



# **Effects of bleeding conditions and storage methods on the quality of Atlantic Cod**

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2016

**Thesis for the degree of Master of Science in Food Science**



**UNIVERSITY OF ICELAND**  
**SCHOOL OF HEALTH SCIENCES**

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FACULTY OF FOOD SCIENCE AND NUTRITION



# **Áhrif blóðgunaraðstæðna og geymsluaðferða á gæði þorsks**

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90 Einingar

Október 2016

Ritgerð til meistaragráðu í matvælafræði



**HÁSKÓLI ÍSLANDS**  
**MATVÆLA- OG NÆRINGARFRÆÐIDEILD**

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Prentun: Háskólaprent  
Reykjavík, Ísland 2016

## Ágrip

Markmið verkefnisins var að rannsaka, í fyrsta lagi, áhrif íslausrar geymslu blóðgaðs og slægðs þorsks fyrir vinnslu við  $-1\text{ }^{\circ}\text{C}$  á gæði flaka (*Gadus morhua*). Í öðru lagi að athuga möguleikann á því að hefja kælingu fyrr í framleiðsluferlinu með því að láta fiskinn blæða í kældum sjó ásamt því að skoða áhrif hreyfingar og endurnýjun blæðingarvatns á gæði flakanna. Í þriðja lagi að skoða áhrif biðtíma fyrir blæðingu á gæði flaka sem geymd eru án íss við  $-1\text{ }^{\circ}\text{C}$ . Hráefni sem notað var í verkefnið kom frá FISK Seafood hf og veitt af ferskfisktogaranum Málmey SK-1. Hann er fyrsta skipið á Íslandi til að geyma afla íslaus í lest við  $-1\text{ }^{\circ}\text{C}$ . Eftir flökun voru sýnin ýmist fryst strax eða léttisöltuð fyrir frystingu og geymd í allt að 6 mánuði. Til þess að hraða á skemmdarferlum þá voru frosin sýni geymd við töluvert hátt hitastig og sveiflur.

Til að meta gæði flakanna voru mikilvægir þættir skoðaðir, s.s. drip, vatnsheldni, suðunýting, litur flaka, magn fosfólípíða, fríar fitusýrur og magn óbundins járns í vöðvanum.

Niðurstöður tilraunar á áhrifum biðtíma fyrir blæðingu á gæði flaka gáfu ekki skýrar niðurstöður vegna galla í sýnatöku.

Niðurstöður mælinga á sýnum sem blædd voru í blæðingarmiðli við mismunandi hitastig gáfu til kynna að kæling við blæðingu gæti valdið því að flök verði rauðari. Þar sem litur flaks er mikilvægur þáttur í gæða og verðmati þorskflaka er ekki heppilegt að byrja kælingarferlið í blæðingartanki heldur láta fiskinn blæða við sjávarhita,  $6\text{ }^{\circ}\text{C}$ . Hitastigsbreytingin hafði meiri áhrif á ósöltuð flök en léttisöltuð. Það hvort einhver hreyfing er á blæðingarmiðli getur haft áhrif á afköst blæðingar. Niðurstöður tilrauna gáfu til kynna að, upp að vissu marki, gæti blæðing í blæðingarmiðli sem sem er á hreyfingu verið afkastameiri en blæðing í óhreyfðu vatni. Þær gáfu líka til kynna að hreyfing blæðingarvatns geti haft meiri áhrif gæði afurðar en hversu hratt vatnið er endurnýjað.

Við mat á áhrifum íslausrar geymslu við  $-1\text{ }^{\circ}\text{C}$  á gæði þorskflaka voru sýni geymd á þrennskonar vegu um borð í togaranum. Þau voru ofurkæld og ísuð, ísuð beint eftir blæðingu eða ofurkæld og geymd við  $-1\text{ }^{\circ}\text{C}$  íslaus. Niðurstöður sýndu að geymsla á ofurkældum þorsk á ís viðhéltað betur gæðum en geymsla án íss við  $-1\text{ }^{\circ}\text{C}$  þegar niðurstöður úr mælingum ósaltaðra flaka voru greindar.

**Lykilorð:** Þorskur, blæðing, geymsluaðstæður, ofurkæling, gæði.



## Abstract

The aim of this project was to study, first of all, the effects of iceless storage at -1 °C on the quality of cod fillets (*Gadus morhua*). Secondly to explore the possibility of starting the cooling process earlier in the processing line by bleeding in cooled bleeding medium as well as the effects of circulation and replacement of bleeding medium during bleeding. Thirdly the effects of prolonged waiting time before bleeding on the quality of fillets stored at -1 °C without ice. All samples were received from FISK Seafood, caught by the fresh fish trawler Málmey, the first Icelandic ship equipped with and utilizing, iceless fish hold at -1 °C. Samples were stored at rather high temperatures during frozen storage in order to speed up quality deterioration.

To evaluate the quality of the fillets various factors were analyzed, for examples drip, water holding capacity, cooking yield, color, phospholipid content, free fatty acid content and heme iron content.

Results of experiment on the effect of waiting time before bleeding on quality of fillets were not conclusive due to a fault in the design of the experiment.

Results of measurements of samples bled at different temperatures indicated that starting the cooling process during bleeding could result in fillets being redder. Since the color of fillets is a large deciding factor when it comes to evaluating the value of fillets this may not be feasible. The circulation of bleeding medium, if any, and how quickly it is replaced can also have a large impact on bleeding efficiency. Results of experiments performed showed that bleeding in bleeding medium that is circulating rather than still is beneficial. It also indicated that, up to a point, the circulation of the bleeding medium can have a larger impact on the bleeding efficiency than how quickly the bleeding medium is replaced.

When evaluating the effect iceless storage at -1 °C has on the quality of the cod fillets samples were kept supercooled on ice, supercooled at -1 °C and iced without any pre-cooling. Results showed that supercooling was beneficial to the quality of the samples, both 500 – 1000 g fillets and + 1000 g fillets, and indicated that it is beneficial to store the fillets supercooled on ice rather than at -1 °C without ice in fish hold.

**Key words:** Atlantic Cod, bleeding, storage conditions, supercooling, quality.



## **Acknowledgements**

This study was conducted at Matís ohf in Reykjavík, Iceland. It was supported by the AVS R&D Fund of Ministry of Fisheries and Agriculture in Iceland (R 068-14) and TþS, Tækniþróunarsjóður, (142667-0611). Their support made it possible to complete these important measurements and analysis.

I would like to thank my supervisors Sigurjón Arason, Magnea Guðrún Karlsdóttir and Sæmundur Elíasson for their invaluable help and guidance during this study. Matís ohf I would like to thank for providing good research facilities and to the staff of Matís Chemical lab for their assistance and guidance. I would like to thank Hólmfríður Sveinsdóttir, Iceprotein, for assistance during sample collections and FISK Seafood hf. for not only providing the material used but for giving the group extensive access to the processing plant and trawler.

Finally, I want to thank my family and friends, especially my boyfriend for all the support and patience throughout my studies, my mother for her unwavering support and my father for his encouragement and valuable help during my study.



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## Abbreviation

$a_w$  = Water activity

DMA = Dimethylamine

FA = Formeldahyde

FFA = Free fatty acids

HI = Heme iron

NHI = Non-Heme iron

PL = Phospholipids

TMA = Trimethylamine

TMAO = Trimethylamine N-oxide

WHC = Water holding capacity

# 1 Introduction

In the whitefish industry bleeding is considered one of the most important steps of the processing line with regards to value and quality of end products. It is generally the first process fish goes through after catching. Insufficient bleeding can affect the shelf life of products through product taste, odor, visual appearance and residual blood can also promote lipid hydration, provide good nourishment for bacteria and cause increased enzymatic activity (Richard & Hutlin, 2002).

When trying to pinpoint a temperature or temperature range that facilitate bleeding, most research has shown mixed results. Some suggest that the amount of blood left in the fillet increases with lowered bleeding media temperature while others suggest using chilled sea during bleeding ensures better bleeding due to slower clotting of blood (Skjervold et al, 2001; Karlsdottir, 2014). The most effective temperature has therefore not been pinpointed yet providing an opportunity to see if it would be possible to combine the bleeding and cooling process of the cod.

After bleeding and cooling the cod is stored until it can be landed. During this time the cod often starts to go through rigor mortis. During that time it is extremely important to make sure that the temperature is low and stable in order to prolong the rigor as long as possible. An experiment performed in Denmark showed that Atlantic cod, both farmed and wild, stored at lower temperature had significantly less shrinkage during rigor mortis and less weight lost during cold storage (Aune et al., 2014). In their experiment Aune and colleagues did not store any group below 0 °C however others have and they reached the same conclusion, that subzero temperature is beneficial down to -2 °C (Erikson et al., 2011).

Supercooling has been researched rather extensively in the last few years. It is a method used to prolong storage life of fresh fish by slowing down chemical reactions, growth of spoilage bacteria and rigor mortis in the flesh. It is widely used within the seafood industry. The general consensus is that the sooner fish can be cooled down to subzero temperatures the better (Lauzon et al, 2009; Gao et al, 2007). Supercooling has not only been used when working with cod but other species as well, for example salmon and mackerel. In a study of the effects of brining, supercooling and modified atmosphere packaging (MAP) on shelf life researchers found that shelf life of fresh cod loins could be prolonged by 3-4 days by supercooling to -2 °C after processing. If both supercooling and MAP was utilized the increase in shelf life was 6-7 days (Lauzon et al, 2009).

The focus of present project is to explore the possibilities of bleeding and storage of wild Atlantic cod. First of all to investigate the effects of the temperature of the bleeding medium in order to see if starting the cooling process during bleeding could be viable, possibly shortening the time it takes to cool down the cod. Also to explore the effects of circulation of the bleeding medium and rate at which it is replaced on bleeding efficiency. Secondly to compare supercooled (-1 °C) storage without ice of gutted, whole cod to storage on ice to study if it is possible to eliminate ice from the process, decreasing both the carbon footprint and cost of the storage. Thirdly an experiment was performed in order to see the effects of prolonged waiting time before bleeding on quality parameters, specifically the bleeding efficiency. Similar experiments have been performed before. The objective of this experiment was to see if the

-1 °C storage without ice would change the effect of waiting time before bleeding on the quality of the fillets.



## 2 Review of the literature

### 2.1 The Atlantic cod

The Atlantic cod (*Gadus morhua*) is one of the most economically important fishstocks in Iceland, accounting for 35-40% of the total seafood export revenue (Gunnarsson, 2001). The Atlantic cod caught in Iceland in 2014 was 27% of the total catch in Icelandic waters or over 220.000 tons (Hagstofa Islands, 2014).

Insuring buyers get a stable quality product is crucial. Therefore a lot of resources have been granted into investigating possible ways to increase the quality and shelf life of cod products. There are many different processes and attributes that can affect the quality and therefore the shelf life of the products.

Atlantic cod (*Gadus morhua*) (Figure 1) is a demersal fish. It is found from just a few meters below the surface down to around 600 m below it. In Icelandic jurisdiction it is most commonly found at 100-400 m below sea level. It prefers temperatures of 4-7°C but can be found in waters varying from 0°C and up to 20°C (Jonsson & Palsson, 2013).

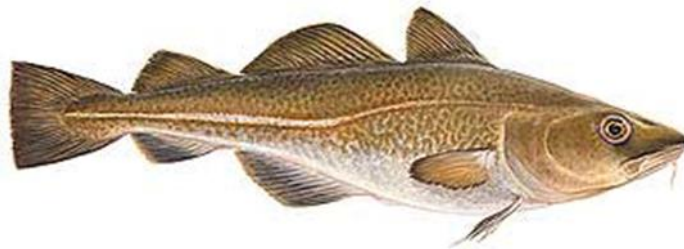


Figure 1 The Atlantic cod (Gunnarsson, n.d.)

The Atlantic cod is omnivorous feeding on invertebrates and fish, its main feed is however capelin. Cod can weigh up to 40 kg but average at 2-4 kg. It can live for up to 24 years and generally reaches sexual maturity at 2-4 years old (Jonsson & Palsson 2013).

### 2.2 Chemical Composition

Cod fillets are made up of, about 80% water, 15-20% protein and usually less than 1% fat, but it fluctuates with seasons and spawn cycle of the specie.

Cod is classified as a lean fish. That means that it deposits most of its fat into its liver, while fatty fish deposit most of its fat cells in the flesh (Murray & Burt, 2001). During processing such as lightly salting the composition changes. The salt content increases as well as the water content, affecting the ratio of other components. Lightly salted cod therefore contains a higher water and salt percentage but lower percentage of fat and protein.

### 2.2.1 Water and water activity

The water content of a product is the total water present in the food. It doesn't matter if the water is bound in reactions or not. As previously stated, water makes up about 80% of the weight of cod fillets. (Murray & Burt, 2001). The water within the muscle has various different roles. It is for example a solvent for salts, enzymes and other water-soluble materials. It plays a big role in deciding the texture of the muscle as well as the quality (Schmidt, 2007). Water within the muscle is categorized into three categories based on how tightly it is bound for example in reactions or in proteins. The terms used are non-bound, loosely bound and tightly bound water (Hui, 2008).

