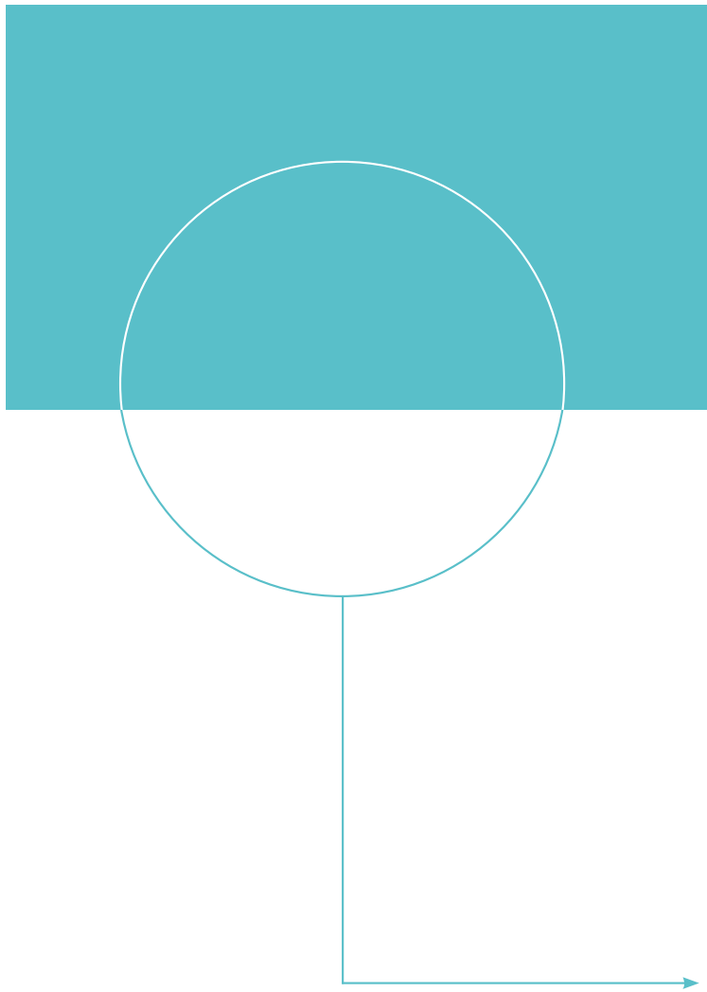

Master's thesis

2021

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NTNU
Norwegian University of
Science and Technology
Faculty of Natural Sciences
Department of Biotechnology and Food Science

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Freezing and thawing of fish

Effect of storage temperature and thawing method on protein denaturation and lysosomal enzymes

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Chemical Engineering and Biotechnology
Submission date: April 2021
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Preface

I would like to give credit and a special thanks to Turid Rustad for practical and theoretical feedback and guidance throughout the master assignment and earlier work. Her expertise in the field of food science have guided me for more than one and a half year, and have no doubt influenced me on my carrier path later on. I would also thank Siri Stavrum for her kind help and guidance in the lab during the experimental work for the thesis. For me, their help was vital to complete this thesis, and I would most likely never manage to do so without their combined help.

I am also grateful for Martin Haider's help at Akrinn when portioning and packaging the fish. I would also express my appreciation to my university, NTNU, for their resources and facilitation.

Abstract

Atlantic salmon is a highly perishable commodity that is traded over large distances and therefore, necessitate the need for good preservation systems. In general, this is often achieved by freezing, which gives long shelf life. While there are many studies that cover the effect of freezing, few exist about thawing of seafood. To achieve fresh-like quality in fish, the freezing and thawing process must be optimized.

Three common thawing methods, immersion in water, thawing in cold room at 4°C and in room temperature was tested for their ability to retain fresh-like quality in Atlantic salmon fillets. The preliminary experiments gave indication that the temperature in the immersion thawing group was too high to achieve fresh-like quality, and the room temperature thawed group's duration was too short to achieve complete thawing of the fillets. Subsequently, the conditions for the water immersion group were changed from 30°C to 25°C and the room temperature group duration was changed from 2,25h to 3h.

Drip loss and lysosomal enzyme leakage was significantly elevated in salmon thawed by immersion at 30°C for 1,25h compared with thawing in room temperature and in cold room. Protein solubility was significantly lower (10,97-12,95%) when thawed by immersion at 30°C for 1,25h compared with immersion thawing at 25°C, for two durations at 1h (22,27%) and 1,25h (19,48%). Reduction of temperature in the immersion group indicated lower protein denaturation, better retention of liquid and minimized lysosomal enzyme activity compared with immersion thawing at 30°C.

The results in this study indicated that immersion thawing at 25°C gave significantly lower lysosomal enzyme leakage (0,079 for 1h) and drip loss (1,72% for 1,25h), while protein solubility (23,27% for 1h) was elevated but not significantly higher compared with thawing in air (room temperature and in cold room at 4°C). Based on the three thawing methods tested, immersion thawing at 25°C for 1h is best suited to achieve fresh-like quality in frozen Atlantic salmon fillets.

Sammendrag

Atlantisk laks er en lett bedervelig matvare som fraktes over store avstander. På grunn av dette trengs det det gode konserveringsmetoder. Generelt sett oppnås dette ved frysing, som gir lang holdbarhet. For å få tilnærmet fersk kvalitet på fryst laks, må både frysing- og tiningsprosessen være optimalisert.

Tre normale tinemetoder, i vannbad, i kjølerom på 4°C og i romtemperatur ble undersøkt med tanke på hvor godt egnet de var til å bevare tilnærmet fersk kvalitet i Atlantisk laksefilet. De innledende forsøkene indikerte at temperaturen i vannbadsgruppen var for høy til å oppnå tilnærmet fersk kvalitet, og at tiden til gruppen som ble tint i romtemperatur var for kort til å oppnå fullstendig tining. Som følge av dette, ble forholdene for vannbadsgruppen ble endret fra 30°C til 25°C, og tiden gruppen som ble tint i romtemperatur ble økt fra 2,25 til 3 timer.

Drypptap og lysosomal enzymlekkasje var signifikant forhøyet i laks som ble tint i vannbad ved 30°C i 1,25t, sammenlignet med tining i romtemperatur og i kjølerom. Proteinløselighet var signifikant lavere (10,97-12,95%) for tining i vannbad ved 30°C i 1,25t, sammenlignet med tining i vannbad ved 25°C, for to tidslengder på 1t (22,27%) og 1,25t (19,48%). Reduksjon av temperaturen i vannbadsgruppen indikerte lavere proteindenaturering, bedre vannbindingsevne (WHC) og minimalisert lysosomal enzymlekkasje, sammenlignet med vannbadstining ved 30°C.

Resultatene i denne studien indikerte at vannbadstining ved 25°C ga signifikant lavere lysosomal enzym lekkasje (0,079 for 1t) og drypp tap (1,72% for 1,25t), mens proteinløselighet (23,27% for 1t) var forhøyet, men ikke signifikant høyere sammenlignet med tining i luft (i rom temperatur og i kjølerom ved 4°C). Basert på de tre tinemetodene som ble testet, var vannbadstining ved 25°C i 1t best egnet til å oppnå tilnærmet fersk kvalitet i frossen Atlantisk laksefilet.

Table of Contents

1 Introduction

Marine foods, such as fish are commodities that are highly perishable and traded over large distances and therefore, necessitate the need for good preservation systems. In 2018, the world fish capture and aquaculture production were estimated to reach 179 million tonnes, with a total first-hand value estimated at 3400 billion NOK. Over the past 80 years, the proportion of fish used for direct human consumption has increased significantly. In the 1960s, it was 67 percent while in 2018 the overall production was 88 percent (156 million tonnes) that were used for direct human consumption. This is equivalent to an estimated annual supply of 20,5 kg per capita. Frozen fish accounted for 35 percent (62,5 million tons), while prepared and preserved, and cured fish accounted for 11 percent (19,7 million tons) and 10 percentage (17,9 million tons), respectively. Norway has for several years has been the second largest seafood manufactures, over 90% of the seafood that is produced/harvested is exported.

Market value and world production of fish is expected to increase in the years to come due to an increased world population, increased global trading, as well as increased seafood intake.

The ocean is one of the main systems of our planetary biosphere. It accounts for almost half of the planet's biological production, yet a much smaller proportion of human food. About 2 percent of overall calorie intake and 15% of protein intake comes from seafood. Increased production and utilization are needed to meet the nutritional demands of a growing population. Fish is a good source of protein and unsaturated fatty acids, which makes it healthy choice for a growing number of consumers that are more conscious of their diet.

According to US department of agriculture, frozen fish have a shelf life from 3 to 8 months, while fresh fish have substantially shorter shelf life depending on initial conditions, temperature and type of fish – In most cases shelf life of fresh fish extends to a couple of weeks to maximum of 17-21 days (Duun & Rustad, 2008), given optimal fish quality and storage conditions. Preservation by freezing increases shelf life and allows for transportation over longer distances, nonetheless biochemical and physical processes that reduce quality still occur (Dawson, Al-Jeddawi, & Remington, 2018). The long shelf life of frozen fish, and preservation of quality generally achieved by freezing makes it possible to export frozen fish over longer distances, e.g., with boats while transportation with planes is avoided. The market can then sell the fish as frozen or thawed with general high quality, if the freezing, storage and thawing process is optimized.

1.2 Fish as raw material

As already mentioned, fish as a healthy food, on the other hand it is a material that is difficult to handle. It perishes quickly due to its high content of water, the content of unsaturated fatty acid that are prone to oxidation, the easily accessible proteins and close to neutral pH that encourages microbiological growth. The water and fat composition varies from species to species, as well as with season. Lean fish is mostly water, up to 83% in some species, while fatty fish is up to 70% water and 15% fat. Texture, colour and taste have been described as important parameters when assessing quality in Atlantic salmon. Due to its low content of connective tissue, myofibrillar fibers are the main textural elements in fish, and is especially important for fresh-like quality including texture and water holding capacity in fish (DUNAJSKI, 1980).

Water-holding capacity (WHC) is the ability muscle tissue to retain moisture. Low WHC can result in unacceptable loss of water, protein and other constituents, which can be measured gravimetrically as the loss of drip (Fennema, Powrie, & Marth, 1973). WHC is dependent on muscle protein's ability to take up and bind water. Temperature, pH, ionic strength, amino acid polarity/hydrophobicity and protein denaturation are parameters that influence muscle protein ability to bind and hold water and are related to WHC. Water accounts for around 75% in muscle tissue, and most of the water in the muscle fiber is trapped within the muscle structure, especially within and between the myofibrils (Huff-Lonergan & Lonergan, 2005). Thus, the mechanism of WHC is especially dependent on protein structures that make up the myofibrillar network.

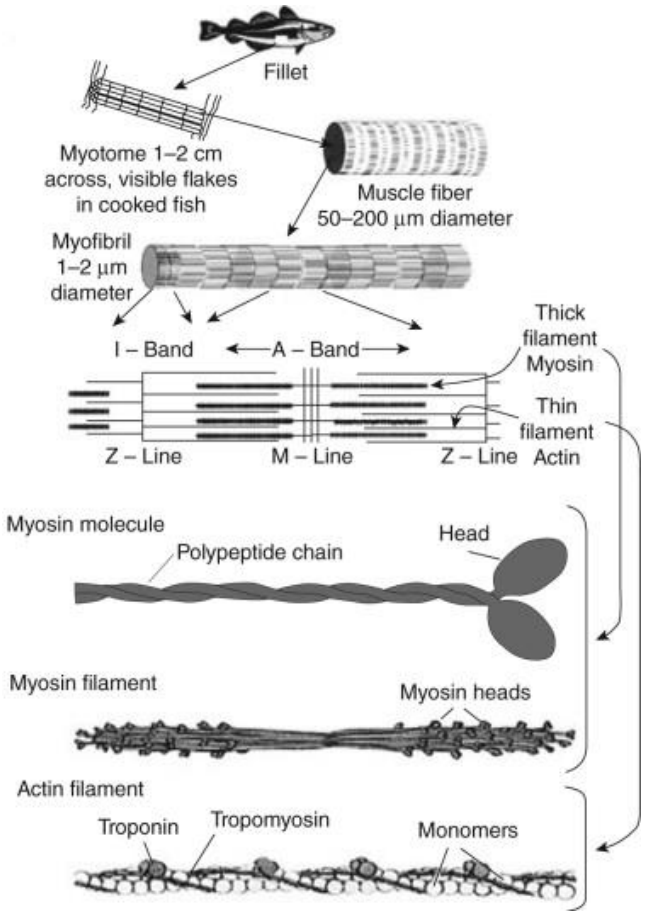

Figure: 1.1 Illustration of muscle tissue structure. Adapted from Bremner, 2002 and http://www.ucl.ac.uk/~sjjgsca/MuscleSarcomere.gif).

