration of time at an low applied shear rate. This could be due to a larger amount of charged particles.
- The CLs became time- independent at large deformation rates.

6.11 Permeability

To the best of the knowledge of the author, no permeability measurements have been reported for fish muscle. In the present study, the permeability of raw cod fish was obtained using two different methods, corresponding to a value of about 2×10^{-16} m^2 (see Figure 5.15). There are very limited available data in the literature for the permeability of whole meat. The permeability of raw beef meat (8 mm thick) has only been reported by Datta (2006), as the range of 10^{-17} to 10^{-19} m^2. It was observed that the permeability varied with the applied pressure. This was not observed within this present study, when the pressure was below 0.3 bar.

Goedeken and Tong (1993) measured the permeability of air through pre- gelatinised flour dough, and found that the porosity was the only parameter that affected the permeability, which varied between 10^{-14} to 10^{-11} m^2. Moist air was forced through partially saturated apple tissues by Feng et al. (2004) to determine the air permeability, which varied between 10^{-13} to 10^{-11} m^2. They found that the permeability increased with porosity. It therefore seems like the porosity of fish muscle tissue is larger than the porosity of whole beef muscle tissue.

A 10 fold increase in the permeability of the fish occurred after cooking at 40 °C, compared to that of the raw meat (see Figure 5.15). Oroszvàri et al. (2006) also measured the permeability when roasting beef burgers and reported a 10 fold increase between burgers roasted at 50 °C and 60 °C. At 70 °C the permeability decreased by 10 fold. They also measured the porosity, and attributed the changes to pore formation or collapse. However, the beef burger example concerned ground meat and it was deduced likely that the increase in permeability would not be as large in whole meat. From the present study, it can be confirmed that the same increase occur, but that the increase occurred at a lower temperature, namely at 40 °C. The pores probably expand as collagen and myosin denatures. Muscle cells of beef meat with a higher permeability in their membranes have shown larger drip loss (Hughes, Kearney, and Warner, 2014). Indeed, the CL increased markedly when cooking at this temperature (see Figure 5.1). Bjørnstad, Meling and Shamirian (2018) found that the liquid holding capacity (LHC) of cod muscle significantly decreased when cooking from 30 to 35 °C, and then further significantly decreased from 35 to 40 °C,

which is in agreement to the observed increase in permeability and CL within the present study.

Furthermore, Skipnes et al. (2008) observed an increase in LHC from 55 °C compared to the LHC at 50 °C, which is consistent with a trend in the decrease in permeability observed within the present study, although not significant. At 60 and 90 °C the pores seem to collapse slightly. It should be noted that the observed decrease in permeability also could be due to a decreased amount of water being available for expulsion. But after the low pressure applied (50 g), a small amount of liquid was expelled, and the fish cooked at 90 °C still contained approx. 70 %water.

Collapsing of pores could be due to SP proteins clogging the pores, or that they stabilise the aqueous phase, as was hypothesised by Ofstad et al. (1993). They found that the amount of extracellular red granulates increased with increasing temperature, in accordance with the transition of SP proteins occurring at 45 °C, 57 °C and 67 °C (Hastings et al., 1985). In some of the experiments done by Hodnefjell (Hodnefjell, 2019), the cod fish actually swelled when cooking at 50- 60 °C, perhaps due to absorbance of more liquid within its structure. If so, this could also cause a decrease in permeability. However, the decrease in permeability is not in accordance to the increased CL at higher temperatures.

Important findings

- Raw fish muscle had a 10 fold larger permeability than beef muscle.
- The permeability increased 10 fold for the fish muscle cooked at 40 °C, compared to raw fish.
- The permeability seemed to decrease from 40 to 90 °C.

Chapter 7

Conclusion

In the presents study, the properties of expelled liquid from Atlantic (*Gadus morhua*) during processing were analysed. The relative amounts, components and properties of cook loss from cod fish muscle were studied as a function of temperature.