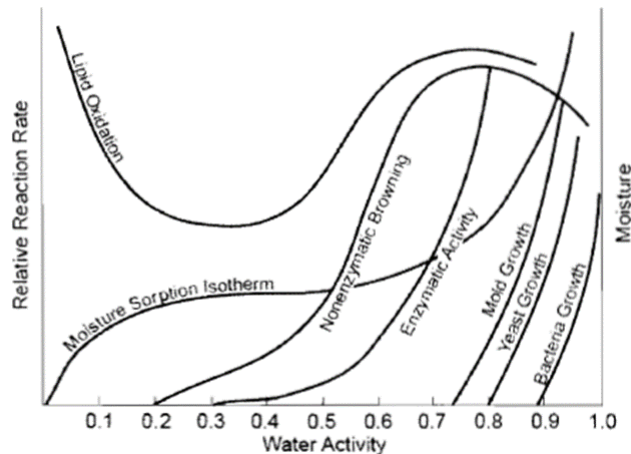
Non-bound water is water that can easily be removed or lost and is between cells within the muscle. The loosely bound water is located inside the muscle cells. It is bound there with capillary force but part of it can be removed. It can take part in chemical reactions. Lastly the tightly bound water is water that is not available for chemical reactions. It is found inside the proteins and forms a strong bound monolayer. It is very difficult to remove from the proteins. After prolonged chilled or frozen storage the proteins lose their ability to retain all the water and some of it is lost as drip (Fellows, 2009).

Because of the many roles played by the water, only part of it is available to engage in chemical reactions or can be utilized by spoilage bacteria. The ratio of free, unbound water can have a large effect on the products shelf life. To evaluate the amount of unbound water in the product water activity ( $a_w$ ) is used. Water activity is defined as “the ratio between the vapor pressure within the muscle ( $p$ ) to the saturated vapor pressure of water at the same temperature ( $p_0$ )” (Fellows, 2009). The  $a_w$  depends on the structure of muscle proteins within the fillet as well as the structure of the muscle proteins that bind and interact with tightly bound water. It is therefore influenced by protein changes (Ramanzin, Bailoni, & Giovanni, 1994; Zayas, 1997).

$$\text{Water activity} = a_w = \frac{p}{p_0}$$

The  $a_w$  plays an important role in determining the shelf life of products. That is why many production processes are focused on lowering it to lengthen the shelf life of products (Fellows, 2009).

Every microorganism has a minimum, optimum and maximum  $a_w$  for growth. Therefore the  $a_w$  can be used by producers to evaluate what factor is most likely to limit the shelf life of his product (Figure 2), for example if a product has  $a_w < 0.85$  the shelf life would not be limited by bacterial growth because bacteria usually does not grow under those conditions. (Damodaran *et al*, 2008; Fellows, 2009; Roos, 1993). This can provide valuable information for those working on research or practice of storage conditions, packaging and shelf life.



**Figure 2 Shows the connection between  $a_w$  and the reactions and organisms limiting shelf life. (Damodaran *et al*, 2008)**

### 2.2.2 Water holding capacity

Water holding capacity (WHC) is the muscle's ability to hold on to its water during application of force. It is influenced by changes in the proteins (Ramanzin, Bailoni, & Giovanni, 1994; Zayas, 1997). There are also some external factors that can affect the WHC of fillets. They are for example catching season and location, feeding patterns, handling post slaughter, size, age and processing methods (Hamm, 1960; Thorarinsdottir *et al.*, 2004). Changes in chemical composition during processing can affect the WHC. Up to a certain point all production methods that include salting increase the WHC (Hamm, 1960; Thorarinsdottir *et al.*, 2004).

WHC is one of the physical properties to evaluate the quality of fish as raw material for further processing. It affects the juiciness and taste of the products as well as the drip loss during thawing. A decrease in WHC can therefore lead to economic losses due to both complications during processing and reduced yield (Fennema, 1990).

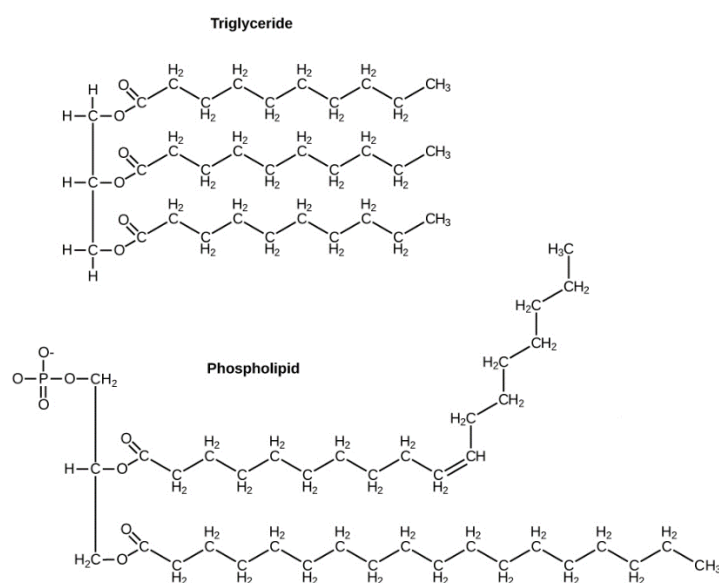
### 2.2.3 Protein

Proteins in cod are made up of three different protein groups. First are the myofibrillar proteins accounting for about 70-80%, second are the sarcoplasmic proteins accounting for about 25-30% and third are stromal proteins accounting for about 3-10%.

Denaturation of proteins during prolonged frozen or chilled storage, mainly myofibrillar proteins, can cause increased drip or water loss during thawing which can lead to for example changes in taste and texture. To evaluate if the proteins in a product are losing their water binding ability it is possible to measure the WHC and drip of the fillets (Careche *et al.*, 1998; Careche *et al.*, 1999; Chichester & Stewart, 1981).

## 2.2.4 Fat

Cod is about 0.4-0.8% fat and the fat is about 90% phospholipids (PL) (Figure 3) (Olley & Lovern, 1960). They are structural lipids found in cell membranes. The rest is triglycerides (Figure 3) which are used as an energy source, mostly found in the muscles responsible for moving the fins and tail, and deposited in the liver (Lauritzsen *et al*, 1999; Damodaran *et al*, 2008). Figure 3 shows the difference between these different types of fat. The triglycerides are saturated fats, three fatty acids with a glycerol head. The PL are however unsaturated made up of a hydroscopic head and hydrophobic end, the fatty acids.



**Figure 3 Structural difference between triglycerides and phospholipids. (Mader & Windelspecht, 2013)**

The fat within the cod products are generally broken down either by lipid hydration or enzymatic activity. The type of fatty acids present play a big role in deciding which process leads (Lauritzsen *et al*, 1999; Damodaran *et al*, 2008). Enzymatic activity is one of the factors likely to limit the shelf life of lean fish like cod during frozen storage (Huss, 1995). Fish is generally rich in poly unsaturated fatty acids (PUFA), those fatty acids are very susceptible to lipid hydration. The elements that play the biggest role in lipid hydration are the amount of double bonds within the fatty acids, the ratio of phospholipids, the location of the fat within the muscle, whether materials that can have a catalytic or halting effect on the lipid hydration are present as well as if outside stimuli such as heat, direct sunlight or air surrounding the product is present. (Khayat & Schwal, 1983). Lipid hydration and enzymatic activity are often deciding factors limiting the shelf life of cod in frozen storage (FAO, n.d.; Khayat & Schwal, 1983). They can for example cause discoloration of the flesh and changes in taste and odors.

One of the main elements affecting if and how much hydration takes place is the ratio of PL in the muscle, since PL have unsaturated fatty acids. They are very susceptible to hydration, with a large

amount of double bonds. They are however also very susceptible to break down due to enzymatic activity (Rougerean & Person, 1991; Hardy *et al*, 1979).

Research has shown that when phospholipids break down due to enzymatic activity the amount of free fatty acids (FFA) increases (Olley & Lovern, 1960; Karlsdottir *et al*, 2014(a)). Measurements of the amount of FFA and PL can therefore be a good indicator of the quality of the material when working with lean fish. Freezing rate and storage temperature can have a large impact on the amount of enzymatic activity as well as the storage time, bleeding efficiency and temperature fluctuations during storage (Olley & Lovern, 1960; Karlsdottir *et al*, 2014).

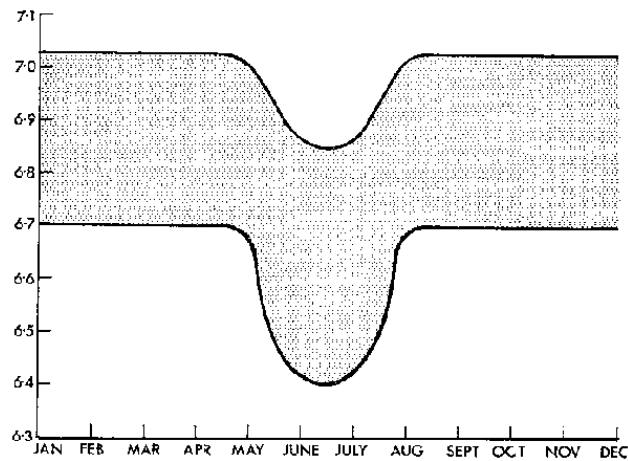
## **2.3 Variability of raw material**

When working with raw materials such as cod it is very important to keep in mind that they are living organisms, and just like people, each individual is in some way different from the next one. Another thing to keep in mind is the variability related to the spawning cycle of the specie, age, gender, season and other factors (Love, 2001a).

The cod sex organs are small during the summer, but start growing rapidly around October. When that happens the cod starts to break down components of its body to supply energy for the changes and to make up for the diminished food supply during that time of year. When the cod starts to break down components of its body in this way it causes changes to its flesh. The muscle is made from hair-like cells which shrink causing the space between them to increase resulting in more fluid within the muscle (Love, 2001a; Page *et al*. 1998; Colton *et al*. 1979). That can cause the texture of the flesh to change. Therefore the condition of the cod is generally considered good in the fall when the muscle is firm and the texture desirable. During spawning, from March until the beginning of May, when access to feed is limited, the muscle therefore becomes soft and watery with low fat content (Raversu & Krzynowek 1991). After spawning the flesh becomes soft and is not able to repair itself until early summer when the food supply increases again. After spawning the cod needs to feed to make up for the energy deficiency during the winter as well as the increased energy needed before spawning. It starts to make that up in early summer. That then causes the flesh to be prone to gaping (Raversu & Krzynowek 1991; Love, 2001a).

Once cod reaches sexual maturity and has spawned for the first time the sex organs start taking up more space within its abdominal cavity. Therefore older cod lose proportionally more body mass during spawning (Love, 2001a). These are factors that all researchers need to keep in mind when deciding at which time samplings should be performed as well as when analyzing data. This is also a factor that producers should keep in mind since they can affect how the cod and its composition is affected by the processing method performed.

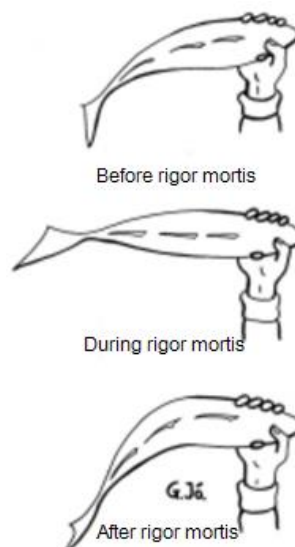
For processors it is possible to monitor this by measuring the pH of the muscle. The degree of acidity goes hand in hand with the nutritional state of the cod. When there is a small amount of glycogen present in the muscle when the cod dies a very small amount of lactic acid is formed during rigor mortis. Then the pH of the muscle is high. The means that when cod starts feeding heavily after spawning the pH of the muscle drops temporarily (Figure 4) (Love, 2001a).



**Figure 4 Average pH values of groups of cod taken from grounds all over the north Atlantic at different time of year (Love, 2001a)**

## 2.4 Rigor mortis

As soon as cod is in post mortem stage specific chemical reactions start occurring. At that point the muscles of the cod are soft and the cod can easily be bent. Gradually the muscles start to tighten up and the cod to stiffen (Figure 5). This is what is referred to as rigor mortis and it can last from a few hours to a few days depending on conditions of the cod and its surroundings. The quicker a cod goes through rigor mortis the more likely it is to suffer damage because of it, most commonly gaping. A cod that is badly bled or chilled goes through the process faster than those sufficiently bled and chilled and supercooling cod slows the process down even further (Gunnarsson, 2001).



**Figure 5 Effects of rigor mortis on stiffness of fish (Gunnarsson, 2001).**

Slowing down rigor mortis is desirable for a number of reasons. Mainly because the cod does not start to spoil until rigor mortis is finished and because of the possible gaping caused by cod going through it too fast. Therefore slowing this process down prolongs shelf life (Bjarnason & Arason, 1998; Gunnarsson, 2001).

## 2.5 Processing and handling

Processing and handling of fish is a large deciding factor in the quality of the final product made from the raw material. Therefore the whole chain from catching until the product lands on the customers' plate needs to be held to the highest possible standard (Gunnarsson, 2001).

Atlantic cod is usually caught in either a bottom trawl or on line. Fish caught on line is generally considered to be of higher quality if all other handling is the same. Line caught fish is considered to experience less stress during catching causing rigor mortis to be prolonged. It is also usually always bled while still alive and if other factors related to the bleeding process are handled well should provide very good bleeding. The largest flaw related to line fishing is that fish caught on line can often contain more ringworms (Gunnarsson, 2001).