Sarcoplasmic protein are water-soluble proteins, e.g., enzymes and chromoprotein, they account for around 20% of total muscle protein. 65-70% of total muscle protein consist of myofibrillar protein forming the contractive bands that consist of myosin, actin, troponin and tropomyosin, which is salt soluble. Water and salt soluble protein are susceptible to freeze denaturation, but fish muscle proteins, especially myosin are more susceptible to abuse conditions of freezing, frozen storage and thawing than those from land animals (Sikorski Z.E., 1994).

The mechanism of WHC is especially dependent on myofibrillar protein, as mentioned above. Muscle protein is affected by processes such as freezing, frozen storage and thawing due destabilization related to cold protein denaturation and protein aggregation. This reduces the solubility of muscle protein and causes colour and flavour changes in fish muscle, as well as diminishing of WHC (Alizadeh, Chapleau, De Lamballerie, & LeBail, 2007; Haugland, 2002). Wang, Luo, Shi, and Shen (2015) reported that 4°C cold storage thawing had less water loss compared to microwave thawing, running water thawing, still water thawing and air thawing, which indicated better water-holding capacity.

Many enzymatic processes take place during storage of fish. These include proteolytic and hydrolytic processes catalyzed by proteases and glucosidases originating from ruptured lysosomes within the cell. Rupturing of organelles is especially prominent during freezing and thawing and is further described in section 1.3 Freezing.

The enzymatic activity is dependent on a specific substrate, in this case a synthetic fluorogenic substrate, Carbobenzoxy-phenylalanyl-arginine-4-methylcoumaryl-7-amide. This substrate is specific for cathepsin B+L-like enzymes.

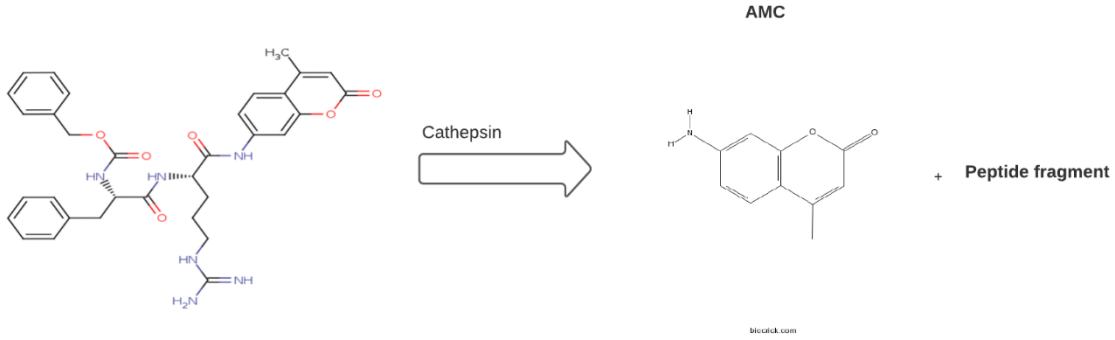

Figure 1.2 Illustrates the enzymatic reaction of cathepsin and it's substate, Carbobenzoxy-phenylalanyl-arginine-4-methylcoumaryl-7-amide, that are cleaved to AMC and a peptide fragment.

A cysteine protease, e.g., cathepsin, hydrolyses the fluorogenic substate, at the Arg-AMC peptide bond, into a peptide fragment and the highly fluorescence substance, AMC.

Other quality-determining factors of fish, is the raw material itself and it is affected by; what the fish have been feeding on, when the fish was slaughtered/season, pollutants in the water, handling on catch, slaughter conditions and type of fish (Alizadeh et al., 2007).

Lipid oxidation is the limiting factor in frozen storage of fatty fish while aggregation of protein is the limiting factor in frozen lean fish (Nilsson & Ekstrand, 1995). However, Atlantic salmon, which is a fatty fish, is very stable to lipid oxidation during frozen storage (Nilsson & Ekstrand, 1995). This might be due to naturally occurring antioxidant, such as endogenous α-tocopherol (Vitamin E), astaxanthin and vitamin C (Lygren, Hamre, & Waagbø, 1999). Measuring oxidation products of lipids is beyond the scope of this study. However, in this study, the effect of thawing on protein stability (sarcoplasmic and myofibrillar proteins solubility), drip-loss and enzymatic activity of cathepsin B+L is measured to assess the quality of Atlantic salmon (*Salmo salar*).

1.3 Freezing

Freezing has been used for a long time as a preservation method and every food object subjected to freezing needs to be thawed before use. From a North European perspective, freezing and thawing was first utilized during the winter in cold areas.

The basic principles of freezing and thawing are the same, both are driven by the temperature difference between the surrounding medium and the product. The difference between freezing and thawing, is that freezing removes thermal energy from a product, while thawing supply thermal energy from the environment to a product. The main challenge in freezing and thawing of seafood products, which contain a high proportion of water (60-80%), is to transform water to ice and vice versa. The process of thawing is further described in 1.4 thawing,

The freezing process converts liquid water to ice - crystallization of ice/freezing of water outside the cells leads to several effects in the tissue of the muscle. Water diffuses from intracellular to intercellular space and freezes out, causing cell rupture, and removal of water from protein structures that leads to freeze-induced protein denaturation (Shenouda, 1980; Xiong & Youling, 1997). Increased concentration of solutes in the unfrozen fraction of water, that is often precent in most commercial frozen storage temperatures, leads to increased enzymatic activity and protein denaturation (Duun & Rustad, 2008; Skåra, Stormo, & Nilsen, 2019). This takes place in the critical zone, as illustrated by figure 1.3.

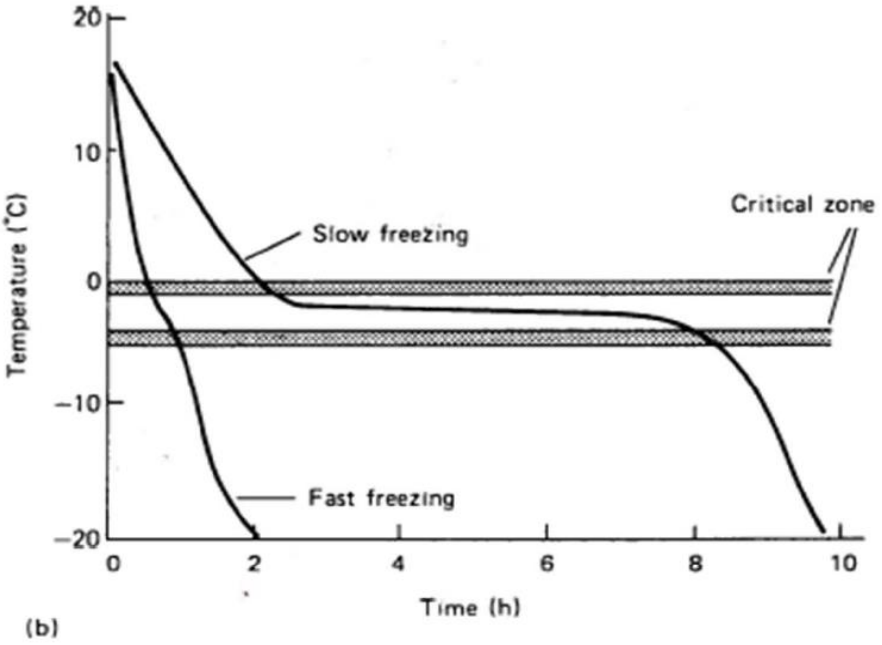

Figure 1.3 Temperature changes through the critical zone (between -5°C and 0°C). In the critical zone, water crystallizes, diffuses and concentration of solutes increases within the muscle. Slow freezing (black line) has a longer time in the critical zone, while fast freezing (black line) has a shorter time in the critical zone. Derived from Leniger and Beverloo (1975)

Freezing and thawing, especially at slow rates, causes leakage of content from organelles, e.g., enzymes from lysosome and mitochondria, where lysosomes seem to be less structured. Lysosomes are single membrane-bounded organelles containing numerous hydrolytic enzymes to digest materials ingested by endocytosis and recycle cellular components (D. & J.G., 1990). The leaked fluid from lysosomes contains enzymes which have been associated with *post-mortem* muscle softening. These enzymes causes proteolysis that degrade the fine structure of muscle myofibrils and intracellular connective tissue (Yamashita & Konagaya, 1991). Proteases are protein degrading enzymes that cleaves peptides bonds within proteins via proteolysis, resulting in smaller polypeptides or single amino acids. In several fish species Cathepsin B (E.C. 3.4.22.1), D (E.C.3.4.23.5), and L (E.C. 3.4.22.15) are considered as the proteases playing the most important role in *post-mortem* muscle softening (Yamashita & Konagaya, 1991). Bahuaud et al. (2010) reported that muscle pH, Cathepsin B and L activity is correlated to myofibril breakages, fillet softening and muscle degradation in Atlantic salmon tissue.

Based on the assumption that tissue damage causes leakage, the amount of cathepsin B+L in the leaked fluid can be used as a marker to assess the damage caused by freezing and thawing. This can be achieved by determine the combined enzymatic activity of cathepsin B+L in the cell tissue fluid (Nilsson & Ekstrand, 1994).

The convenience of prolonged shelf life and fresh-like quality achieved by freezing/thawing makes it possible for frozen fish to compete with fresh fish in the marked. Some products are thawed and rightly, sold as thawed fish, but some products are thawed and wrongly, sold as fresh fish. This emphasizes the importance to distinguish fresh from thawed fish to prevent fraud.

In earlier studies, there have been failed attempts to distinguish between thawed and fresh fish fillet by studying the presents of certain enzymes and their activity that are released during freezing and thawing, e.g., the mitochondrial form of glutamate-oxaloacetate-transaminase (E.C.2.6.1.1) which is used to determine freezing and thawing of beef and pork. The mitochondrial enzyme found in pork did not translate as an marker enzyme in carp due existence of the corresponding isoenzyme in the cytoplasm, and the high degree of destruction of mitochondria during iced storage of carp fillets (Rehbein, Kress, & Schreiber, 1978). However, the investigation of lysosomal enzymes seemed to be very promising due to fish muscle contains various lysosomal enzymes, and some of these enzymes are released during freezing/thawing within the fillets. The activity in the acid pH range of lysosomal enzymes is different from enzymes that catalyze the same reaction. This makes lysosomal enzymes readily distinguishable from cytoplasmic, mitochondrial and bacterial forms of the same enzyme.

Significant activity of cathepsin D (E.C.3.4.23.5), β-glucuronidase (E.C.3.2.1.31), acid phosphatase (E.C.3.1.3.2), α-glucosidase (E.C.3.2.1.20) and β-N-acetylglucoasminidase (E.C.3.2.1.30) were found in cod fillets. Rehbein, Kress, & Schreiber (1978) found that the two latter enzymes proved to be the most suitable because of their considerable latency and the high sensitivity of enzyme assays measuring absorbance. However, other sensitive methods of measuring enzymatic activity can be achieved, e.g., measuring fluorescence of a product cleaved in an enzymatic reaction with a fluorogenic substrate, which is used in this report.

Fast freezing is considered optimal for fish due to retention of fresh-like quality parameters, these include low lipid and protein oxidation, high solubility of proteins, low drip-loss and bacterial growth (Tressler et al., 1968). This can be achieved with freezing method's that have a high heat transfer coefficient, like cryogenic freezing, plate freezing and immersion freezing, which is discussed later in this section.