Using scanning electron microscopy (SEM), it was observed that inter-cellular collagen was expelled at 30 °C, in accordance to the observation of high molecular weight proteins in the SDS-PAGE (sodium dodecyl sulfate -polyacrylamide gel electrophoresis). This expulsion did not cause any substantial water loss. It was found that the water loss increased markedly at 40 °C, which occurred simultaneously with the expulsion of a large amount of proteins. The permeability of cod muscle cooked at 40 °C markedly increased, compared to the permeability of raw cod muscle. Expansion of pores in the muscle structure could have facilitated the large liquid loss observed at this temperature.

The amount of cook loss did not increase when cooking cod muscle between 40-60 °C. At 60 °C protein losses reduced, which could include less expulsion of actin, as was observed by differential scanning calorimetry (DSC). It was hypothesised that aggregation of sarcoplasmic proteins stabilise the aqueous phase. Fewer native proteins were expelled in the cook loss at 40 and 60 °C, which could indicate that these cook losses are more prone to aggregation. As found by SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis), large molecular weight proteins were expelled below 40°C and above 60°C.

In present study, it was found that the water loss increased markedly between 60-90 °C. It was observed by SEM-images that more fibrous proteins were expelled at higher temperatures, and SDS-PAGE analysis indicated that these proteins included the myosin heavy. The permeability of cod muscle was slightly lower at 60 °C and 90°C, compared to the permeability at 40 °C. This could explain why the water loss did not increase at 60 °C, but the decrease in permeability do not correlate well with the substantial increase in water loss at higher temperatures. Explanations for the loss of water at higher temperatures could be to thermodynamic changes in capillary forces. But from the surface tensile strength measurements, it was found that the fish was completely wetted, which indicates that such changes did not occur.

Thermophysical properties were measured for mass transfer modelling purposes. It was found that the density of the cook loss was larger and that the specific heat capacity was lower, when compared to pure water. The apparent viscosity of the cook losses illustrated time independent shear-thinning non-Newtonian behaviour. The complex viscosity of the cook losses increased substantially according to denaturation of the proteins present. The 80 °C cook loss exhibited time dependent creep relaxation behaviour at low shear rates, resulting in a large increase in apparent viscosity with duration of shearing. It was hypothesized that presence of myosin heavy chain could contribute to this behaviour. It is likely that the effect of cooking on the viscosity of the liquid is similar to that of draining under gravity, which usually corresponds to a shear rate between 0.1 and 1 $\frac{1}{s}$. This shear rate corresponds to a viscosity in the linear viscoelastic and/or shear thinning region of the flow curve, in which it was found that the viscosity was substantially larger than that of pure water.

The results from the present work support the hypothesis present in the literature, which concerns formation of aggregated sarcoplasmic proteins between 40-60 °C. The results from the present thesis does not give any clear answers as to why the water losses increase with higher temperatures. The pH of the fish did increase between 40 and 90 °C, which is in correlation to an increased water loss, but this is not a sufficient explanation. In the vacuum pouch used, the water vapour is saturated, and therefore vapour diffusion mechanisms can be eliminated. The expulsion of large particles could occur due to liquid diffusion or due to a convective pressure gradient. In either case, the particles would probably need to break through the already formed sarcoplasmic gel network. From the present thesis, it has been shown that cook losses from higher temperatures exhibited a large viscosity, could gel, and for the fish the permeability was at its lowest point. The only result that could indicate some of the increased water loss was the fragmentation of fibrils that occurred at higher temperatures, which could, for example, contribute to the formation of a pressure gradient.

The data provided from this master thesis may be used for later improvement of models for cooking of fish and optimization of the cooking process. By providing detailed information on the effect of heat load on muscle proteins, processing can be tailored and optimised to maintain the best quality of the fish product.

Chapter 8

Future Perspectives

In the future it would be interesting to:

- Investigate further the mechanisms that causes the large expulsion of water at 70-90 °C, as this information is needed to properly model mass transport phenomena during cooking.
- Investigate the observed structure break down of fibrils that occurred during cooking at higher temperatures, as this information could explain the expulsion of water at higher temperatures.
- Study the cook losses by the use of transmission electron microscopy, as this technique allows for looking at the proteins in their natural form in solution, which could give valuable information in regards to aggregation phenomena that occurs in each cook loss.
- Model the viscosity of the cook losses using the Carreau model, and possibly use this to improve the mass transfer model.

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