Fish caught in bottom trawl (Figure 6) can be exhausted, depending on the haul time. During trawling the fish is in a way hoarded into the net. The fish can therefore become rather stressed. It can also become exhausted trying to get away. This can affect how well the fish bleeds, how quickly it goes through rigor mortis and therefore have a big impact on the quality of the fish. The best way to prevent this from becoming an issue is to limit the haul time as well as the size of each haul. That way it is more likely that the fish is still alive when it gets on-board and thus gives better quality raw material (Gunnarsson, 2001).

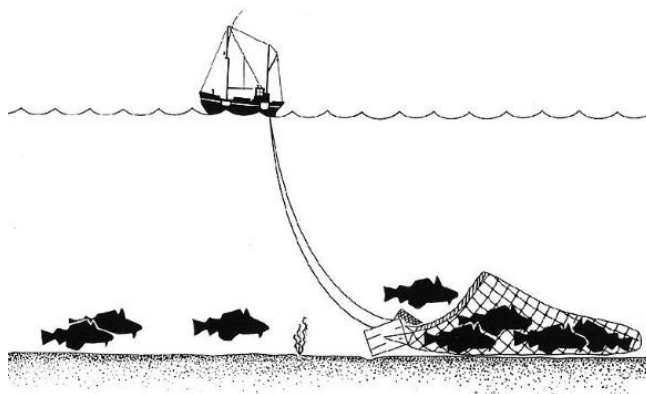
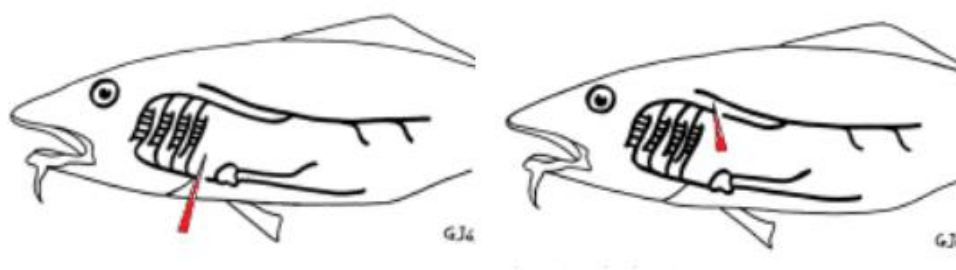


Figure 6 Bottom trawling (Wikipedia, 2016)

### 2.5.1 Bleeding

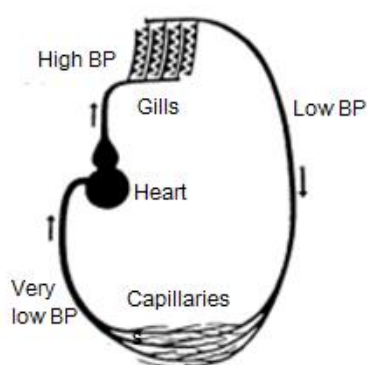
Bleeding is generally the first process the fish goes through after catching. The process is carried out in a way that it eliminates most of the blood from the muscle. In the whitefish industry, bleeding is considered one of the most important steps of the processing line with regards to value and overall

quality of the products. It can affect the shelf life of products, taste and visual appearance. Badly bled fish loses quality faster because of the affect residual blood has in the muscle. Residual blood is a large factor in inducing development of undesirable flavor, odor and promoting lipid hydration (Fletchermacher, 1975; Richard & Hutlin, 2002). Generally the objective is to bleed everything as quickly as possible, however, how it is done exactly varies. When bleeding cod, it is traditional to cut the vein between the gills and the heart and/or the jugular veins on each side of the spine (Figure 7) (Adalbjornsson & Vidarsson, 2010; Thordarson, Hognason, & Gestsson, 2012). Often the esophagus is also cut. It is however important to keep in mind that researches have also shown that the time that passes before a fish is bled has more effect on how well blood is drained than if the fish is gutted before bleeding or not (Karlsdottir *et al*, 2014(a)).



**Figure 7 Demonstration on where cod is traditionally cut to ensure sufficient bleeding. (Gunnarsson, 2001)**

The pressure within a cod circulatory system varies (Figure 8). The pressure is very high between the heart and gills but drops drastically beyond the gills. That is why cutting the veins between the heart and gills causes the blood to pour out quickly (Gunnarsson, 2001).



**Figure 8 Schematic of blood pressure fluctuations within the cod circulatory system (Gunnarsson, 2001)**

Blood generally makes up about 1.5 – 7% of a fishes total body weight depending on the species (Huss, 1995). Majority of the blood is found in the internal organs of the fish and only about 20% in the

muscular tissue, varying on the stress level of the fish and species (Huss, 1995). Blood serves as good nutrition for spoilage bacteria and can speed up chemical reactions. It also affects the visual aspects of the flesh (Gunnarsson, 2001).

Bleeding and gutting are often done simultaneously. Whether catch is bled first and then gutted or bled and gutted at the same time can impact the quality of the fish. When deciding what procedure to follow it is important to keep in mind how long it will take to finish bleeding the whole haul if catch is bled and gutted simultaneously. Bleeding and gutting at the same time could lead to part of the catch being bled after death. An upside to this method would be more efficient bleeding since a large amount of the blood present in fish is found in the guts. Research has shown that the time that passes before a fish is bled has more effect on the blood that is not drained than the way the fish is bled and gutted (Karlsdóttir *et al*, 2014(a)).

Muscle contractions play a big role in draining of the blood from the fish during bleeding. That is the reason why bleeding the fish alive is better. Fish are usually bled in sea water with a constant flow through the container. Sometimes however they are let bleed out in air which does not provide a product of the same quality. (Gunnarsson, 2001)

Most researches have shown that it is more important when it comes to bleeding that the fish is bled alive than that it is bled out in liquid media (Valdimarsson 1981; Valdimarsson & Gunnarsdóttir 1982; Karlsdóttir *et al*, 2014(a)). No definite temperature has been pinpointed yet that proves to be the most efficient temperature during bleeding of cod. Researches have however showed contradictory results. Some suggested that the amount of blood left in the cod after bleeding increases with lower temperature while others suggest that it is important to cool the cod down during bleeding to slow down clotting of the blood (Skjervold *et al*, 2001; Karlsdóttir *et al*, 2014(a)).

Bleeding is a very important processing step to ensure that the cod quality is not lost after catching. Badly bled cod can be discolored and therefore of lower value. Another important part is in general the overall handling of the cod during processing on-board. If the processing line can in some place cause the cod to fall down or get hit with any kind of force it can cause bruising. Bruising affects the value of the fillets dramatically.

Bleeding cod is important for a number of reasons. Not doing it properly can shorten the shelf life and decrease the quality of the final product as previously stated. It affects the color of the fillets which is very important when considering the value of the product. Consumers are not likely to buy fillets with stains or other visual impairments. Agents found in blood can stimulate chemical reactions, for example, lipid hydration and formation of FFA due to increased enzymatic activity. The blood of cod also contains more TMAO-ase activity than muscle causing it to speed up the breakdown of TMAO in to formaldehyde and dimethylamine during frozen storage or into TMA in fresh fillets (Karlsdóttir *et al*, 2014(a)). TMAO is thought to help stabilize proteins against denaturation and other changes. However TMAO breakdown products, DMA and FA have been linked to textural problems during frozen storage and an increase in drip loss. FA may produce cross-linking of the muscle proteins causing these changes (Jeremiah, 1996).

### 2.5.2 Supercooling

Supercooling is a technique used to increase the storage life of fish. Supercooling of fish is used to lower its temperature below the melting point of ice, the ideal temperature varies with species and composition of the flesh but usually fish is supercooled down to -1 °C. When fish is chilled to that temperature ice crystals start forming. It is important to control the temperature when supercooling to make sure not to freeze the material. Freezing food when the intention is to supercool could cause loss of quality. It could cause formation of large ice crystals which cause damage within the muscle, for example increased drip loss and changes in texture (Bauhuud, D., 2008; Waterman, J.J. & Taylor D.H., 2001).

Research has shown that supercooling prolongs the shelf life of the product by slowing down growth of spoilage bacteria as well as chemical reactions that cause spoilage, such as changes in total volatile bases (TVB-N) and trimethylamine (TMA) formation, and prolong rigor mortis (Lauzon *et al*, 2009).

This method to prolong the shelf life of fish has been thoroughly researched in the last few years. It has not only been used when working with cod but other species as well. For example salmon and mackerel (Gao *et al*, 2007). The shelf life of fresh cod fillets was prolonged by 3-4 days by supercooling to -2 °C in a study of the effects of brining, supercooling and modified atmosphere packaging (MAP) on shelf life. If both supercooling and MAP was utilized the increase in shelf life was 6-7 days (Lauzon *et al*, 2009).

### 2.5.3 Lightly salted cod

Using salt to prolong the shelf life of cod products has been done for a long time. At first the main goal was to stabilize the product where due to limited access to acceptable cold storage. By salting the product, water activity was lowered enough to get a shelf life of months instead of days (Andrés *et al*, 2005). The method is based on a transfer of salt and water, where the cod loses water and takes up salt. It affects the texture and taste of the cod. Today salting of cod is done mostly to get those changes in taste and texture and not as much to prolong the products shelf life (Andrés & Rodríguez-Barona, 2002; Martínez-Alvarez & Gómez-Guillén, 2006).

When working with salted cod it takes some time to rehydrate the fillets before they can be utilized. That does not suit the lifestyle of the western world so a new product entered the market about 15 years ago. This was lightly salted cod containing only around 2% salt which is obtained by injecting and/or bringing the fillets. The brine is made up of water, salt and sometimes phosphates and the brining time is often 24-48 hours (Thorkelsson *et al.*, 2008; Xiong, 2005).

Lightly salted cod is a product that can be produced in less than 10% of the time it takes to produce salted cod, and can therefore be sold at a lower price. This product does vary a lot from the traditional product. It does not share the same textural characteristics or flavor but has still gained popularity, especially in Spain (Gudjonsdottir *et al*, 2010; Lindkvist *et al*, 2008).

While lightly salted cod has become increasingly popular it is important to note that there is another significant difference between it and salted cod that is not related to taste or texture, that is

water activity. Since the product has only been lightly salted, the water activity has not been lowered like it would be during traditional salting and therefore lightly salting is not used as a way to prolong storage life. The fillets do still need to be frozen (Gudjonsdottir *et al*, 2010).

## **2.6 Study objective**

The main aim of this project was to evaluate the possibility of starting the cooling process earlier in the processing line by bleeding in cooled medium. To investigate how iceless storage compares to other methods like icing and supercooling and icing and lastly the effect of circulation of bleeding medium as well as how quickly it is being replaced during bleeding on the bleeding efficiency.

As previously stated, research has shown that there are clearly a lot of advantages to supercooling a product. The sooner it is possible to start that process the better with regards to spoilage bacteria, chemical reactions and rigor mortis. There have been performed experiments with mixed results, some indicating that supercooling cod during bleeding can be beneficial while others find it would not be. This is therefore still an unanswered question



### 3 Material and methods

#### 3.1 Experimental design

All fish used in the project was provided by FISK Seafood. The cod was caught by the fresh-fish trawler Málmey SK-1. Málmey SK-1 is based in Sauðárkrókur. Skaginn and 3X Technology are companies based in Iceland that designed Málmey's bleeding, cooling and storage systems. It is a rotex system designed to bleed, and supercool all fish equally and then store it all without ice in the fish hold at -1 °C (Figure 9).

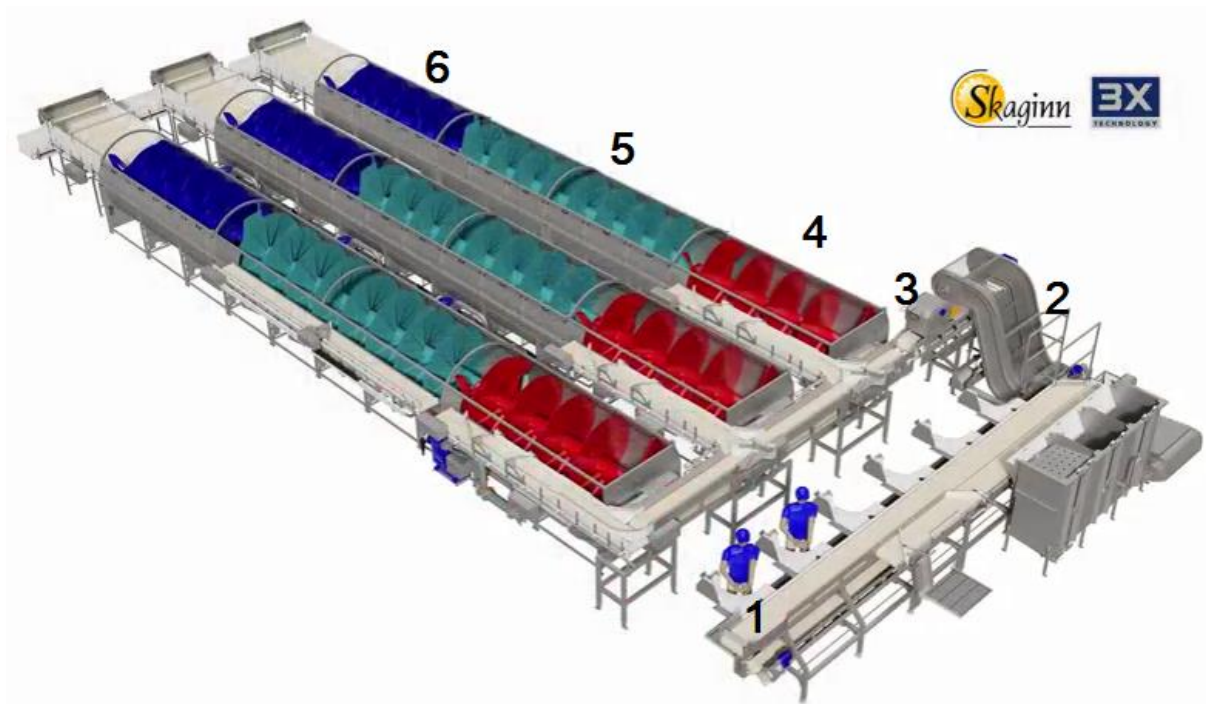
This project consists of two main experiments performed in July 2015 and March 2016. Each one can then be separated into three smaller ones. In total 171 fillets were used. 108 in the experiment performed in July and 63 in the other one.