Slow freezing and prolonged storage, e.g., low velocity blast freezing or cold storage freezing in still air, can cause extended ice crystal growth and result in larger ice crystals compared with fast freezing, which have been shown to produce smaller crystals. In addition, slow freezing can cause tissue damage due to longer exposure in the latent zone/critical zone, as illustrated by figure 1.3 (Fennema et al., 1973).

As a frozen commodity, deterioration is drastically decreased compared to unfrozen fish due to immobilization of water and low temperatures, which causes a reduction in reaction rates and water activity. In unfrozen fish, these factors accelerate bacterial growth, oxidation of lipid and denaturation of protein, thus contribute to shorter shelf life compared to frozen fish.

Freezing pre-rigor or post-rigor has an effect on muscle quality, Einen, Guerin, Fjæra, and Skjervold (2002) found that drip-loss was not significantly affected by pre- or post-rigor filleting, pH and gaping scores in pre-rigor fillets was significantly lower than post-rigor fillets, however frozen/thawed fillets had a trend of higher gaping scores compared with unfrozen fillets, indicating that freezing and thawing causes gaping in fish muscle. Freezing and thawing induces damage in muscle tissue causing mechanical cell disruption and loss of membrane integrity. This can cause lysis of organelles, e.g., lysosomes, due to formation and reformation of intra- and extracellular ice crystals, as mentioned earlier. Though most of the formation is of extracellular origin in frozen meat (Devireddy, Smith, & Bischof, 2002). Membrane lysis can cause fluid to leak from the cell and result in a loose disorganized muscle structure (Karvinen, Bamford, & Granroth, 1982). Optimal freezing and thawing can reduce fillet softening and other factors related to quality; lipid oxidation, protein denaturation, drip loss and bacterial growth (Tressler, Van Arsdel, Woolrich, & Copley, 1968).

There are several preservation methods similar to freezing, such as superchilled and ice storage. Superchilling reduces the temperature of food to the freezing point and 1-2°C below this (in the critical zone, as illustrated by figure 1.3), causing ice formation in the outer few millimeters depending on method (Fennema et al., 1973). Superchilling have been reported to double shelf life of Atlantic salmon. Ice chilled salmon of premium grade, stored in vacuum bags had a shelf life of 17-21 days (Duun & Rustad, 2008). Sveinsdottir, Martinsdottir, Hyldig, Jørgensen, & Kristbergsson, (2002) reported a similar sensory shelf life (20 days) of salmon stored in ice boxes (0-2°C), based on evaluation of cooked salmon .

Fillet softening during unfrozen storage is a problem for the fish industry. This causes a reduction in hardness which is the most important textural characteristics of fish (Duun & Rustad, 2008). Freezing can increase hardness compared with other preservation method's such as superchilling and icing. Duun and Rustad (2008) found an increase in hardness of superchilled Atlantic salmon stored at -3,6°C compared with superchilled (-1,4°C), ice chilled and frozen references, however there was a reduction in hardness after prolonged storage in all groups. Aubourg, Torres, Saraiva, Guerra-Rodríguez, and Vázquez (2013) found an increase in hardness of Atlantic mackerel (*Scomber scombrus*) after freezing, and a lowering of hardness after 3 months of frozen storage, indicating that superchilling and freezing increases hardness, and chilled, frozen or superchilled storage over time reduces hardness.

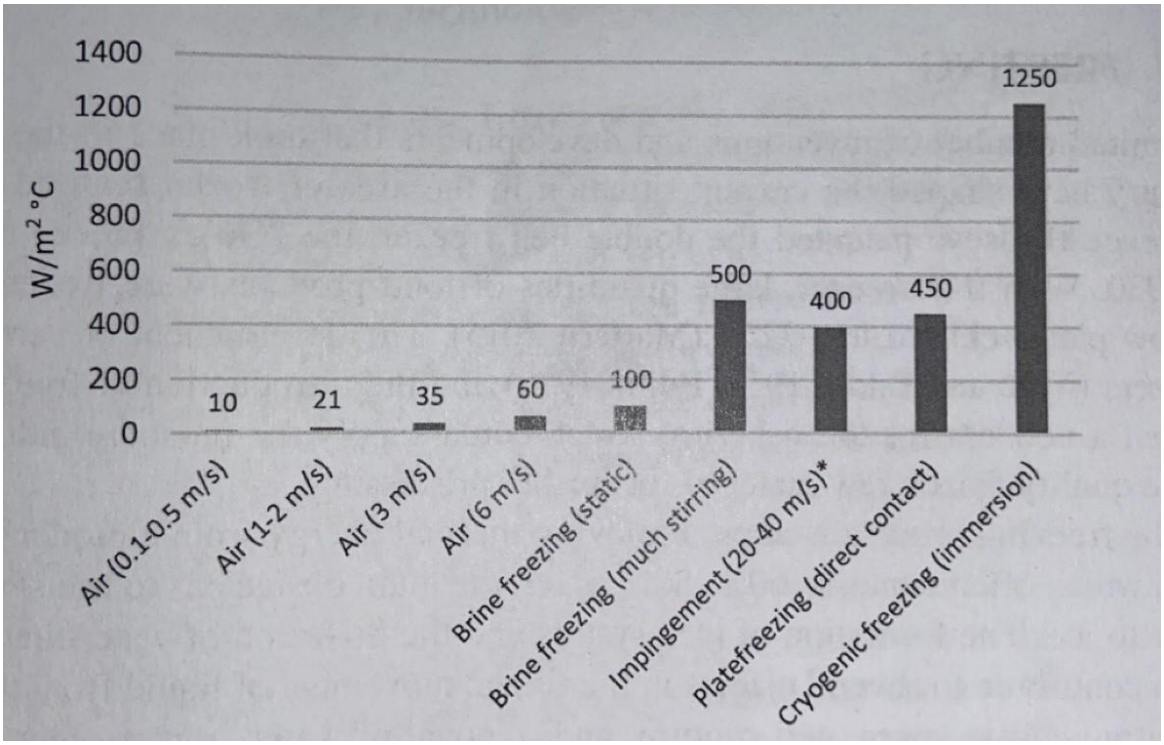

Figure 1.4 Typical values for heat transfer coefficient ($W/m^2°C$). The velocity of air for the air method is marked in the brackets. Adapted from James S.J and James, C., Chilling and freezing of foods, in *Food processing*: *Principles and Applications,* ed. S. Clark, S. Jung, and B. Lamsal. John Wiley & sonds, Hoboken, NJ 2014).

As mentioned, freezing conditions such as freezing rate and storage temperature affect the final eating quality of frozen fish. Freezing rate is dependent on the freezing method's heat transfer coefficient. The heat transfer coefficient is the proportionality constant between the heat flux and the thermodynamic driving force of the flow of heat, i.e., the temperature difference. Hence, a freezing method with a large coefficient is efficient at transferring heat from a product to the environment (e.g., freezing medium), especially at large temperature differences.

It is evident that, although being very common freezing medium, air (gas) has a poor heat transfer coefficient, as illustrated by figure 1.4. This can be elevated to some extent by increasing the airflow around the product, thereby continuously replacing the air that have been warmed by

the product with cold(er) air. When taken to extreme levels (impingement) this becomes effective because the barrier layer of warmer air is completely removed. (air) Blast freezing is a freezing method that is commonly used in the pelagic sector, where the products are packaged as blocks and filled in racks for overnight freezing in large (freezing) tunnels. This is a high-capacity solution, yet the freezing rate is limited to a large extent and it has been reported that it takes more than 10 h to reach a core temperature of -5°C in whole fatty mackerel within the blocks (Widell, Stavset, & Nordtvedt, 2015).

Plate freezers have been more common on fishing vessels as they are compact and very efficient, in contrast to blast freezing with low air velocity, yet oversized fish are often air frozen in the cold storage unit with respect of the size limitations of the plate freezer, which is suboptimal due to slow freezing rate.

Immersion freezing is also to some extent used commercially; the most known use is freezing tuna at sea. Due to large total contact area and high heat transfer in liquid, compared to gas, immersion is very efficient, particularly for irregular shaped products. The freezing medium can be water with salt, sugar or alcohols or other nontoxic mixtures that have a low freezing point. There are some technological limitations; however, salt (NaCl) brine has a low freezing point, thus limiting the maximum temperature difference between the product and the brine, product-to-brine volume, temperature control and brine stirring velocity can are also factors that can limit freezing efficacy.

Cryogenic freezing uses either evaporation of liquid nitrogen or sublimation of dry ice (solid carbon dioxide) on a single belt freezer to remove large amount of thermal energy on the surface of the products. This results in elevated heat transfer coefficient, as illustrated by figure 1.4

1.4 Thawing

Generally, thawing occurs more slowly than freezing due to the heat conductivity of water (above 0°C) is far lower than that of ice. The heat conductivity of liquid water at room temperature is about 0,6-0,7 W m−1 K−1, whereas it is 2,1-2,2 W m−1 K−1 for ice at 0°C. As illustrated by figure 1.5, the heat conductivity of salmon above 0°C is 0,5 W m−1 K−1, at this temperature point all the water present in the fish is liquid, compared with 1,2-1,3 W m−1 K−1 for frozen salmon at -20°C. The heat conductivity of frozen salmon and cod increases when temperature is reduced, as more of the liquid water is frozen to ice.

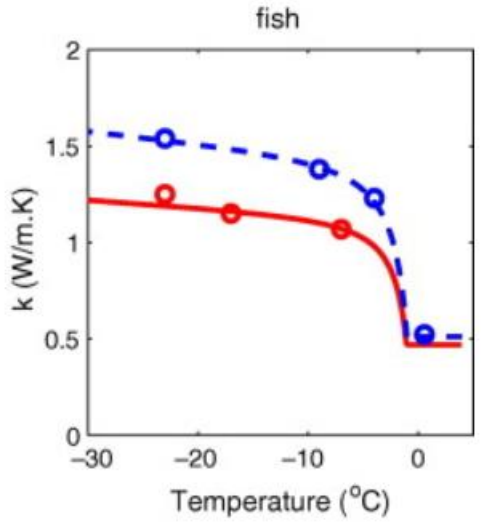

Figure 1.5 Heat conductivity of fish (circles) compared with a modeled mathematical prediction (lines) of salmon (solid red line) and cod (blue dashed line). From van der Sman (2008), Prediction of enthalpy and thermal conductivity of frozen meat and fish products from composition data.

When thawing, the internal product temperature reaches a critical zone, also called the latent zone as illustrated by figure 1.3. When the product reaches this zone in the thawing process, several physical and biochemical changes. An extended period in this zone can promote bacterial growth in the outer layers of the fish, where the surface reaches temperature as high as the surrounding medium, when the core still is in the thawing process (Ragnarsson & Viðarsson). Activity of leaked enzymes can increase, causing protein denaturation and degradation. It is found that shortening the time in the latent zone retains quality, enhances product yield and reduces drip loss (Haugland, 2002; Nilsson & Ekstrand, 1995).

Like freezing, thawing is driven by the temperature differences between the product and the surrounding medium, generally air or water. For fish and seafood, this temperature difference is limited by these product's low tolerance of elevated temperatures, which may promote bacterial growth and protein destabilization and thus compromise food safety. Consequently, industrial standards (Codex Alimentarius 1989) and legal regulations limit the maximum temperature differences that can be used for thawing of fish.

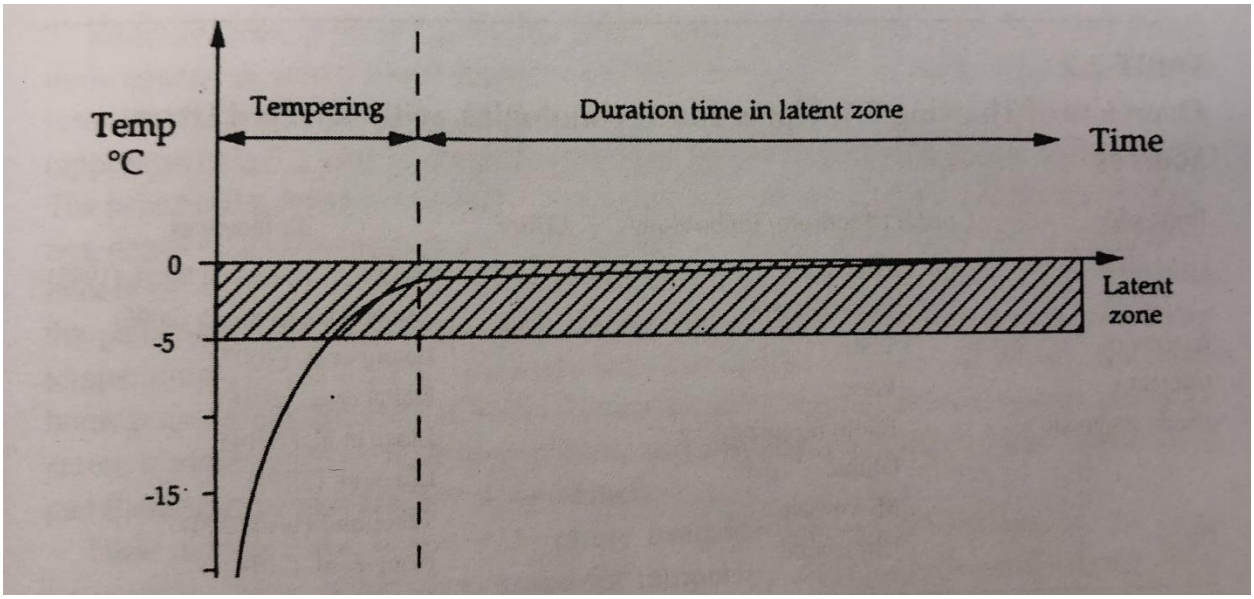

Figure 1.3 Thawing curve illustrating latent zone (between -5°C and 0°C). From Nilsson and Ekstrand (1994).

The American Meat Science Association (AMSA) recommends meat thawing in a refrigerator at 2 to 5 °C until internal temperature reaches 2 to 5°C. Long thawing time, in air at 5°C have been associated with higher lysosomal activity and increased volume of cell tissue fluid (CTF) compared with fast thawing by immersion, in water at 25°C, independent of storage time (Nilsson & Ekstrand, 1995). Thawing rate is therefore related to retaining fresh-like quality of frozen salmon due to low leakage of CTF and high integrity of muscle tissue cells. Domestic thawing is manly achieved in the refrigerator overnight (Skåra et al., 2019).

In recent years, there have been reports of increasing sales of thawed fish, sold in the marked. It is apparent that the quality of thawed fish is acceptable for the consumer. It is expected that this trend will continue to increase as the delivery of frozen fish products is more stable than fresh fish delivery, can be scheduled in the long term, especially in the seasons where fresh fish delivery has failed due to shortage of catch (Solheim, 2012).

1.4.1 Thawing methods

Many traditional and novel thawing method exists, that can be classified in terms of how heat is supplied into the fish muscle. This can be divided into two main groups; The first group is suppling heat through the surface e.g., convection, conduction and radiation, which is the most used group and what the three methods in this report is based on. The other is by generating heat within the product, thus making the thawing a more uniform process e.g., microwaving, ultrasound and electrical resistance. These methods are often not possible to apply due to the scale of the production and the high degree of complexity.

The industry mostly relies on water and air related thawing due to low cost, efficiency, and effect on the quality of fish. Implementation of thawing method is largely dependent on batch size e.g., a restaurant is capable of thawing using a microwave, however a large company will find it difficult to achieve this and may instead use water or air thawing.

The main method of thawing principles is listed in table 1.1.

Table 1.1 Overview of thawing principles and technologies, with selected literature. Derived from table 2.2 in Advances in freezing and thawing, Skåra et al. (2019)

Principle	Contact medium/Technology	Reference
Gas (air)	Humid air Impingement	Mannapperuma and Singh (1988)
Immersion	Water	Anderson and Singh (2006)
Contact	Plate	Leung et al. (2007)
Electromagnetic	Radio frequency	Roiha et al. (2007)
	Ohmic	Llave et al. (2017)
	Microwave	Liu et al. (2017)
Other	Ultrasound	Hong et al. (2014)

The principles listed in table 1.1 is implemented by the industry depending on cost, efficiency, complexity and scalability. Still air thawing is widely used due to how easy it is to implement on a large scale, in addition still air thawing has low initial and operating costs. Thawing in still air is a slow process, however large block of whole fish or whole gutted fish can be thawed at the

same time. However, drying at the surface of the block at constant high temperatures is a problem and can cause bacterial growth and irreversible muscle protein damage. Heat is transferred from the surroundings to the fish, this creates a boundary layer of liquid water that results in slow thawing rate, which is explained in the beginning of this section.

Air blast thawing involves circulation of air around the product, this removes the boundary layer of still air around the product. This increases the convection coefficient (heat transfer coefficient) from around 15-30 up to 100 w/m^{2}k. Hence, an overall increase in efficiency and thawing rate is achieved. However, this can have a negative impact on drip loss if the fish was not properly frozen. Unproper frozen fish muscle subjected to slow thawing have been shown to reabsorb some of the water that has been lost during freezing. However, as most fish product is frozen with a high freezing rate, slower thawing is not recommended. In addition, bacterial growth and irreversible protein damage is minimized as compared to sill air thawing due to elevated thawing rate (Ragnarsson & Viðarsson).

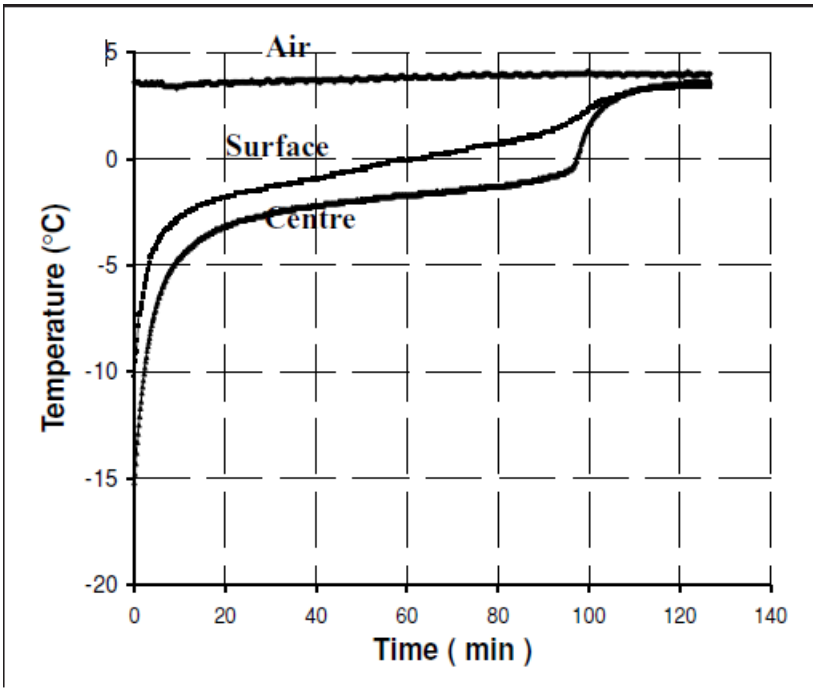

Figure 1.2 Temperature changes in central and surface area of Atlantic salmon sample during air-blast thawing (4°C, 4m/s). The sample was frozen with air-blast freezing (-30°C, 4 m/s). From Alizadeh et al. (2007).

Immersion in water is the most common method of seafood thawing, and it often used by commercial companies that aim to thaw large quantities and deliver high quality fish products (Skåra et al., 2019). Immersion thawing is often achieved with water as the contact medium, which do not have the same limitations as immersion freezing, as the temperature is above the freezing point of water. This allows for greater thawing rate due to higher heat conductivity of water compared with air, which reduces muscle protein damage and bacterial growth (Ragnarsson & Viðarsson). Temperature control is also important in immersion thawing, if the

medium is too cold or too warm it can have negative impact on the final quality and any gain in muscle quality or bacterial growth can be lost, compared to air thawing. The operation and initial cost of immersion systems differs depending on the type of system implemented and complexity, e.g., running water, temperature control, fish tanks, rotating tanks, continuous belt systems, agitation of water is some of the specifications that can be implemented in such a system.

To obtain high quality in thawed fish it is necessary that both the freezing and thawing processes, as well as the raw material quality is optimal. The aim of this study was therefore to investigate the effect of 3 different thawing methods on the quality of Atlantic salmon. To study this, the freezing method was unchanged and at constant temperatures (-20°C), and the raw material was of similar quality (premium grade, frozen after about 5 days slaughter). Immersion thawing in water at 25°C for 1h and 1,25h and 30°C for 1,25h, thawing in room temperature for 2,25h and 3h, and thawing in a refrigerated room at 4°C for 24 hours was tested for its ability to retain quality in frozen Atlantic salmon.

2 Material and methods

2.1 Sample preparation

Farmed Atlantic salmon (*Salmo Salar)* was purchased from Vikenco at Aukra as iced filleted fish and delivered to Akrinn 5 days after slaughter. The skin was removed, the skinned fish was cut in appropriate samples sizes (80g) and vacuum packed in a Webomatic SuperMax-C machine. After vacuum packaging, the sample was transported to Gløshaugen and stored in a conventional freezer at -20° C.

2.2 Experimental set-up

Table 2.1 Thawing method with parameters, and date/period

Thawing method	Period/date	Thawing principle and parameters
1	19.11.2020-9.3.2021	Cold room (Air),4°C, 24 h
2.1	19.11.2020-18.2.2021	Water immersion, 30°C, 1,25h
2.2	9.3.2021	Water immersion, 25°C, 1,25h
2.3	9.3.2021	Water immersion, 25°C, 1h
3.1	19.11.2020-18.1.2021	Room temperature (Air), 2,25h
3.2	25.1.2021-9.3.2021	Room temperature (Air), 3h

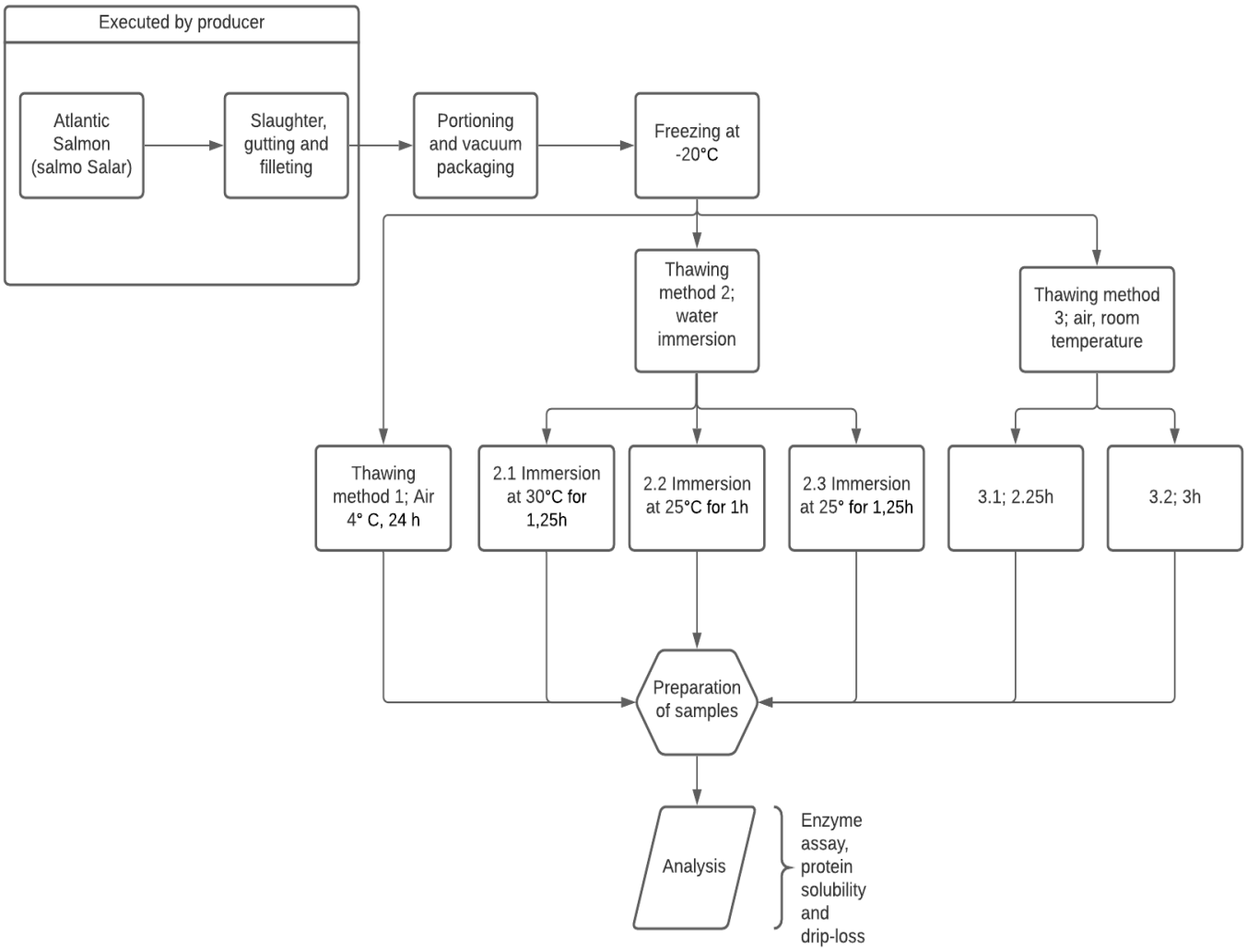

Figure 2.1 Flow chart of the experimental set-up, step by step. Steps executed by producer is marked. The rest of the steps in the flow chart is executed at the process lab at Akrinn and at the food chemistry lab at Gløshaugen.