**Table 1 Experiments performed on the project.**

<b>Experiment:</b>	
<b>Bleeding temperature</b>	July 2015: Bleeding samples in water at sea temperature (6 °C), 2 °C and -1 °C.
<b>Storage method</b>	July 2015: Stored on ice ,supercooled and iced or supercooled stored without ice
<b>Waiting time</b>	July 2015: Cod waiting 0 mins, 30 mins, 60 mins or 90 mins before being bled.
<b>Rotex system</b>	March 2016: Explore effect of circulation of water and how quickly bleeding medium is replaced on bleeding efficiency.
<b>Storage of large fillets</b>	March 2016: 1000-1500g fillets stored on ice or supercooled stored without ice.
<b>Circulation</b>	March 2016: Samples bled in tubs with or without a pump circulating bleeding medium.

#### 3.2 The bleeding and supercooling processes

After catching, the cod was transferred onto a conveyer belt to the bleeding station, marked 1 on figure 9. The fishermen bleed and gutted each individual cod and slid it onto the conveyer belt under the table. From there the cod went onto the first stage of bleeding and cleaning, marked 2 on figure 9. There the cod was cleaned with high water flow and pressure. The next stage of the process was grading, marked number 3 on figure 9. The cod is automatically graded with a vision grading system, graded both by species and size. This controlled into which rotex the cod was guided. It continued on a conveyer belt ending up in the bleeding chamber of the rotex, marked number 4 in figure 9. There it stayed for approx. 20 mins in bleeding medium at sea temperature. That of course varies but during our experiments the sea temperature in July 2015 was 6 °C and in March 2016 was 5 °C. When it moved on to the cooling chamber of the rotex, number 5 on figure 9, the sea in the tank has been cooled down to about -1 °C. Lastly the cod entered the second cooling chamber set at temperature around -3 °C. When the cod left the rotex system it had been cooled down from sea temperature to -1 °C in 45 - 60 mins.



**Figure 9 Schematic demonstration of the setup of bleeding station and rotex systems on board the fresh fish trawler Málmei (Youtube, 2015)**

Each rotex is about 1.6 m in diameter. This is a fully automatic system where each chamber has individual heat exchangers. Each chamber of the rotex system holds about 150 kg of cod at a time.



**Figure 10 Rotex on-board Málmei used in present project. (Photo S. Elíasson)**

Due to the systems design it can be guaranteed that each individual cod is treated equally and that the “first in – first out” rule is followed. The bleeding tanks are all about 7,000 L but the power of the circulating pump and how fast water in the tank is replaced varies between the three systems (Table 2).

**Table 2 Information regarding circulation and condition of water in bleeding tanks of the rotex systems used in present study.**

Placement of Rotex	Pump	Water replaced per. min	Whole tank replaced every:
Port side	8 kW	235 L / min	30 mins
Middle	3 kW	585 L / min	12 mins
Starboard side	3 kW	390 L / min	18 mins

Following bleeding and supercooling, cod was stored in tubs (Figure 11). They were packed straight into the tubs without ice, stored iceless and kept at -1 °C until unloading.



**Figure 11 Cod being transferred into tubs in hold in Málmey (Youtube, 2015).**

### **3.2.1 The July 2015 sampling**

The catch used for the samplings performed in July 2015 was caught on July 3<sup>rd</sup> and 4<sup>th</sup>. Three experiments were carried out at this time (Figure 12). The first experiment was to explore the effects of different bleeding temperature on fillet quality. The samples for this experiment were caught on the 3<sup>rd</sup> of July. The hauling time during catching was 180 min. and the hauling size was 9.7 tons. The samples were bled at three different temperatures, sea temperature (6 °C) in the middle rotex, at 2 °C and at -1 °C in the rotex on starboard side.

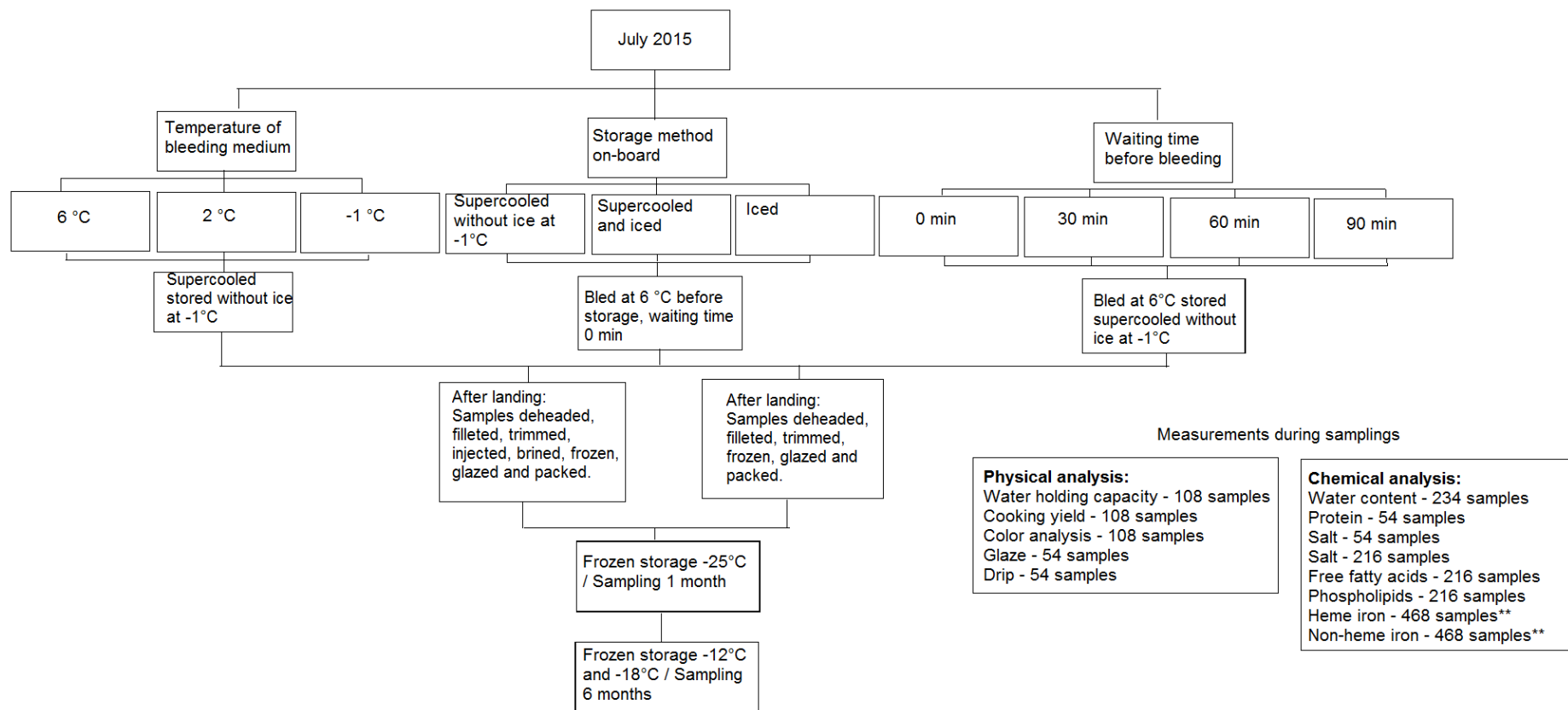
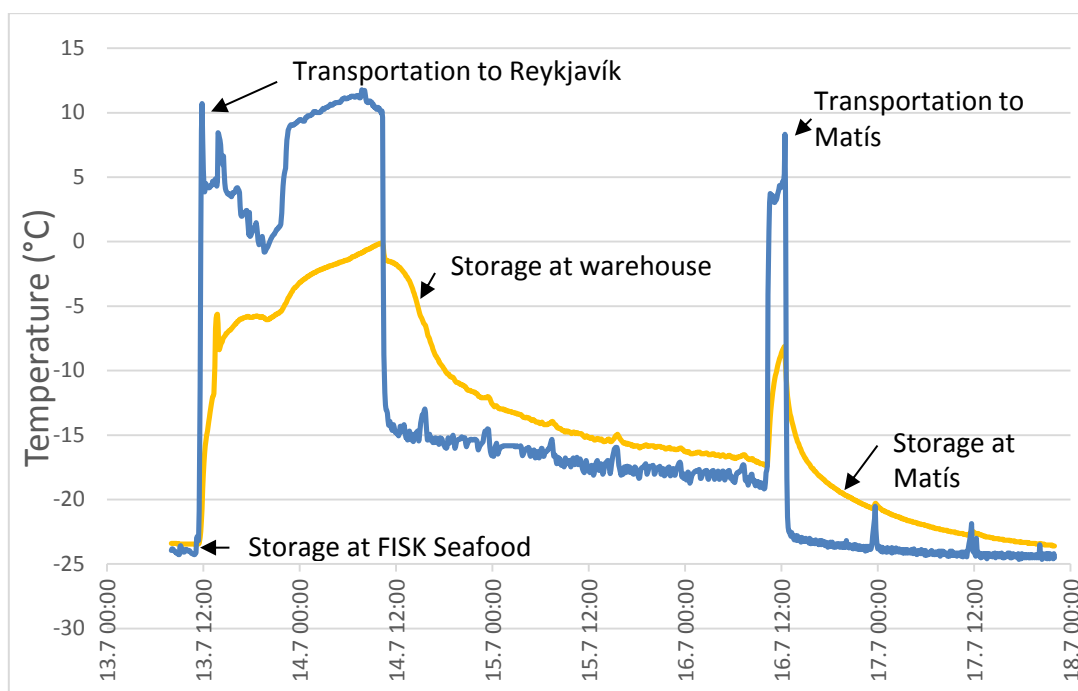


Figure 12 Setup of the July 2015 experiment and amount of samples measured in the July 2015 experiment. Analysis were performed after 1 and 6 months of storage and figure shows total number of samples measured. \*\*Water, Heme Iron and Non-Heme Iron was in all parts of the fillets, tail, middle part, loin and belly flap in the storage method and the waiting time experiment. Other chemical analysis were performed only on middle part.

The other two experiments were performed on samples caught on July 4<sup>th</sup>. These experiments were performed to investigate the effects of waiting time before bleeding and storage methods on-board on fillet quality. The hauling time was in this case 157 min. and the haul size was 8.23 tons. Three different storage methods on-board were analyzed. Firstly, the collected samples went through the standard Málmey procedure, i.e. supercooling and storage without ice. Secondly, the samples went through the supercooling rotex but were then stored on ice on deck. Thirdly, the samples were removed from the rotex after the bleeding (before pre-cooling) and stored in tubs with ice on deck. When investigating the waiting time before bleeding, all the samples went through the standard Málmey procedure, but were taken at different times into the process, having waited for 0, 30, 60 and 90 min before bleeding (Figure 12).

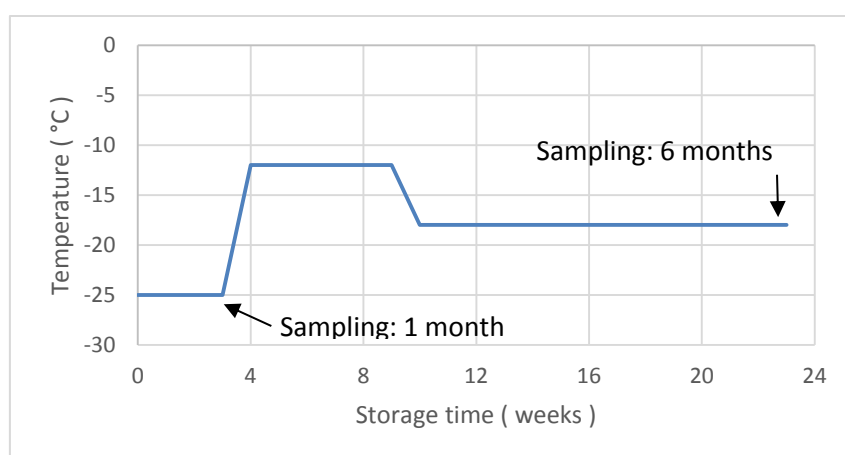
After landing, the cod was kept in storage at -1 °C until it was processed. The samples from the experiment of different bleeding temperatures were processed on the 7<sup>th</sup> of July. The cod were headed, filleted and trimmed. Half of the fillets were injected and brined in a 4.5% brine for 45 hours before being IQF frozen and subsequently glazed and packed in plastic bags and carton boxes. The freezing took 116 min and the final temperature of the fillets was -24 °C. The other half was frozen directly without any further treatment.