2.3 Drip-loss

The drip-loss of each sample was measured after thawing. The whole sample, with fish and packaging was weighed on a digital scale with an accuracy range of 0,01g. Then the sample were unpackaged and drained. The fish fillet was lightly blotted with a paper towel to remove excess drip at the surface and weighed. Empty packaging was blotted dry and weighed. The drip loss was calculated and reported as percentage according to eq. 2.4.

Mass of drip = Fish in bag - (blotted fish + dry bag) (2.1)
Initial mass of product = Fish in bag - dried bag (2.2)

$$\frac{weight\ of\ drip}{Initial\ weight\ of\ product} \times 100\ \% \qquad (2.3)$$

2.4 Preparation of cell tissue fluid

Approximately 20 g of fish muscle was weighed accurately and placed in a SS-34 centrifuge for 30 minutes at 27000g .

The supernatant were pipetted out and placed in pre-weighed Eppendorf tube and weighed again. The CTF filled Eppendorf tubes was later frozen for later determination of enzymatic activity.

2.5 Protein extraction

The method used in this report is a modified method of Anderson and Ravesi (1968) and Licciardello et al (1982) as described by Hultmann and Rustad (2004).

4 g of salmon fish fillet was homogenized (10 sec) with Ultra-Turrax in a phosphate-buffer solution (80 mL, 0,05 M, pH=6,95), the solution was then centrifuged (8000 g, 20 min, 4-10°C). The supernatant was decanted through glass wool, the volume was adjusted to 100 ml with phosphate-buffer.

The precipitate was homogenized (10 sec) in the phosphate-buffer solution with salt (80 mL, 0,06 M KCl in 0,05 M KH_2PO_4, pH=6,95), and centrifuged again (8000 g, 20 min, 4-10°C). The supernatant was decanted through glass wool, the volume was adjusted to 100 ml with phosphate buffer with salt.

The protein concentration in water and salt soluble extracts were determined by the Biorad method (Bradford, 1985), with a Bovine gamma globulin (BGG) standard.

Suitably diluted samples were prepared, and Biorad-solution was added (5 ml), the solution was then placed on a Vortex for proper mixing. The absorbance was measured in a spectrophotometer (Ultrospec 2000) at a wavelength of 595 nm. A standard curve of bovine gamma globin (BGG) was used to determine protein concentration.

2.5 Enzymatic activity

The activity of cathepsin B + L was measured against a synthetic fluorogenic substrate, Carbobenzoxy-phenylalanyl-arginine-4-methylcoumaryl-7-amide as described by Stoknes and Rustad (1995)

Cell tissue fluid (CTF) or water-soluble protein extract was diluted with distilled water to a protein concentration around 0,5-1,0 mg/mL. Assay buffer (100 µL, 150 mM bis-Tris, 30 mM EDTA, 6mM DTT, pH=6-7) was added to a test tube. Suitable diluted enzyme extract/protein extract (100 µL) was also added to the test tube, the blank was made with distilled water. Three parallels was made for each sample/blank.

The solution was incubated for 15 min to reach 4°C. This temperature was chosen because it is close to the temperature of a refrigerator where fish often is stored. The reaction was started by adding substrate working solution (100 µL) to all samples/blank and the mixture was incubated for an additional 30 min at 4°C. After 30 min stopping solution (3,0 mL, 1% SDS, 50 MM bis-Tris, pH=7) was added to stop the reaction. All the samples, including blank was run as triplets. Fluorescence was measured at 360 nm excitation and 460 nm emission using a Techan Spark 20M machine. The increase in fluorescence was used to determine enzymatic activity.

3 Results and discussion

Three commonly used thawing methods for fish were compared to assess their effect on drip-loss, protein solubility, amount of cell tissue fluid (CTF) and enzymatic activity in CTF and homogenate. However, in the preliminary experiment the following thawing methods where first tested:

Table 2.2 Thawing methods and parameters

Thawing method	Parameters
1	Cold room, 4°C , 24 h
2.1	Water immersion, 30°C, 1,25h
2.2	Water immersion, 25°C, 1,25h
3.1	Room temperature, 2,25h

The results indicated that the first chosen immersion method was not suitable to retain quality in Atlantic salmon, the temperature appeared to be too high, and the duration appeared to be too long for the parameters that were tested (30°C, 1,25h). This was reflected in the results - protein solubility was low (10,97-12,95% for M2.1 at 19. Nov and 18. Feb, as illustrated by figure 3.5 and 3.6

), and relative enzymatic activity (0,39 for M2.1 at 18. Feb, as illustrated by figure 3.9) and drip loss (5,27% for M2.1 as illustrated by figure 3.3) was elevated in the first immersion group at 30°C for 1,25h compared with the other group thawed in room temperature for 2,25h and 3h, as illustrated by figure 3.1, 3.2, 3.3, and 3.9. Subsequently, the temperature and duration of the immersion group was later changed (9. March) to 25°C and for two durations, 60 and 75 min. The group thawed in room temperature was also changed from 2,25h to 3h as the core of the fillet was still frozen after thawing. Thawing in room temperature were adjusted to fit a core temperature above the freezing point.

Table 3.1 Surface temperature on the surface of the vacuum bag for each method at the end of thawing for method 1, method 2.1 and method 3.2.

Method	Temperature [°C]
1. Coold room	1,5
2.1. Immersion	28,0
3.2 Room temperature	8,7

The surface temperature was 28,0°C for the water immersion group thawed at 30°C thawed for 1,25h (M2.1), far above 0°C. The room temperature group thawed for 3h (M3.2) and the group thawed in cold room (4°C, 24h) had a lower surface temperature, 8,7 °C and 1,5 °C, respectively. This resulted in high enzymatic activity and drip-loss for the immersion group thawed at 30°C for 1,25h compared with other groups, as illustrated by figure 3.8 and 3.2, respectively. Due to the duration and temperature of the immersion group (30°C, 1,25h) a core temperature above the recommended 2 to 5 °C interval (by AMSA) was most likely reached. The soluble protein and enzymatic activity results is discussed later in this section.

The surface temperature was recorded for the immersion group thawed at 25°C and the group thawed in room temperature, as illustrated by figure 3.1. Duration in the latent zone is far longer for the group thawed in room temperature for 3h than for immersion thawing at 25°C for 1h and 1,25h, however the surface temperature of the vacuum bag was recorded, there are no temperature measurement of the core temperature of the fillet, thus the duration in the latent zone was not recorded and statements about the duration in the latent zone are based on surface temperature, which is in the range between -2,5-4,6°C for the group thawed in room temperature and 7-21°C for the immersion group (25°C).

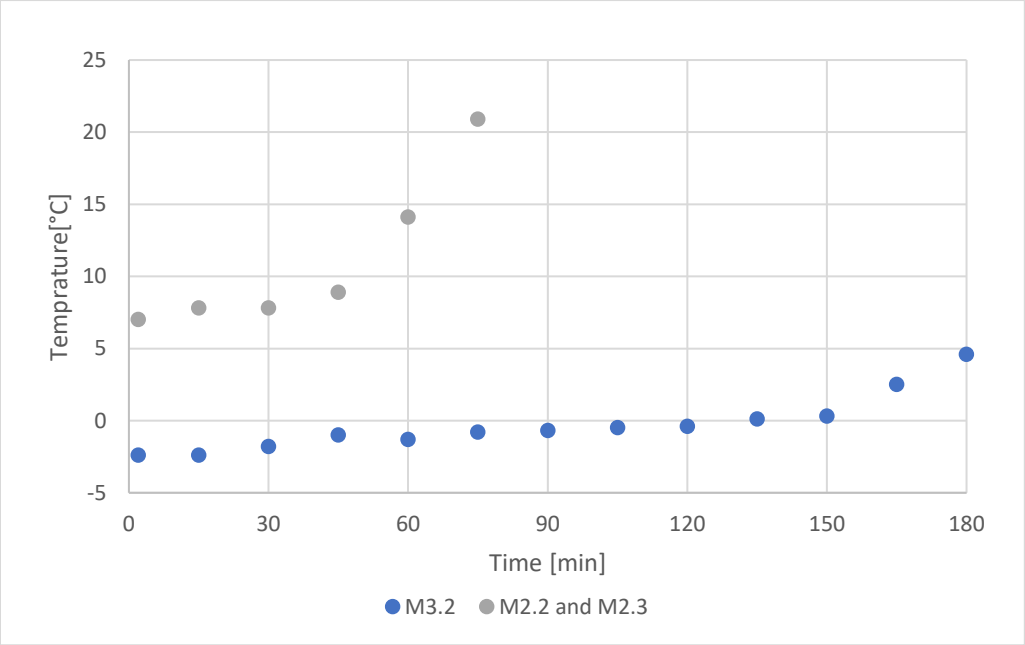

Figure 3.1 Surface temperature of the vacuum bag of the fish thawed in room temperature (blue circles) for a duration of 3h (M3.2) and the immersed group (gray circles) at 25°C for a duration of 60 and 75 min, (M2.2 and M2.3).

Drip-loss was largest (5,27%) for the water immersion group thawed at 30°C for 1,25h (M2.1). The salmon thawed by water immersion at 25°C (1,25h, M2.3) had the lowest drip-loss, 1,72 % which is a third that of M2.1 (immersion in water at 30°C for 1,25h). The group thawed at 4°C in

cold room (M1) had a drip-loss at 4,13%. Einen et al. (2002) found increased drip loss in frozen Atlantic salmon compared with unfrozen fillets, indicating correlation between freezing and drip loss in Atlantic salmon. Their fillets was frozen (post-rigor) in nitrogen for 21 min after 5 days of cold storage, stored in -25°C for 4 days, thawed in a refrigerated room for 30h at 4°C. Their reported drip-loss was 0,8%. In comparison, the drip-loss of the cold stored group (4°C,24 h) in this experiment were over four times as high.

The immersion group thawed at 25°C for 1,25h (M2.3) yielded the lowest drip loss (1,72%) of all the groups, indicating that decreasing the temperature of the (water) medium from 30°C to 25°C resulted in better WHC and better retention of liquid within the fillets. The elevated drip loss in the immersion group thawed at 30°C for 1,25h is positively correlated with low protein solubility and high enzymatic activity found in fish muscle for this group. Thus, indicating fiber shrinkage, cell damage, low WHC, protein denaturation and aggregation during freezing and thawing, especially at higher thawing temperatures (Mackie, 1993; Sikorski & Pan, 1994).