The samples from the other two experiments, storage method on-board and waiting time before bleeding experiments, were processed on the 8<sup>th</sup> of July. The cod were processed the same way as described above with the exception that the fillets were brined for 44 hours compared to 45 hours. After freezing and glazing, all samples were packed in plastic bags and carton boxes and stored in the frozen storage facility at FISK Seafood at -24 °C until it could be shipped to Reykjavík (the 13<sup>th</sup> of July). Figure 13 shows data from temperature loggers used to monitor the temperature during the transportation to Reykjavík with a truck. The fluctuations were extensive during transportation as the figure shows.



**Figure 13** Temperature profile during shipping of July samples from Sauðárkrókur to Reykjavík. Temperature loggers were placed on the inside and outside of boxes. Blue line represents the surrounding temperature and the orange line the temperature inside the boxes center.

At arrival to the laboratory on the 16<sup>th</sup> of July, the samples were placed in frozen storage (-25 °C), and stored for 4 weeks prior to analysis (1 month sampling point). Subsequently, the samples were moved first to -12 °C storage for 6 weeks followed by -18 °C for 8 week before being taken out for analysis (Figure 14). The fluctuations of storage temperature and high storage temperature were chosen to accelerate the quality deterioration.



**Figure 14** Schematic overview of the storage temperature of samples collected in July 2015 and samplings points.

Sampling points were two for all experiments as shown on figure 12, after storage for 1 month and 6 months. At the beginning of each sampling point, 3 fillets from each group were taken out of the freezer. Glaze was removed and glazing recorded (only at 6 month sampling point). They were then thawed at 2 – 0 °C for 48 hours on racks so drip could leak away but covered with plastic to prevent drying. After thawing all physical analysis was performed and the fillets divided as shown in figure 16.

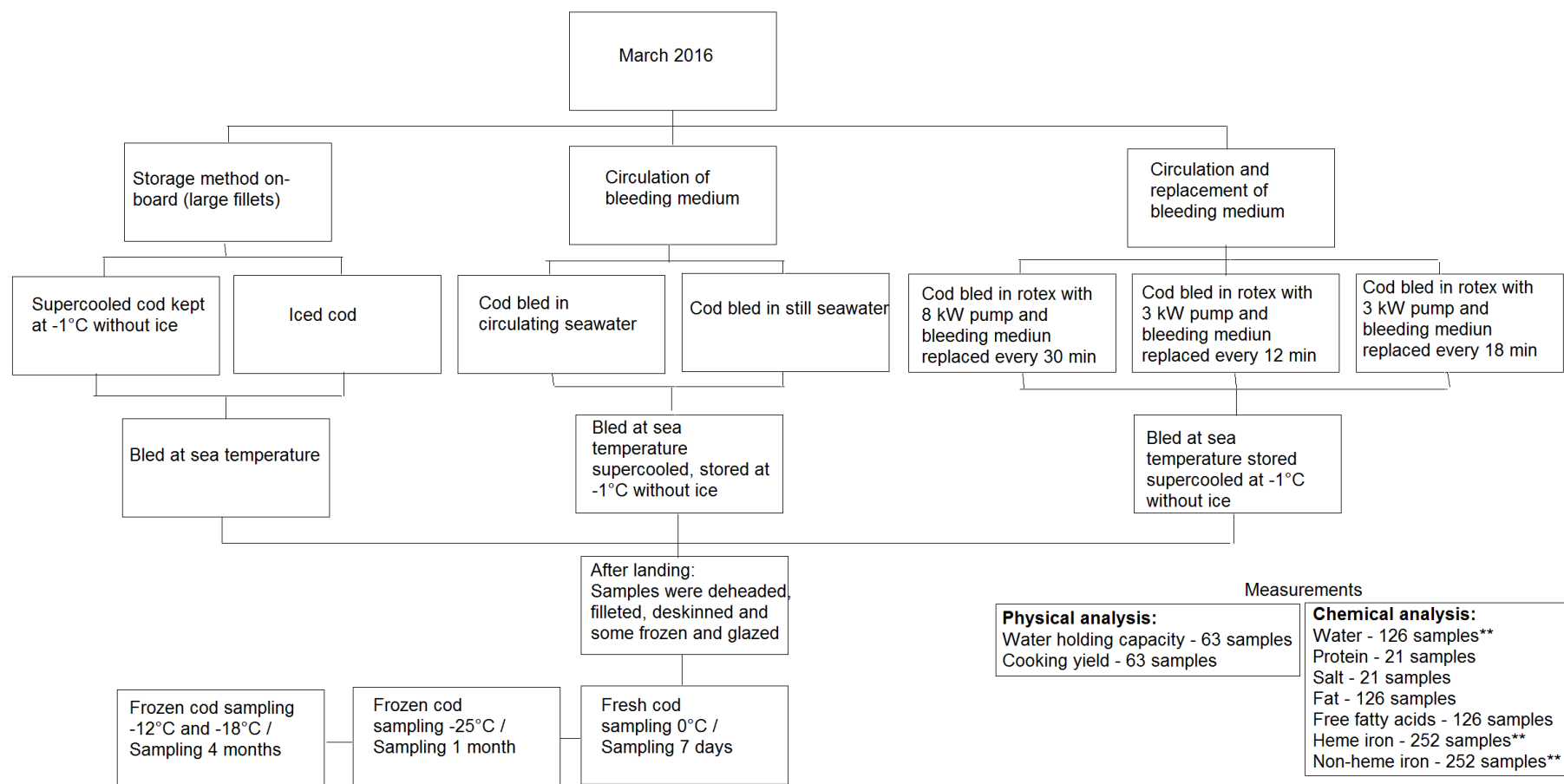


**Figure 15 Fillets were divided prior to chemical analysis. (1 = Middle part / Cooking yield, 2 = Middle part / Analysis, 3 = Loin, 4 = Belly flap, 5 = Tail).**

Minced muscle was stored, at -80 °C until chemical analysis could be performed.

### **3.2.1 The March 2016 sampling**

Three experiments were performed on-board Málmey on material caught on the 4<sup>th</sup> and 5<sup>th</sup> of March 2016. Figure 16 shows setup of experiments performed in March 2016 on Málmey.



**Figure 16 Setup of the March 2016 experiment and amount of samples measured in the March 2016 experiment. \* Analysis was performed after 1 and 6 months of storage and figure shows total number of samples measured. \*\*Water, Heme Iron and Non-Heme Iron was measured in all parts of the fillets, tail, middle part, loin and belly flap in storage method and waiting time experiment. Other chemical analysis were performed only on middle part.**

Material for the first two experiments was caught on the 4<sup>th</sup> of March. The material came from a haul of 5.3 tons with a hauling time of 90 min. The first experiment was designed to explore the effects of both circulation of bleeding medium as well as how quickly it is replaced during the bleeding process on bleeding efficiency. All samples went through the same standard procedure but were placed in different rotex systems, one on the port side, one in the middle and one on the starboard side (Figure 9). Information on the difference between the rotex systems can be found in table 2. The aim of the second experiment was to explore the effects of water circulation during bleeding on the fillet quality. The samples were placed in two tubs where each tub contained approx. 100 kg of cod and 200 kg of sea, at sea temperature, 5 °C. In one of the tub a pump was installed to circulate the water while the other had no pump (still water) (Figure 17). The samples were bled for 20 min and the water was replaced once after 10 min during the bleeding.



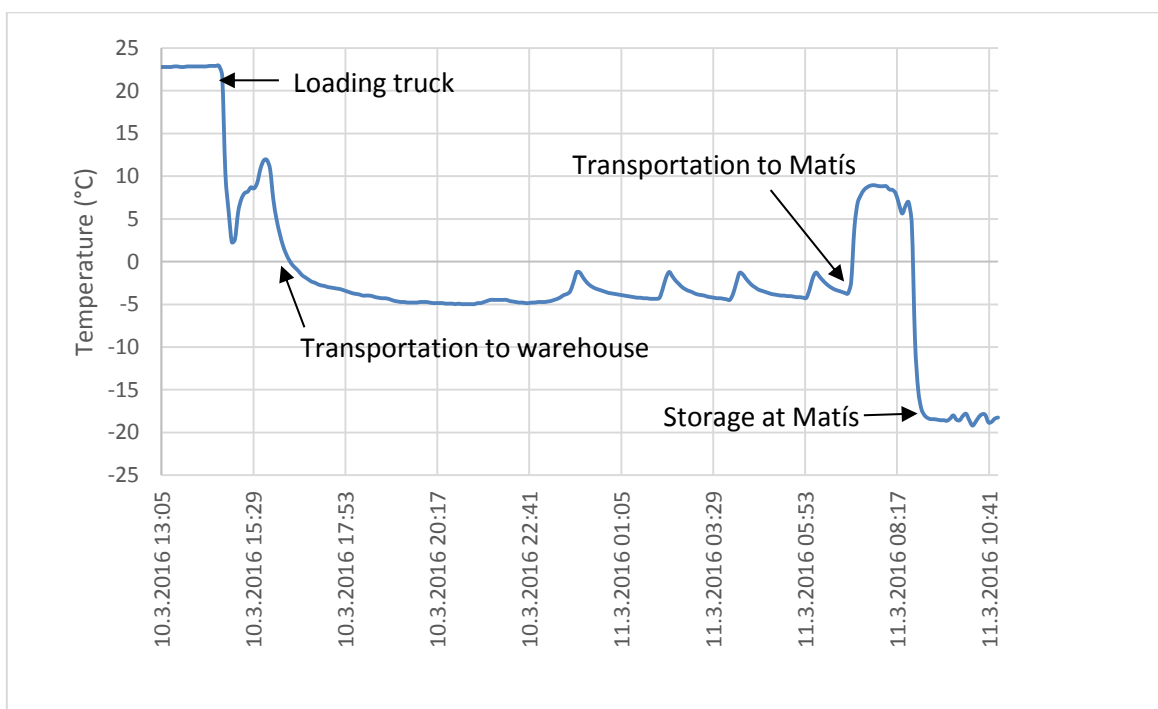
**Figure 17 Bleeding of samples from March 2016 circulation experiment, on the right fish being bled in circulating water and on the left in still water (Photo by S. Elíasson)**

The third experiment was to investigate the effects of iced cod compared to supercooled storage without ice on fillet quality. Samples from that experiment were frozen as previously described. The material for this experiment was caught on the 5<sup>th</sup> of March 2016 in a haul of 9.4 tons with a hauling time of 25 min.

After landing, the cod were stored at -1 °C prior to processing. Samples caught on the 5<sup>th</sup> were processed on the 9<sup>th</sup> of March. The cod were headed, filleted and fillets trimmed and skinned. The fillets were then transported fresh in EPS boxes to Reykjavík at 0 °C. At arrival to the laboratory, part of the fillets were kept at 0 °C storage for 2 days before being analyzed while the rest were frozen down to -25 °C with blast freezer (Ilsa, 20 T 2/1 POS/NEG REMO, Italy). The freezing time was 3.5 hours. The samples caught on the 4<sup>th</sup> of March, were processed on the 10<sup>th</sup> of March. The cod were headed, filleted and skinned. The fillets were not trimmed. Part of the fillets were packed into EPS boxes with ice and cooling mats and were transported directly to Reykjavík. The rest frozen in an IQF

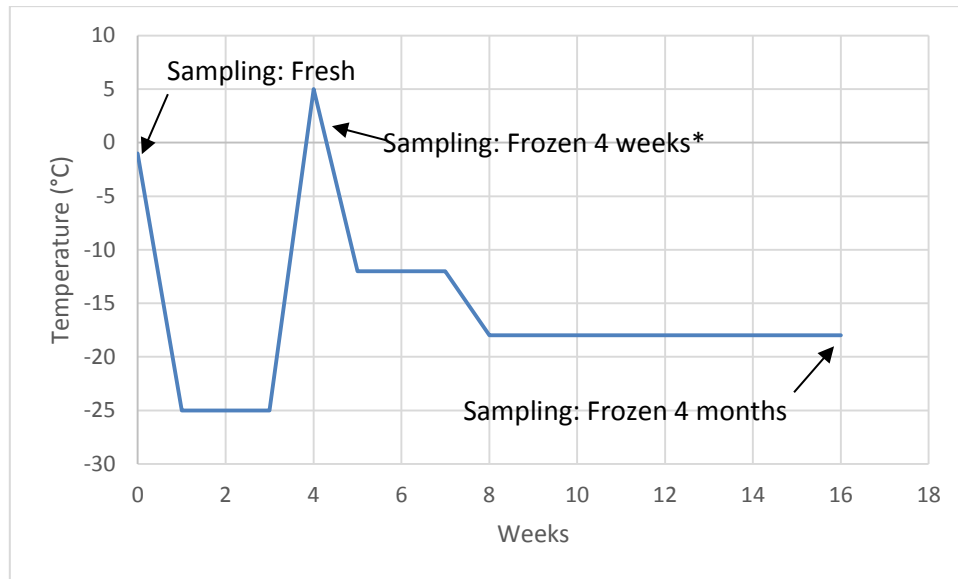
freezer and glazed before being transported later. The frozen samples were packaged in plastic bags and cardboard boxes.