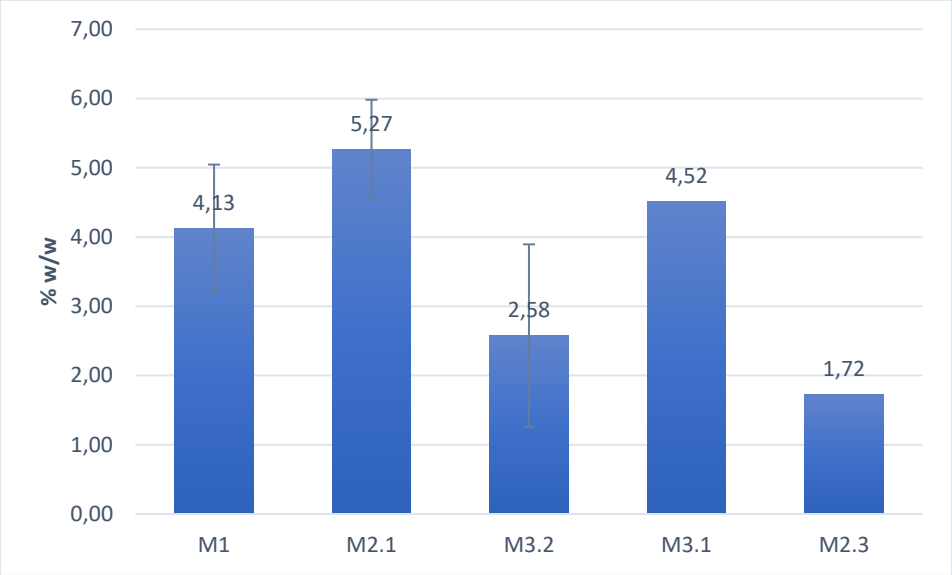

Figure 3.2 Average drip-loss for method 1, method 2.1, and method 3.2. Drip-loss for method 3.1 and method 2.3 was obtained at 19. Nov and 9. March, respectively, and are single measurements. Drip-loss for all the method is illustrated as weight per weight percentage (blue pillars), SD are represented with error bars, n=4. Method 1 (M1) represent salmon thawed at 4°C for 24h. Method 2.1 (M2.1) represent salmon thawed at 30°C for 1,25h. Method 3.2 (M3.2) represents salmon thawed at room temperature for 3h. Method 3.1 (M3.1) represents salmon thawed at room temperature for 2,25h, with no SD. Method 2.3 (M2.3) represents salmon thawed for 1,25h at 25°C, with no SD.

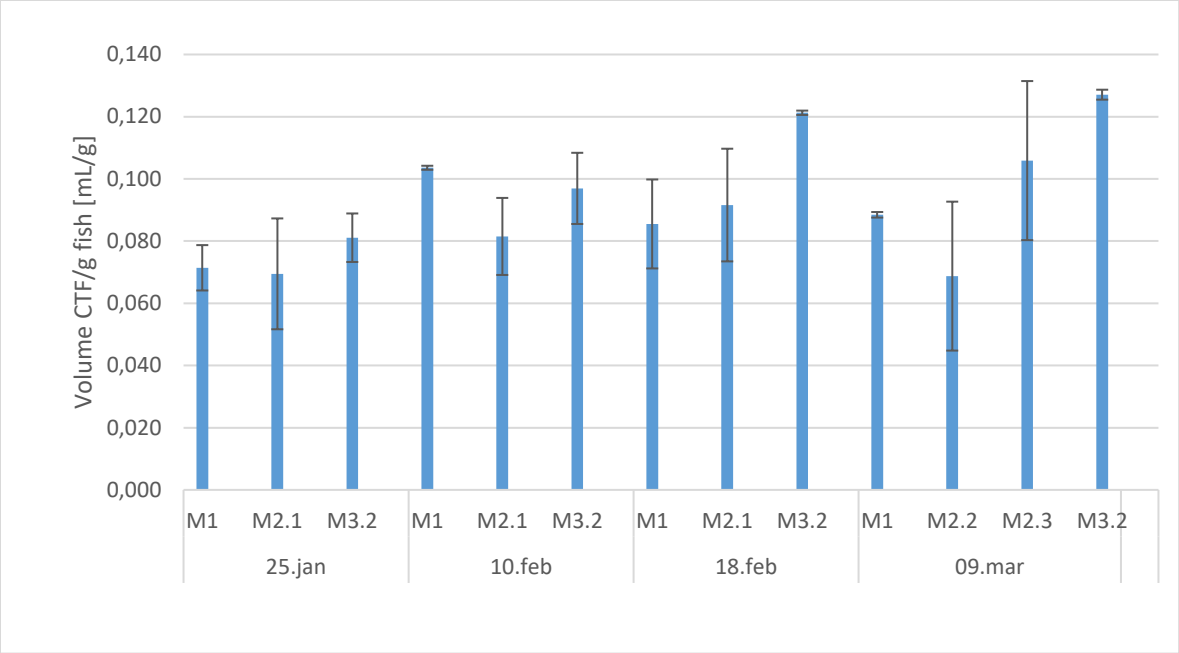

Figure 3.3 Average CTF volume per gram wet weight grouped by date. The CTF volume was calculated from the measured weight of CTF, assuming a liquid density of 1g/mL. M1 illustrates the refrigerated group (4°C, 24h), M2.1/immersion at 30°C for 1,25h, M2.2/immersion at 25°C for 1h, 2.3/immersion at 25°C for 1,25h, and M3.2/room temperature for 3h. SD (n=2) is displayed as error bars. The results are categorized by date, as shown on the x-axis.

The CTF volume gives an indication of loss of liquid, resulting from damage during the freezing and thawing process. Nilsson & Ekstrand (1994) found that slow thawing, in air at 4°C for 5,5h, increased CTF volume compared with fast thawing, in water at 7,5°C for 1,5h, from 0,81-1,23 mL/10g of fish muscle. Compared with the result in this study, the room temperature group thawed for 3h gave the largest volume of CTF (0,125 mL/g for M3.2), and the immersion group thawed at 25°C for 1h gave the lowest volume of CTF (0,07 g/mL). This is similar to the result in Nilsson & Ekstrand (1994), the fast thawed group gave the lowest volume of CTF. However, it was not found that the slow air thawed group (4°C) gave the largest CTF.

Presumably, there could be a correlation between drip loss and CTF, assuming that fish fillet with elevated drip loss, would also release more liquid when put under a centrifugal force. Drip-loss was elevated in the immersion group at 30°C for 1,25h, but the volume of CTF for the same group was not elevated. The results from drip loss, illustrated by figure 3.2 do not seem to correlate with the result from volume of CTF, illustrated by figure 3.3.

Figure 3.4 Picture of fluid collected after CTF extraction, the top phase is oil extract, and the lower phase is water based CTF extract in an Eppendorf tube.

As seen in figure 3.4, there is an oil- and water phase collected after centrifugation of Atlantic salmon fillets. The oil phase was not separated from the CTF before weighing, however the pipette tip was placed in the water phase when collecting enzymatic extract for determination of water-soluble protein and enzymatic activity. Atlantic salmon is a fatty fish, and in general oil in the supernatant after centrifugation is expected.

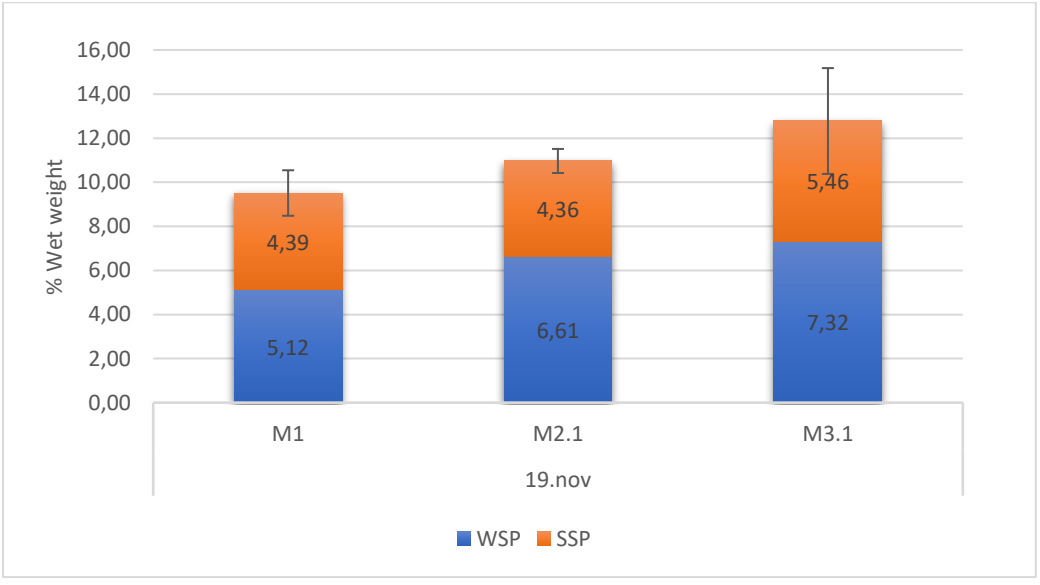

Figure 3.5 Total soluble proteins displayed as wet weight percentage grouped by date of experimentation, blue pillar displays water-soluble protein and orange pillars displays salt-soluble proteins. SD, n=2.

Method 1 (M1) represent salmon thawed in cold room at 4°C for 24h. Method 2.1 (M2.1) represent salmon thawed at 30°C in water for 1,25h. Method 3.1 (M3.1) represents salmon thawed at room temperature for 2,25h. Soluble protein was determined using a BGG standard from appendix A

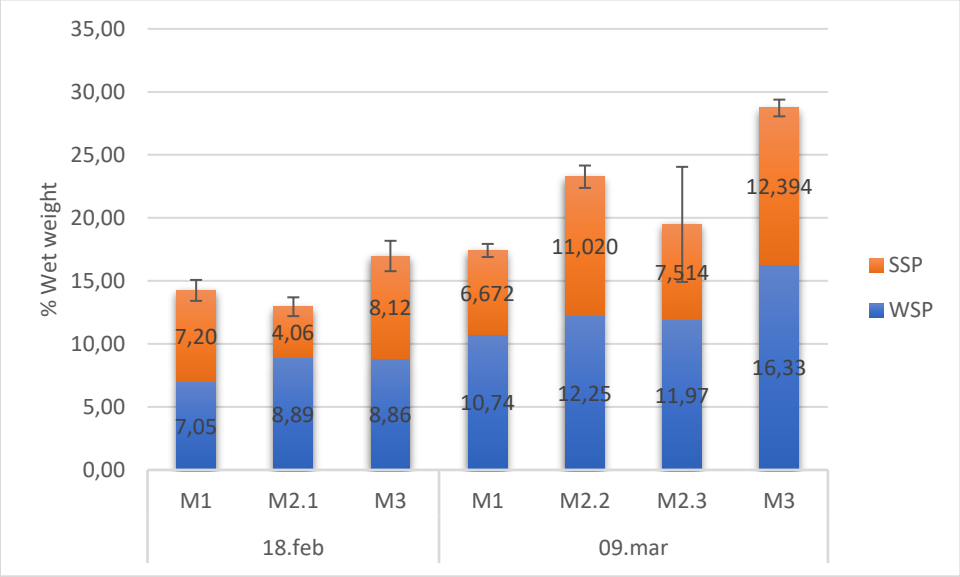

Figure 3.6 Total soluble proteins displayed as weight per wet weight percentage grouped by date of experimentation, blue pillar displays water-soluble protein and orange pillars displays salt-soluble proteins. SD, n=2. Method 1 (M1) represent salmon thawed at 4°C for 24h. Method 2.1 (M2.1) represent salmon thawed at 30°C for 1,25h. Method 3.1 (M3.2) represents salmon thawed at room temperature for 3h. Method 2.2 (M2.2) represents salmon thawed at 25°C in water for 1h. Method 2.3 (M2.3) represents salmon thawed at 25°C in water for 1,25h. Soluble protein was determined using a BGG standard from appendix A

The refrigerated group at 19. Nov has the lowest percentage of total soluble protein (9,51%) of all the groups at that date. The results from the immersion group at 30°C for 1,25h (M2.1) at 19. Nov (10,97%) and 18. Feb (12,95%) compared with immersion at 25°C (9. March, M2.2 and M2.3) indicates that M2.1's duration and temperature lead to a reduction of soluble protein, especially salt soluble protein, compared with immersion at lower temperature and shorter duration. A reduction of duration, from 1,25h to 1h, and of temperature, from 25°C to 30°C, gave a soluble protein concentration at 22,27% for immersion thawing at 25°C for 1h and 19,48% for immersion thawing at 25°C for 1,25h, illustrated by figure 3.3, indicating that temperature over 25°C for that given duration (1,25h) leads to increased protein denaturation in Atlantic salmon. Salt soluble protein (SSP) makes up a large proportion (65-70%) of total muscle protein, e.g., myofibrils. Hence, the percentage of SSP is especially important for texture in fish muscle (Duun & Rustad, 2008; Mi, Qian, Zhao, Liu, & Mao, 2013).