Samples frozen at Sauðárkrúkur were transported with a frozen storage truck like the samples from July 2015 sampling. Temperature loggers were placed outside and inside of the boxes during transportation. Unfortunately, the loggers inside the box malfunctioned so only information on surrounding temperature was recorded during the transport (Figure 18). Other samples were transported fresh and temperature loggers used to monitor the transportation. The temperature inside the EPS boxes was at all time below 0.5 °C during the transport both in the corner and middle of boxes.



**Figure 18 Data from temperature logger monitoring surrounding temperature during transport of frozen samples to Reykjavík. Logger was placed on the outside of a box.**

Samples were stored in a similar way as the July 2015 samples (Figure 19). The main difference was due to malfunction that occurred in the freezing facilities. Because of that it was not possible to evaluate drip loss or glaze amount. The fillets were divided as demonstrated earlier prior to analyzing (Figure 15). Measurements of physical characteristics were performed and mince stored at -80 °C until further analysis could be completed.



**Figure 19 Storage of samples from Málmey March 2016 experiments and sampling points.**  
**\*Thawing due to malfunction shown in figure 60, appendix D.**

### 3.3 Physical analysis

#### 3.3.1 Glazing content

The Codex standard was used for instruction on how to evaluate the amount of glazing present on fillets (Codex Alimentarius, 2001). Frozen fillets were weighed before being placed under and spray of cold water. They were sprayed until all glaze that could be seen or felt had been removed. The fillets were dabbed with tissue to dry adhering water and then weighed again. Glazing was then calculated according to following equation

$$\text{Glazing (\%)} = \frac{\text{g frozen fillets} - \text{g fillets after gentle spray of cold water}}{\text{g frozen fillets}} \times 100$$

#### 3.3.2 Drip loss

Frozen fillets were weighed before thawing, after glaze had been removed, and then again after thawing. During thawing fillets were covered with plastic to prevent drying and laid on grated racks to ensure that fillets do not lie in the drip. The fillets were thawed for 48 hours at 2 - 0 °C. It was then calculated as weight loss during the thawing time.

$$\text{Drip loss (\%)} = \frac{\text{g frozen fillets without glazing} - \text{g thawed fillets}}{\text{g frozen fillets}} \times 100$$

### 3.3.3 Cooking yield

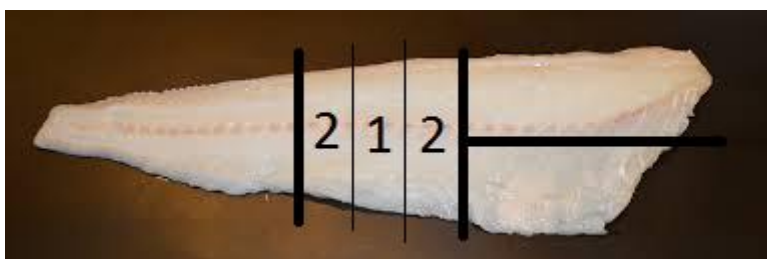
Cooking yield is the amount of liquid lost during cooking. The middle part of the center, marked number 1 on figure 21, was cooked in a preheated steaming oven at approximately 100 °C for 6 min (Convotherm, Elektrogeräte CmbH, Eglfing, Germany).



**Figure 20** The oven (Convotherm, Elektrogeräte CmbH, Eglfing, Germany) used to cook samples when determining cooking yield.

After cooking samples were drained and left to cool for at least 15 min or until they had reached room temperature before being weighed again.

$$\text{Cooking yield (\%)} = \frac{\text{g cooked sample}}{\text{g raw sample}} \times 100$$



**Figure 21** Fillet separation before cooking yield analysis and what parts were used.

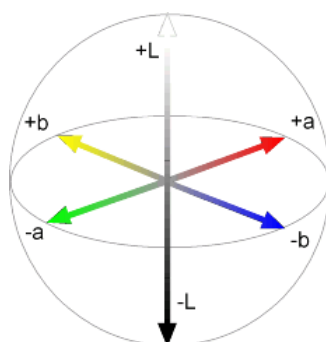
### 3.3.4 Water holding capacity

Water holding capacity (WHC) was determined by a centrifugation method (Eide, Børreses, & Strøm, 1982). The sample glasses were made from cylindrical plexi-glass, 62 mm high with an inner diameter of 19 mm and outer 25 mm and a membrane (100 µm) on the bottom. Approximately 2 g of the minced samples were weighed into the glasses and centrifuged at 1350 rpm for 5 mins at 4°C (Biofuge Stratas, Thermo electron corporation, Germany). The weight lost during centrifugation was then recorded and WHC calculated.

$$\text{WHC (\%)} = \frac{(\% \text{water} \times \text{g sample}) - (\text{g weight loss during centrifugation})}{\% \text{water} \times \text{g sample}} \times 100$$

### 3.3.5 Color

The color of the surface of raw cod fillets was measured using a color machine vision system (CMVS), consisting of a light box and a CCD color camera connected to a computer. Cod fillets were placed in the light box and the digital camera captured a picture of them. Images were captured on the CMVS software program LensEye (Engineering & Cyber Solutions, Gainesville, FL, USA), and color results obtained based on the CIE L\*a\*b\* system. L\* describing lightness (L = 0 for black, L = 100 for white), a\* describing intensity in red (a\* > 0), b\* describing intensity in yellow (b\* > 0) (Figure 22) (Hutchings, 1999).



**Figure 22 Description of the meaning of values gotten through using the CIE L\*a\*b\* system (Hutchings, 1999).**

The CMVS was calibrated using ColorChecker® (X-Rite, Grand Rapids, MI, USA), an array of 24 scientifically designed colored plates. Average L\* (lightness), a\* (redness), b\* (yellowness) values of the muscle surfaces were calculated using the LensEye software. In addition, whiteness of the samples was calculated as described by Park (1994):

$$\text{Whiteness (\%)} = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$$

This was performed only in July 2015 experiment due to problems with the equipment. When calculating results whole fillets were examined as well as belly flaps and loins individually.

### **3.4 Chemical analysis**

#### **3.4.1 Water content**

The water content was determined by accurately weighing 5 g of minced sample into a ceramic bowl. The sample was then dried in an oven at approximately 103 °C for 4 hours and then let rest in a desiccator for 30 min before weighing again. The weight lost was used to calculate the water content and the results expressed as a percentage (ISO 6496. 1999, 2007).

#### **3.4.2 Salt content**

The salt content was determined by the method of Volhard according to AOAC 937.18 (2000). Approximately 5 g of minced sample was weighed into 250 mL plastic bottles and then 200 mL of distilled water added. The bottles were shaken for 45 min in an electric shaker. Bottles were allowed to stand while waiting for sedimentation. Next 20 mL of the solution were pipetted into a 100 mL beaker along with 20 mL of HNO<sub>3</sub> solution. The solution was then titrated with 0.1 N AgNO<sub>3</sub> in a 716 DMS Titrino device.

#### **3.4.3 Protein content**

Protein content was established using the method described in ISO 5983-2:2005 using Tecator. The method was followed with two exceptions, first of all sulfuric acid was used instead of hydrochloric acid and sample size for protein content from 3 to 30% was 1.5 – 2.0 g instead of 1.0 – 1.2 g (ISO 5983-2:2005).

#### **3.4.4 Lipid extraction and measurement**

The lipid extraction of the samples was based on a method by Bligh and Dyer (1959) with adaption. With samples containing 80% water or more 25 g of minced sample was weighed out into a 250 mL centrifuge bottle. If the mince contained less than 80% water the amount weight would need to be adjusted.

25 mL of chloroform and 50 mL of methanol were added to the bottle and the sample homogenized for 2 min. Another 25 mL of chloroform was added and the sample mixed for 1 min. 25 mL of 0.88% KCl was then added to the mixture and mixed for 1 min. Next the sample was centrifuged at 2500 rpm for 20 min at 4°C.



**Figure 23 Centrifuge (Beckman Coulter, TJ-25 Centrifuge, United States of America) used during fat extraction, PL analysis and heme iron analysis.**

After centrifugation, the lower chloroform phase containing the fat, was absorbed with glass pipettes and filtrated on a glass microfiber under suction. The suction flask content was then poured into a 50 mL volumetric flask and the suction flask rinsed with chloroform added to the volumetric flask to ensure the whole sample had been collected. Every trace of the upper phase was removed and the 50 mL volumetric flask filled with chloroform to the mark. The content was then poured into a 50 mL test tube and stored in freezer at -80 °C for later analysis. The chloroform phase was used further for determination of total lipid content, phospholipid content and free fatty acid content.

The extraction was only done once for each sample but duplicates were done when concluding the lipid content. 3 mL of chloroform from the lipid extraction was transferred into 2 screw cap glass tube and the solvent removed at 55 °C using a nitrogen jet. The weight of the glass tube was recorded before adding the extract and after all of the solvent had been removed. The difference in weight of the glass tube before adding the extract and after all of the solvent had been removed was used to determinate the fat content.

**Total fat content in 3 mL of extract** = Weight of the empty glass tube –  
weight of the tube after all of the solvent had been removed

$$\text{Fat (\%)} = (\text{Total fat in 3 mL extract} \cdot \frac{50}{3}) / \text{Weight of minced muscle used during extraction}$$

### 3.4.5 Free fatty acid content

The method used for determination of free fatty acids (FFA) content was the method from Lowry and Tinsley (1976) with modification by Bernárdez, Pastoriza, Sampedro, Herrera and Cabo (2005). The duplicate sample in the 2 screw cap glass tube with the removed solvent from the lipid extraction (Bligh & Dyer, 1959) were used. 3 mL of cyclohexane and 1 mL of cupric acetate-pyridine reagent were added. The mixture was vortexed for 40 seconds and centrifuged at 2000 g for 10 min at 4 °C (Biofuge Stratas, Thermo electron corporation, Germany). The upper layer of the sample was read at

710 nm (Amersham Pharmacia Biotech, Ultrospec 3000 pro, United Kingdom). Quantification was based on a calibration curve constructed from oleic acid standards.

$$\text{Free fatty acid content (\%)} = \frac{\text{Oleic acid} \times 282.46 \times 10^{-6}}{\text{g lipid in the sample}} \times 100$$

Oleic acid in the equation stands for  $\mu\text{mol}$  of oleic acid and 282.46 is its molecular weight.

### 3.4.6 Phospholipid content

The method used for estimation of phospholipids content (PL) was the colorimetric method based on the formation of a complex between phospholipids and ammonium ferrothiocyanate (Stewart, 1980). Duplicates were made of each extract from the lipid extraction. 2 mL of chloroform was added to 15 mL plastic tube with a screw cap. 10  $\mu\text{L}$  of the lipid extract was then added to the tube as well as 1 mL of thiocyanate reagent. The mixture was vortexed for 1 min and centrifuged at 2000 rpm for 5 min at 4 °C. (Beckman Coulter, TJ-25 Centrifuge, United States if America). The lower layer was read at 480 nm (Amersham Pharmacia Biotech, Ultrospec 3000 pro, United Kingdom) And compared with known amounts of a standard phospholipid solution.

### 3.4.7 Iron detection

To evaluate the amount of blood still present in the muscle after bleeding methods detecting iron were used. Two methods were chosen, heme iron and non heme iron measurements. They were specifically chosen in hopes of being able to detect not only the amount of iron present in the muscle but also how the blood would be breaking down during storage. Measurements of heme iron would give an indication of blood present while non heme iron measurements the amount of iron not bound to hemoglobin and then hopefully how much of the heme iron was breaking down during storage.

#### 3.4.7.1 Heme iron content

The heme iron content was determined according to method of Gomez-Basauri and Regenstein (1992) with a slight modification with the main reagents being 40 mM phosphate buffer (pH 6.8) (Disodium hydrogen phosphate). A grounded sample, 2 g, was weighed into 50 ml centrifuge tube and 20 ml of cold 40 mM phosphate buffer was added. The content was homogenized at 13,500 rpm for 10 sec and then centrifuge at 3000 g for 30 min at 4 °C (Beckman Coulter, TJ-25 Centrifuge, United States if America). The supernatant was filtered using Whatman No. 1 filter paper and then the filtrate read at 525 nm (Amersham Pharmacia Biotech, Ultrospec 3000 pro, United Kingdom). Myoglobin content was calculated from the millimolar extinction coefficient, 7.6, and a molecular weight of 16,110. Heme iron was calculated based on Myoglobin, which contains 0.35% iron. The heme iron content was expressed as mg/100 g sample.