In comparison, Duun and Rustad (2008) reported a salt-soluble protein concentration at 4,5% and a water-soluble protein concentration at 7% approximately, giving a total wet weight

percentage at 11,5%, in Atlantic salmon frozen in a freezing tunnel at -30°C with 2 m/s air velocity, stored at -40°C for 37 days and thawed in room temperature. In their study, soluble protein was analyzed using bovine serum albumin as standard, while in this experiment bovine gamma globin was used as standard. The percentage of total protein at 19. Nov is similar to that of Duun and Rustad (2008). However, BSA (Bovine serum albumin) was used as standard protein reagent to determine the standard curve. The standard curve for BSA and BGG, with data points is plotted in Appendix B.

If the calculated relationship presented in Appendix B is calculated with the result from Duun and Rustad (2008) then the salt-soluble protein concentration is at $1{,}69x4{,}5\% = 7{,}6\%$ and a water-soluble protein concentration at $1{,}69x7\% = 11{,}83\%$, approximately giving a total wet weight percentage at 19,4%. This is very similar to the results from the immersion group at 30°C thawed for 1,25h (M2.3 at 9. march).

Martinez, Salmerón, Guillén, and Casas (2010) reported a salt-soluble protein concentration at 1,8 % wet weight (w/w) after 2 days of storage at 4°C for Atlantic salmon fillets that where smoked and salted before freezing at -25°C for 24 h and thawed for 24h at 4°C. Their reported values for water-soluble protein (6,15-6,79%) is comparable to the refrigerated group, however smoking, salting and 2 days of storage at 4°C can affect protein solubility, and possibly change protein stability in fish muscle.

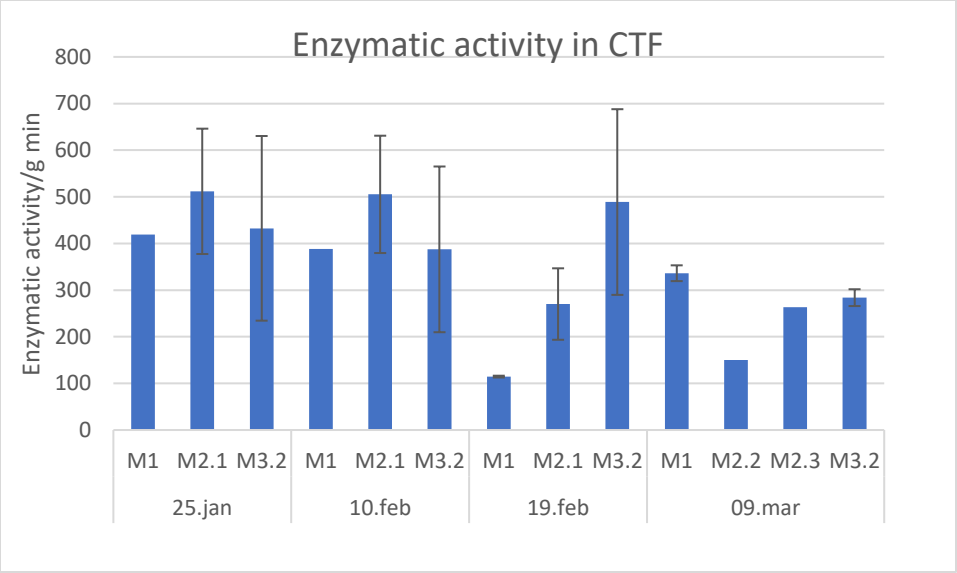

Figure 3.7 Enzymatic activity in cell tissue fluid per gram wet weight×minutes of incubation time. The enzymatic activity is illustrated for M1/the group thawed in cold room (4°C, 24h), M2.1/immersion thawing at 30°C for 1,25h, M2.2/immersion thawing at 25°C for 1h, M2.3/immersion at 25°C for 1,25h and M3.2/room temperature for 3h. SD (n=2) is displayed as error bars for the groups illustrated as averages. The results are categorized by date, as shown in the x-axis.

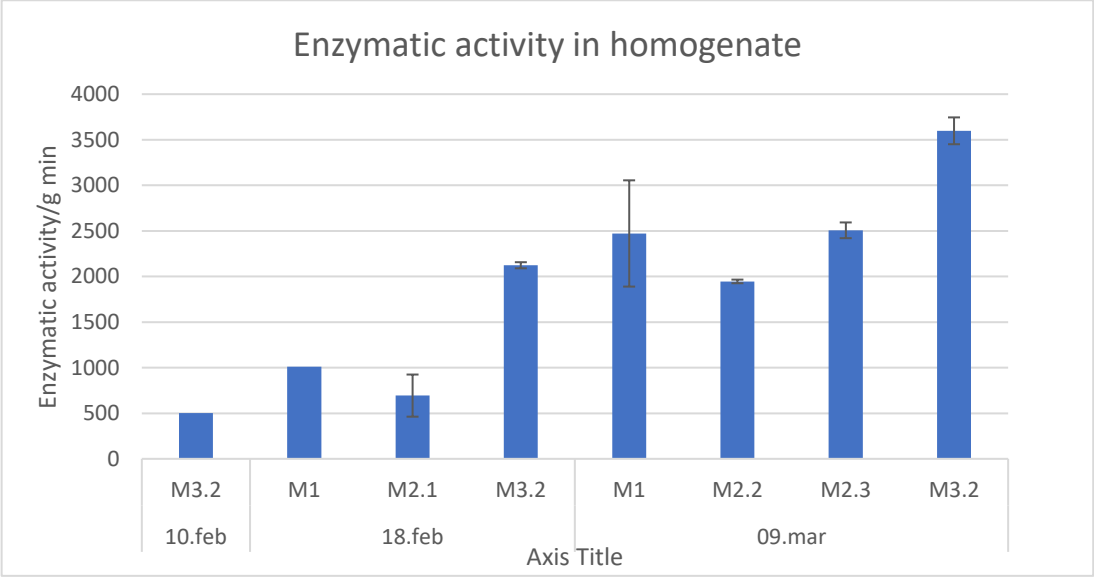

Figure 3.8 Enzymatic activity in homogenate per gram wet weight*minutes of incubation time. The enzymatic activity is illustrated for M1/the group thawed in cold room (4°C, 24h), M2.1/immersion thawing at 30°C for 1,25h, M2.2/immersion thawing at 25°C for 1h, M2.3/immersion at 25°C for 1,25h and M3.2/The group thawed in room temperature for 3h. SD (n=2) is displayed as error bars for the groups illustrated as averages. The results are categorized by date, as shown in the x-axis.

The results from the enzymatic activity assay for CTF and homogenate are illustrated in figure 3.7 and 3.8, and the relationship between the enzymatic activity in CTF and homogenate are expressed in figure 3.9. There were not any measurements taken on 25. January because the water-soluble protein extract was left for over two days in the cold room and was not suited for further determination.

The enzymatic activity of cathepsin B+L in the CTF is used as a marker to assess leaked tissue fluid originating from ruptured lysosomes. This can give an indication of the damage that have occurred during freezing, storage and thawing. A difference in the relative enzymatic activity between the methods indicates how the thawing methods affects tissue leakage and the following release of enzymes from the lysosomes within muscle cells. Enzymatic activity in homogenate reflects the total enzymatic activity because homogenization of muscle tissue causes all the lysosomes within the cell to rupture and subsequently releasing the enzymes present – The total sum of cathepsin B+L are then released.

The enzymatic activity is dependent on a specific substrate, in this case a synthetic fluorogenic substrate, Carbobenzoxy-phenylalanyl-arginine-4-methylcoumaryl-7-amide. This substrate is specific for cathepsin B+L-like enzymes. These enzymes are cysteine proteases that have the ability to cleave muscle protein that contain the amino acid cysteine. The cathepsin's play an important role in intracellular proteolysis and originate in the lysosome within cells. However, when the lysosome membrane disintegrates during freezing and thawing, the enzymes are released, and uncontrolled proteolysis can occur. In several fish species Cathepsin B, D and L are considered as the proteases playing the most important role in *post-mortem* muscle softening, as described in the introduction (section 1.2 Fish as raw material).

$$\frac{\Delta arbitrary\ fluorescence\ value\ of\ CTF \times \min -1}{\Delta arbitrary\ fluorescence\ value\ \ of\ homogenate \times \min -1} \tag{3.1}$$

In order to investigate if the temperature and duration chosen for immersion thawing at 30°C for 1,25h (M2.1) was causing the elevated relative enzymatic activity, as illustrated by figure 3.7, the temperature was lowered to 25°C and the duration was unchanged at 1,25h (M2.3) and changed to 1h at 25°C for M2.2. The result from immersion thawing at 25°C gave the lowest enzymatic activity compared with the other groups at 9. March, which is conclusive with the results from drip-loss and soluble protein when reducing the temperature for the immersion method. Drip loss and protein solubility did indicate that immersion thawing at 30°C for 1,25h was not suited to achieve fresh-like quality of Atlantic salmon.

It should be mentioned that a change in enzymatic activity can either originate from an increase in quantity of enzymes that have been released as lysosome leakage or it could be due to a change in the activity of each enzyme during thawing. The first one is assumed.

The large difference in relative enzymatic activity between the dates for all groups are perhaps due to better preparation and execution of the enzymatic assay, the standard deviation is also

substantially lower for the group thawed in cold room (4°C for 24h) and room temperature (3h) at 9. March compared with 18.feb.

Fast thawing gave the best result regarding relative enzymatic activity, which is in line with theory. Nilsson and Ekstrand (1994) reported that slow thawing rate increased cell tissue fluid volume and enzymatic activity in CTF more than fast thawing, they studied the effect of tempering rate, which is partial thawing, where not all the ice has been converted to water, in rainbow trout. In their work, β-N-acetylglucosaminidase and α-glucosidase, which originates from the lysosome, where studied regarding enzymatic activity.

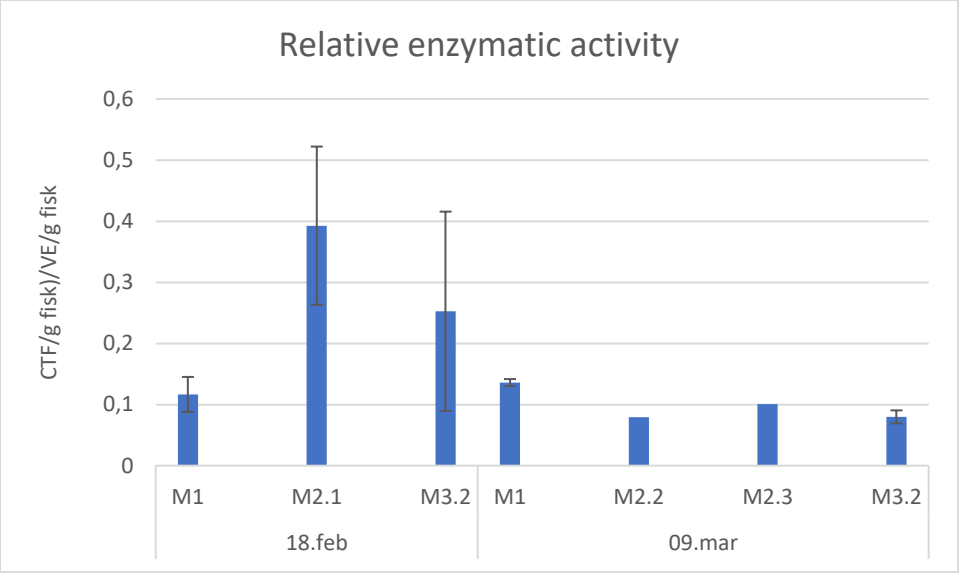

Figure 3.9 Displays enzymatic activity, activity in cell tissue fluid (CTF)/ activity in homogenate (H) for each method. SD (n=2). M1 illustrates the refrigerated group (4°C, 24h), M2.1/immersion at 30°C for 1,25h, M2.2/immersion at 25°C for 1h, 2.3/immersion at 25°C for 1,25h, and M3.2/room temperature for 3h. SD (n=2) is displayed as error bars. The results are categorized by date, as shown on the x-axis.