### **3.4.7.2 Non-Heme iron content**

The non heme iron content was determined according to the method describe by Schricker et al.(1982) with the main reagents being 0.39% (w/v) sodium nitrate, 40% trichloroacetic acid solution, 6 M HCl solution and an iron color reagent. The color reagent was prepared by mixing a 1:20:20 ratio (w/v/v) of Bathophenanthroline (0.162 g dissolved in 100 ml of double deionized water with 2 ml thioglycolic acid (96- 99%)), double deionized water and a saturated sodium acetate solution. A minced sample (1 g) was weighed into 15 ml screw cap test tube and 50 µl of 0.39% (w/v) sodium nitrate added as well as 4 ml of a freshly prepared mixture (ratio 1:1, v/v) of 40% trichloroacetic acid solution and 6 M HCl solution. The tubes were tightly capped and placed in an incubator shaker at 65 °C for 22 hours. After the 22 hours, the mixture was allowed to cool down to room temperature for 2 hours in a dark place. The supernatant (400 µl) was mixed with 2 ml of the iron colour reagent (freshly prepared), vortexed and let stand for 10 min. The absorbance was measured at 540 nm (Amersham Pharmacia Biotech,Ultrospec 3000 pro, United Kingdom). The non-heme iron content was calculated from the iron standard curve created. The iron standard solution, ranging from 0 to 4 ppm (400 µl) was mixed with 2 ml of the non-heme iron colour reagent. The concentration of non-heme was expressed as mg/100 mg sample.

After finishing the measurements of the July 2015 experiment it became clear that the results were not reliable. It seemed that for some reason the absorbance of the samples changed very quickly making the order in which they were measured a very influential factor as well as the age of the color reagent. The small size of the sample used could possibly be the reason or one of the chemicals used. All chemicals that could be replaced were replaced before the analysis was tested again but the results were still unreliable.

After performing the measurements again with new chemicals and not seeing a change experiments were performed to try to scale up the method by using 5 g of mince instead of 1 g and doubling the amount of liquids used. In order to see if this change would influence the amount of time needed in incubator an experiment was designed. 5 samples were prepared with 1 g following protocol and 25 samples where 5 g of mince were used and the liquid was doubled. Due to the increase of liquid these samples were put in 50 ml red cap tubes instead of the 15 ml. Samples were all put into the incubator at the same time and after 22 hours the 1 g samples were taken out as well as five of the 5 g samples. They were let cool for 2 hours in the dark before being measure. The 5 g samples were then taken out in 4 hour intervals from 22 hours to 40 hours. The most reliable results came from samples incubated for 36 hours. The experiment was then repeated. This time five 1 g samples were prepared and incubated for 22 hours but the 5 g samples were incubated for 32 to 38 hours, measuring at 2 hour intervals. This time the data obtained did not seem reliable. There was still an enormous difference between the samples even though they all came from the same mince.

The last attempt was to use a different method to analyze iron content to compare to the results from the heme iron and non-heme iron analyzis. The results from the iron content analysis performed were not available at this time so it will not be possible to finish this comparison here.

Because of the inconsistencies in the data and method, whatever the reason, the results from the non-heme iron measurements will not be omitted.

### **3.5 Statistical analysis**

Values are presented as mean  $\pm$  standard deviation. SPSS for Windows (12.0.1; SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. During analysis a difference at the level of  $p < 0.05$  is considered statistically significant. The significance of difference between groups was determined with one-way ANOVA. Correlations analysis was performed with SPSS as well using Pearson's correlation. Calculations of correlation were performed for all experiments, those not shown in chapter 5, Results and Discussions, are shown in appendix.

## 4 Results and discussions

### 4.1 Effect of bleeding medium temperature on cod fillet quality: July 2015

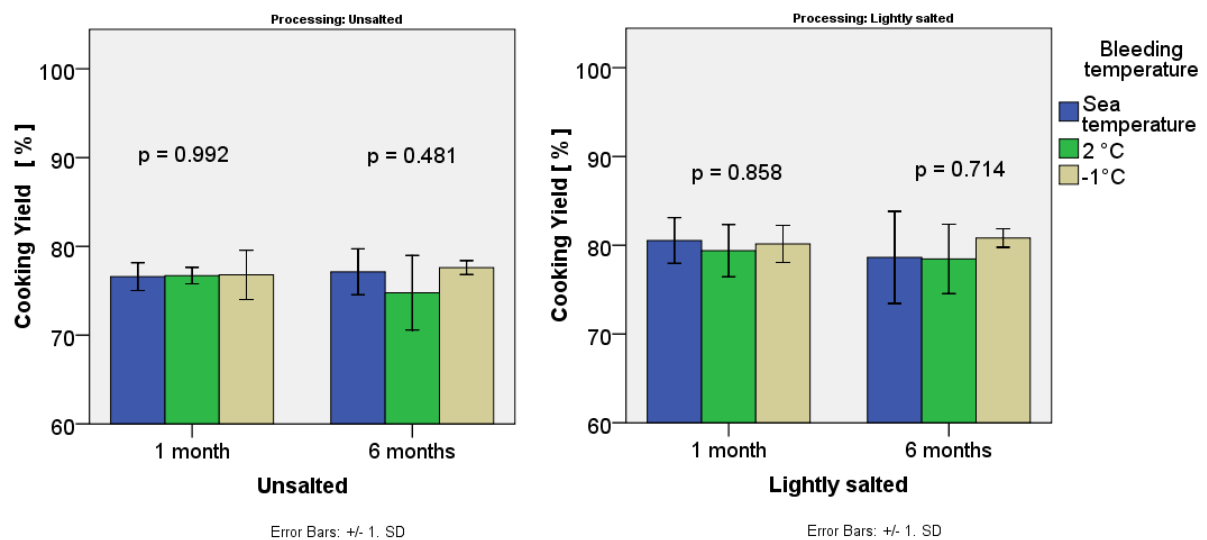
In this experiment 3 different temperatures of bleeding medium during bleeding of cod were examined. The experimental groups were bled at sea temperature (6 °C), 2 °C and -1 °C.

The water, fat, protein and salt content of the samples was measured. No significant changes were observed between groups at all sampling points (Appendix A). The chemical composition of the material, when an average value is calculated for all samples in experiment regardless of groups, can be found in table 3.

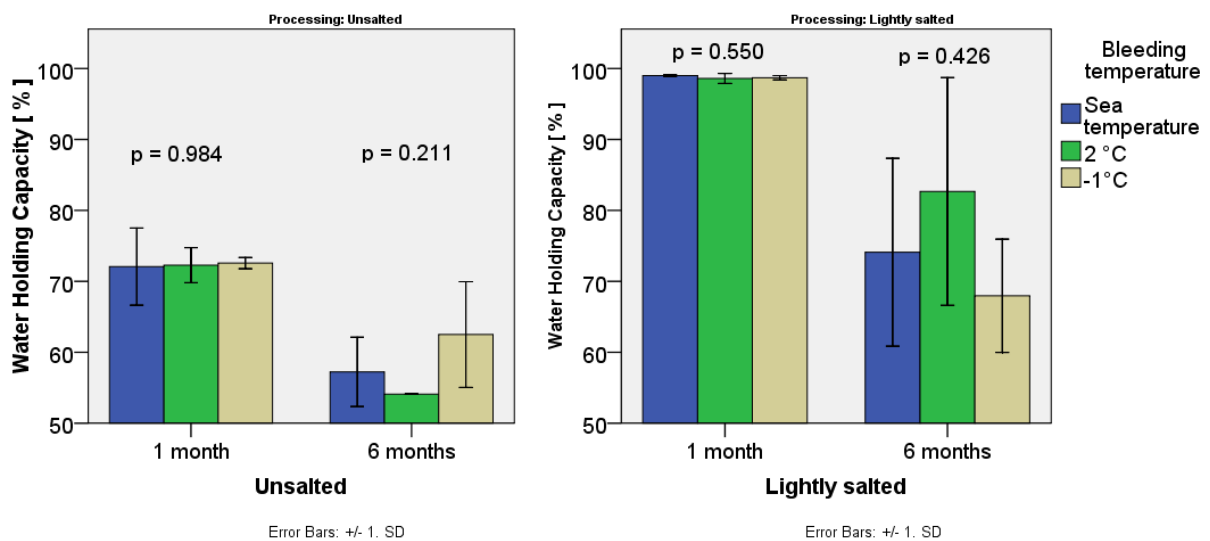
**Table 3 Chemical composition of the raw material used in the experiment of temperature of bleeding medium. Samples were analyzed after 1 month of frozen storage. Table contains an average value for all samples in all groups.**

	Unsalted samples	Lightly salted samples
Water [%]	81.2±0.5	85.6±0.4
Fat [%]	0.6±0.0	0.4±0.1
Protein [%]	18.0±0.6	12.5±0.5
Salt [%]	0.3±0.0	1.6±0.1

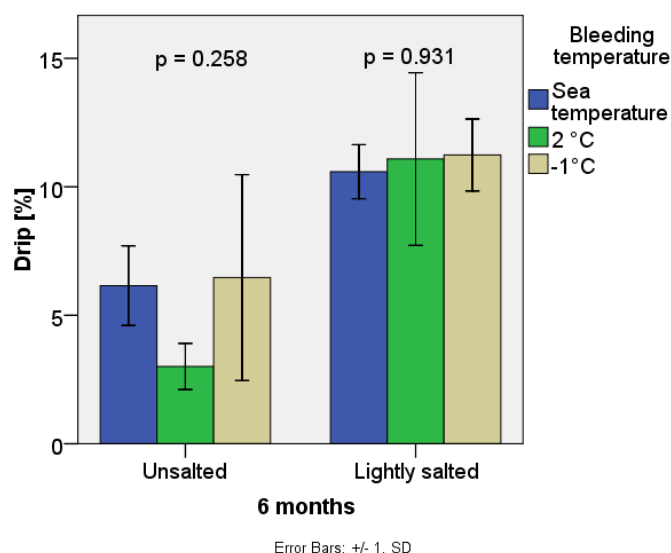
Water holding capacity, cooking yield and drip of samples was analyzed (Figure 24, Figure 25, Figure 26). No significant difference was present between groups in any of the samplings, indicating that temperature of bleeding medium does not influence the water binding ability of the fillets. There was however a decrease in WHC of unsalted cod with prolonged storage ( $p<0.05$ ) as expected (Careche *et al.*, 1998; Careche *et al.*, 1999; Chichester & Stewart, 1981). When analyzing the results of these results the extreme temperature fluctuations and temperatures samples were kept at need to be taken into considerations. Especially when looking at results of drip content which can change dramatically dependent on f.ex. placement of fillets within boxes during storage.



**Figure 24** Cooking yield (%) of unsalted and lightly salted cod fillets from cod bled at different temperature stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 14) (n=3). At the time of sample collection sea temperature was 6 °C.



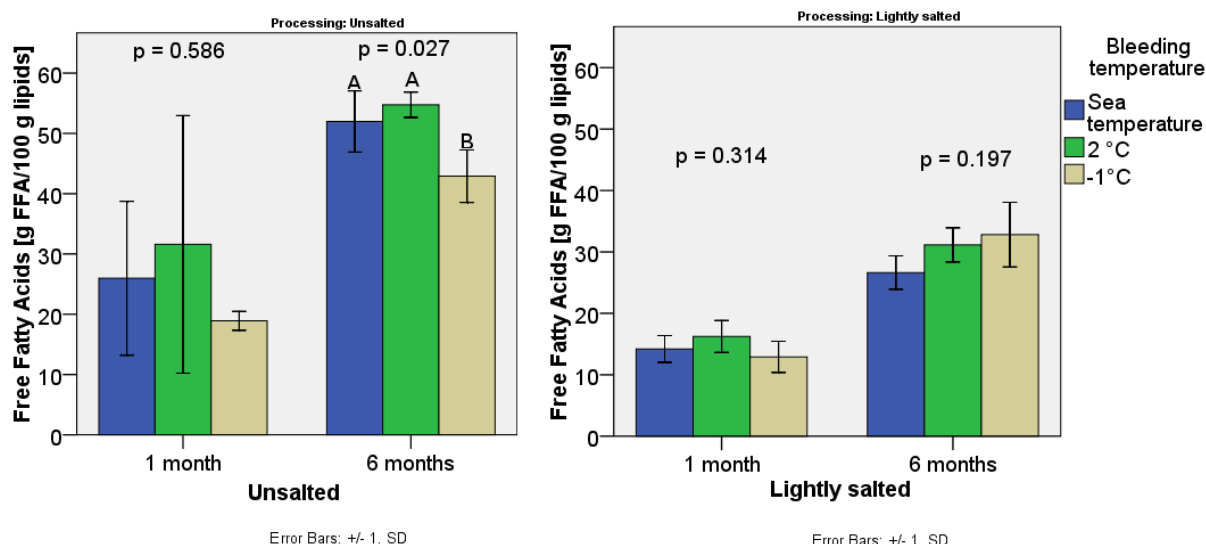
**Figure 25** Water holding capacity (%) of unsalted and lightly salted cod fillets from cod bled at different temperature stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 14) (n=3). At the time of sample collection sea temperature was 6 °C.