4 Conclusion

Three common thawing methods, immersion in water, thawing in cold room at 4°C and in room temperature was tested for their ability to retain fresh-like quality in Atlantic salmon fillets. The preliminary experiments gave indication that the temperature in the immersion thawing group was too high to achieve fresh-like quality, and the room temperature thawed group's duration was too short to achieve complete thawing of the fillets. Subsequently, the conditions for the water immersion group was changed from 30°C to 25°C and the room temperature group duration was changed from 2,35h to 3h.

Drip loss and lysosomal enzyme leakage was significantly elevated in salmon thawed by immersion at 30°C for 1,25h compared with thawing in room temperature and in cold room. Protein solubility was significantly lower (10,97-12,95%) when thawed by immersion at 30°C for 1,25h compared with immersion thawing at 25°C, for two durations at 1h (22,27%) and 1,25h (19,48%). Reduction of temperature in the immersion group indicated lower protein denaturation, better retention of liquid and minimized lysosomal enzyme activity compared with immersion thawing at 30°C.

The results in this study indicated that immersion thawing at 25°C gave significantly lower lysosomal enzyme leakage (0,079 for 1h) and drip loss (1,72% for 1,25h), while protein solubility (23,27% for 1h) was elevated but not significantly higher compared with thawing in air (room temperature and in cold room at 4°C). Based on the three thawing methods tested, immersion thawing at 25°C for 1h seems to be best suited to achieve fresh-like quality in frozen Atlantic salmon fillets.

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Abbreviations

WSP – Water-soluble proteins

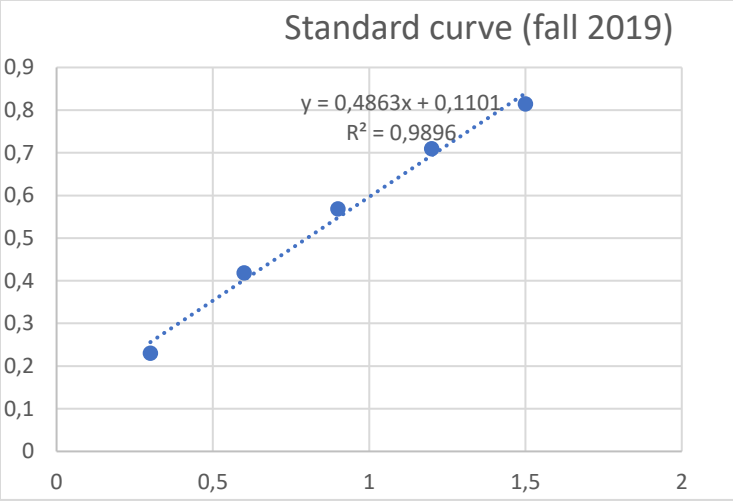
SSP – Salt soluble protein

WHC – Water holding capacity

CTF – Cell tissue fluid

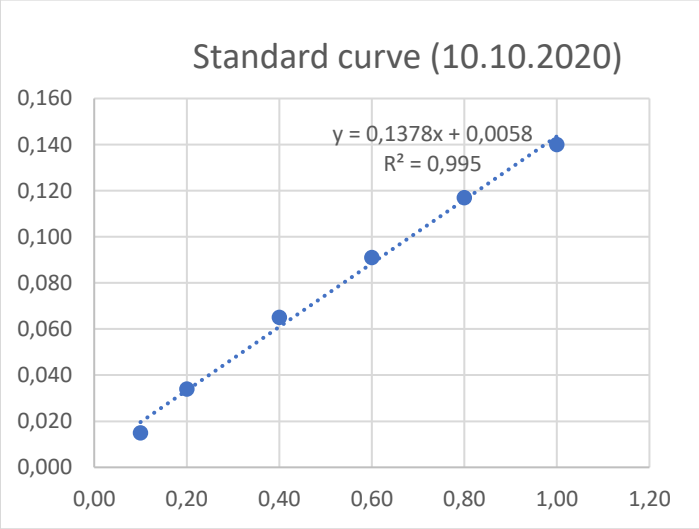
Appendix A

BGG datapoints and plotted standard curve used for determination of salt soluble protein and water-soluble protein

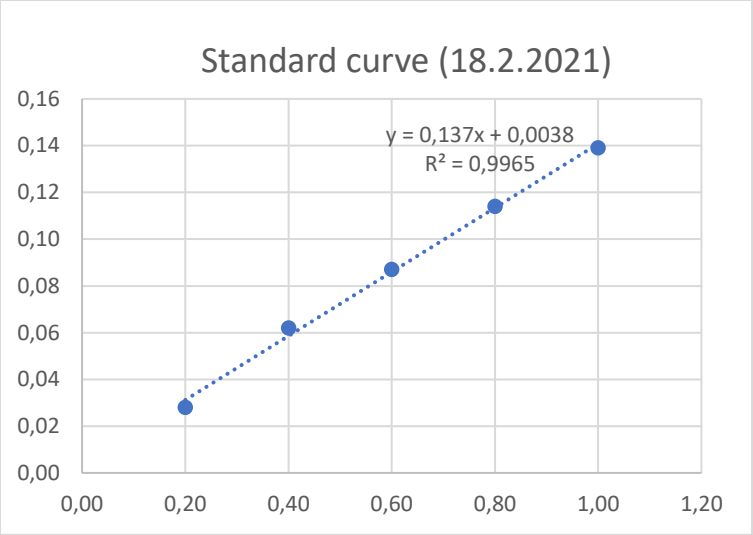


BGG(Fall 2010)

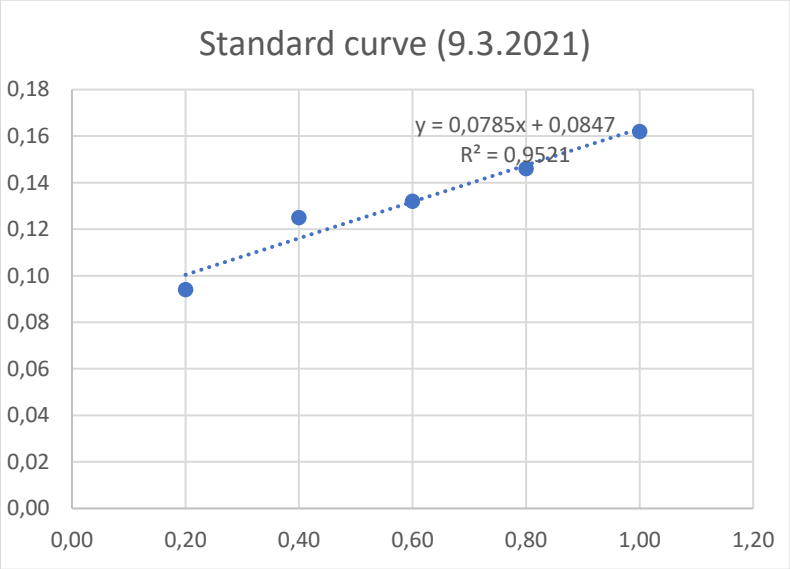
OD595	Kons.(mg/mL)
0,23	0,3
0,418	0,6
0,568	0,9
0,709	1,2
0,814	1,5



BGG standard curve 10.10	
OD595	Kons
0,015	0,10
0,03	0,20
0,07	0,40
0,091	0,60
0,12	0,80
0,14	1,00



BGG (18.2)	
OD595	Kons
0,03	0,20
0,06	0,40
0,087	0,60
0,11	0,80
0,14	1,00



BGG (9.3)	
OD595	Kons
0,09	0,20
0,13	0,40
0,132	0,60
0,146	0,80
0,162	1,00

Appendix B

Cell Tissue Fluid

BSA standard					
rør #	mg/ml	OD_{595}			snitt
1	0,2	0,247	0,249	0,261	0,252
2	0,4	0,461	0,454	0,462	0,459
3	0,6	0,614	0,679	0,982	0,758
4	0,8	0,832	0,841	0,843	0,839
5	1	0,979	1,017	0,978	0,991

Cell Tissue Fluid

BGG standard					
rør #	mg/ml	OD_{595}			snitt
1	0,1	0,167	0,163	0,149	0,160
2	0,3	0,312	0,324	0,316	0,317
3	0,6	0,498	0,498	0,491	0,496
4	0,9	0,668	0,675	0,678	0,674
5	1,5	0,824	0,802	0,828	0,818

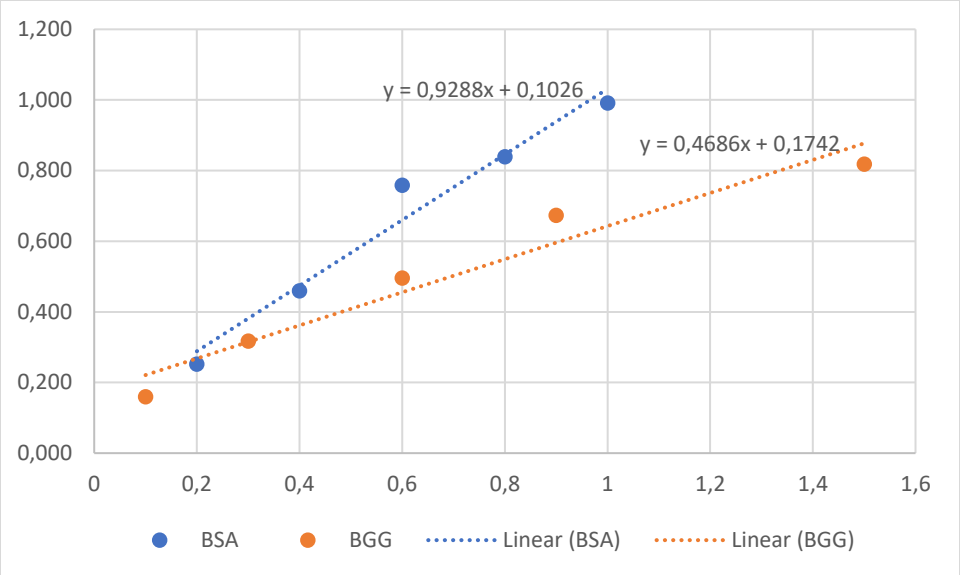


Figure B: Illustrated datapoints for BSA (blue circles) with plotted standard curve (blue dotted lines) and illustrated datapoints for BGG (orange circles) with plotted standard curve (orange dotted lines). Taken datapoints are collected from datasheet in Appendix B.

To compare the two regression lines, absorbance was set to 0,6

$Y = 0{,}6$

1: Y=0,9288X+0,1026 (3.1)

$X(y = 0{,}6) = 0{,}535 \rightarrow 0{,}535\ mg/mL$ BSA (3.2)

2: $Y = 0{,}4686x + 0{,}1742$ (3.3)

$X(y = 0{,}6) = 0{,}909 \rightarrow 0{,}908\ mg/mL$ BGG (3.4)

Then, by comparison the x-values, this we get this relationship.

$$\frac{X_{BGG}}{X_{BSA}} = 1{,}69 \rightarrow 1\ mg\frac{BSA}{mL} = 1{,}69\ mg\frac{BGG}{mL} \quad (3.5)$$