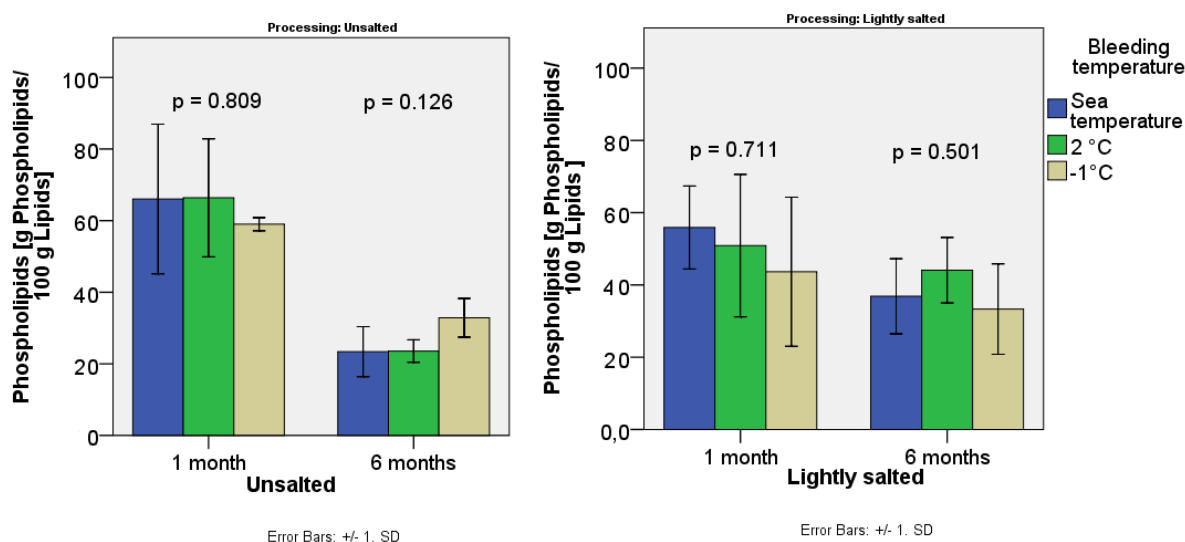
**Figure 26 Drip (%) of unsalted and lightly salted cod fillets from cod bled at different temperature stored in frozen storage for 6 months at a temperature range from -12 °C to -25 °C (Figure 14) (n=3). At the time of sample collection sea temperature was 6 °C.**

Analysis of free fatty acids (FFA) and phospholipid (PL) content was performed (Figure 27, Figure 28). FFA content of lightly salted fillets increased with prolonged storage time ( $p < 0.05$ ). The FFA of the unsalted fillets increased however due to the large variance between samples the increase was not significant for all groups, only unsalted fillets bled at 6 °C and -1 °C. After 6 months of frozen storage the FFA content of unsalted fillets bled at -1 °C was lower than of those bled at higher temperatures ( $p < 0.05$ ). The lightly salting affected the rate at which FFA formed within the muscle during storage, there was less FFA present in lightly salted fillets after 6 month storage than unsalted fillets ( $p < 0.05$ ). The results of changes in PL content mirrored those of the FFA content indicating that PL are mostly breaking down due to enzymatic activity in the fillets (Olley & Lovern, 1960; Karlsdottir *et al*, 2014). That is that when the FFA increased the PL decreased. The difference between groups within a sampling point is not significant.

Unsalted cod fillets bled at -1 °C had significantly less FFA and had more PL on average ( $p > 0.05$ ) than those bled in bleeding medium at higher temperatures. Present results suggest that bleeding cod at -1 °C could be beneficial.

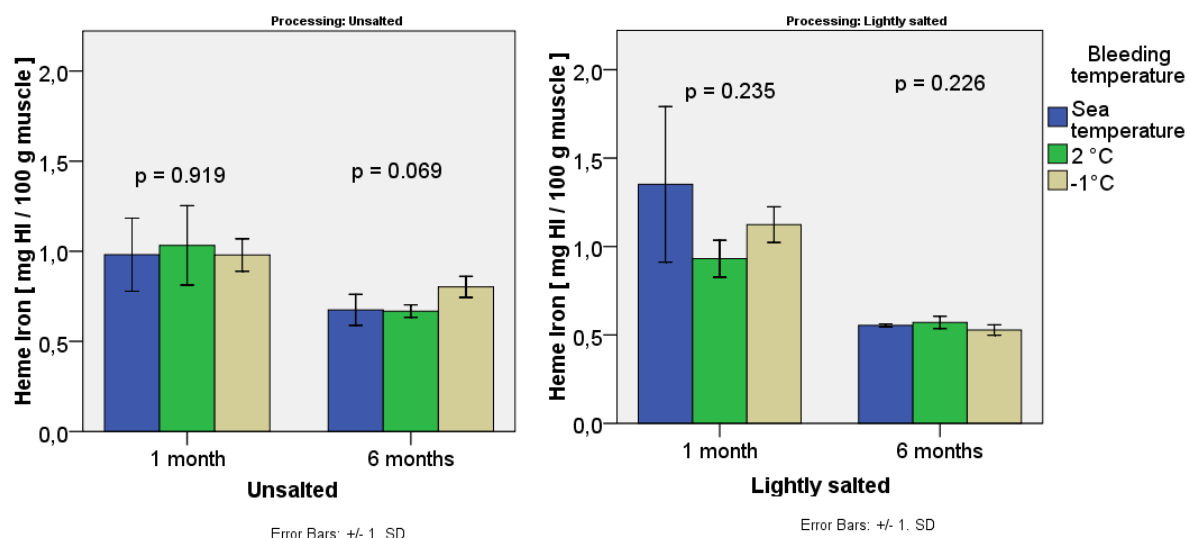


**Figure 27** Free fatty acid content (g FFA/100 g lipids) of unsalted and lightly salted cod fillets from cod bled at different temperature stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 14) (n=6). Columns marked with the same letter are not significantly different. At the time of sample collection sea temperature was 6 °C.



**Figure 28** Phospholipid content (g phospholipids/100 g lipids) of unsalted and lightly salted cod fillets from cod bled at different temperature stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 14) (n=6). At the time of sample collection sea temperature was 6 °C.

Heme iron content was measured in the middle part of the fillets (29). No significant difference was observed between groups in the samplings. The heme iron content, however, decreased for both unsalted and salted fillets with prolonged storage ( $p < 0.05$ ) indicating that the heme iron is breaking down during storage (Gomez-Basauri & Regenstein, 1992).

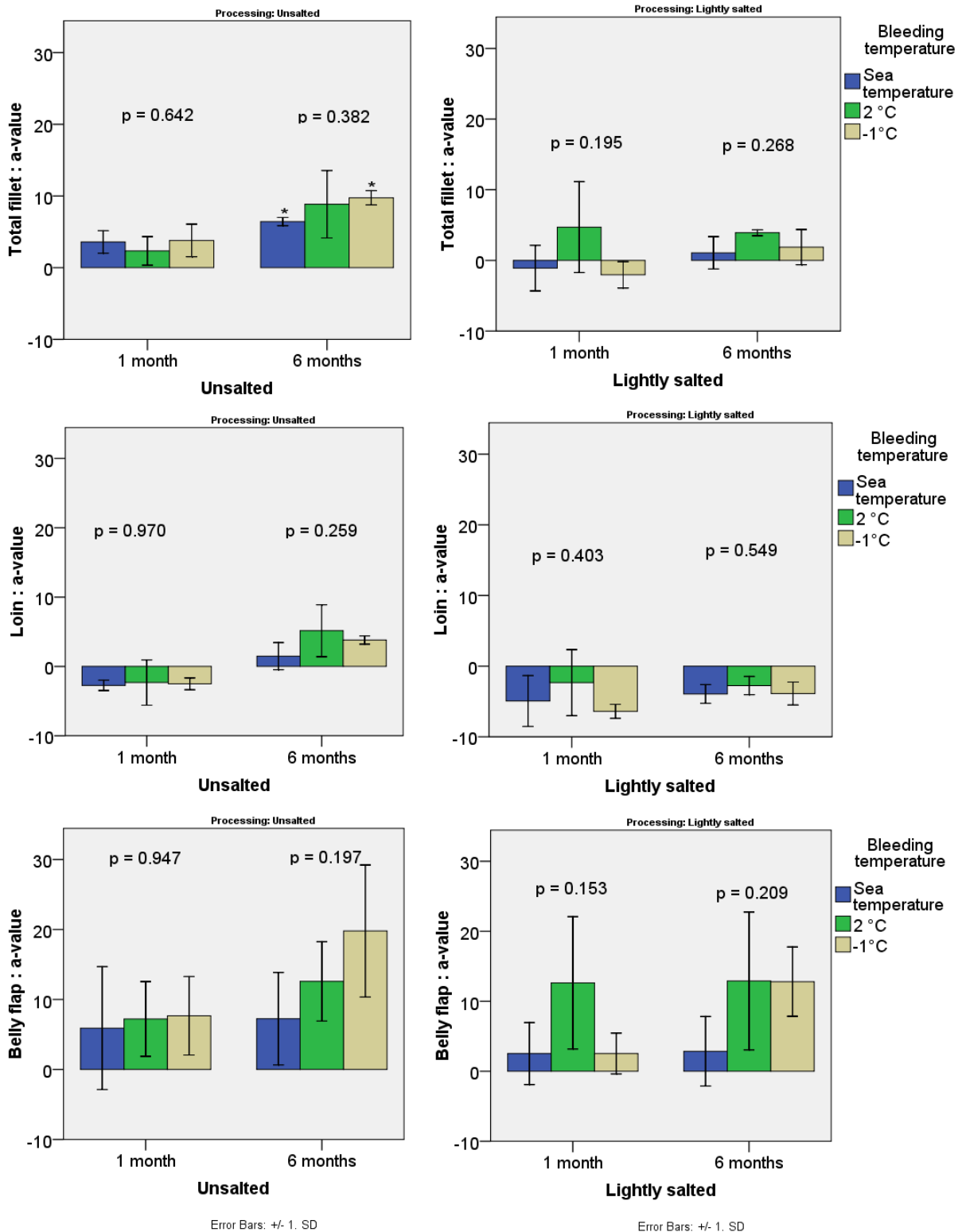


**Figure 29 Heme iron content of unsalted and lightly salted cod fillets from cod bled at different temperature stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 14) (n=6). At the time of sample collection sea temperature was 6 °C.**

The surface color of the collected fillets was recorded according to the CIA  $L^*a^*b$  (Appendix A). The redness or a-value indicated presence of red to blue color where increasing positive values indicate red color darkening and a decreasing negative values indicate a darkening blue color. The results showed the redness of the unsalted cod fillets increased with prolonged storage (Figure 30). The difference between unsalted samples was not in any cases statistically different when using ANOVA analysis. However when a T-test was performed on results for whole fillets between groups bled at 6 °C and -1 °C the difference was significant. That may reinforce the suggestion that cod bled at -1 °C may be redder than those bled at other temperatures.

Lightly salting the fillets affected these changes. The redness of lightly salted samples did not change dramatically with the exception of the belly flap of samples bled in bleeding medium at -1 °C (Figure 30).

When looking at figure 31 taken of fillets after filleting and trimming the difference in appearance between the groups is rather obvious. The samples bled in temperature lower than sea temperature are clearly redder, mainly on the belly flap and the cod bled at -1 °C seems to be even redder than the one bled at 2 °C.



**Figure 30** The redness values, a-values, from lightly salted and unsalted fillets whole and loin and belly flap individually stored for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 14) (n=3). \*Columns marked were not found to be statistically different with ANOVA analysis but when T-test was performed  $p < 0.05$ . At the time of sample collection sea temperature was 6 °C.



**Figure 31 Photo taken of unsalted samples 4 days after catching, bled in bleeding medium at three different temperatures shown in the figure, before freezing (Photo by S. Eliasson).**

The results from analysis of FFA and PL content suggested that bleeding cod at subzero temperature could be beneficial. However images taken of the samples during processing as well as results of color analysis indicate that it could affect the color of the fillets, mainly the belly flap of the fillets but not the loin part. As soon as fillets had been injected and brined the difference between the groups was minimal and all spoilage were slowed down. There were indicators however that the lower temperature, mainly -1 °C, of bleeding medium may not be beneficial. After storage the lightly salted samples bled at -1 °C had the highest FFA ( $p>0.05$ ) and the belly flap of the samples were much redder than of those bled at sea temperature. Further experiments would need to be performed in order to determine the viability of this method with more extensive chemical analysis and perhaps sensory evaluation.

## **4.2 Effect of waiting time on cod fillet quality: July 2015**

In this experiment the effect of waiting time before bleeding on cod fillet quality were examined. All sampling groups were treated the same with the exception of waiting time prior to bleeding, 0 min, 30 min, 60 min or 90 min.

Information stated in chapter 0 should be kept in mind while looking over the results from this experiment. The fact that the samples collected were not all from the same place within the haul can have great effect on the results of these measurements. That is mainly because the placement of the cod within the troll can have detrimental effect on its state during bleeding. It can affect f. ex. the stress level of the fish and the distribution of blood within the flesh. When performing an experiment it is important to limit the amount of variables that can possibly affect the result and in this case this variable was not controlled as well as possible. Therefore no conclusion could be drawn from analysis of results. There would be no way in particular to determine which variable in handling caused a

difference between groups. Therefore the results of analysis in this experiment will not be presented but can be found in appendix B. The fact that the samples were caught in July, a time where the variability between individuals could be more than at other times due to the cods spawning cycle (Raversu & Krzynowek 1991; Love, 2001a).

Similar experiments have been performed before, however, none where samples were kept at -1 °C without ice on-board. The results showed that the length of waiting time clearly is connected to the quality of the fillet. (Eliasson, 2013; Olsen *et al.*, 2014) To verify that this also applies when different methods are used during the handling and storing of catch, as on Málmei, further researches are needed.

### 4.3 Effect of storage method on cod fillet quality: July 2015

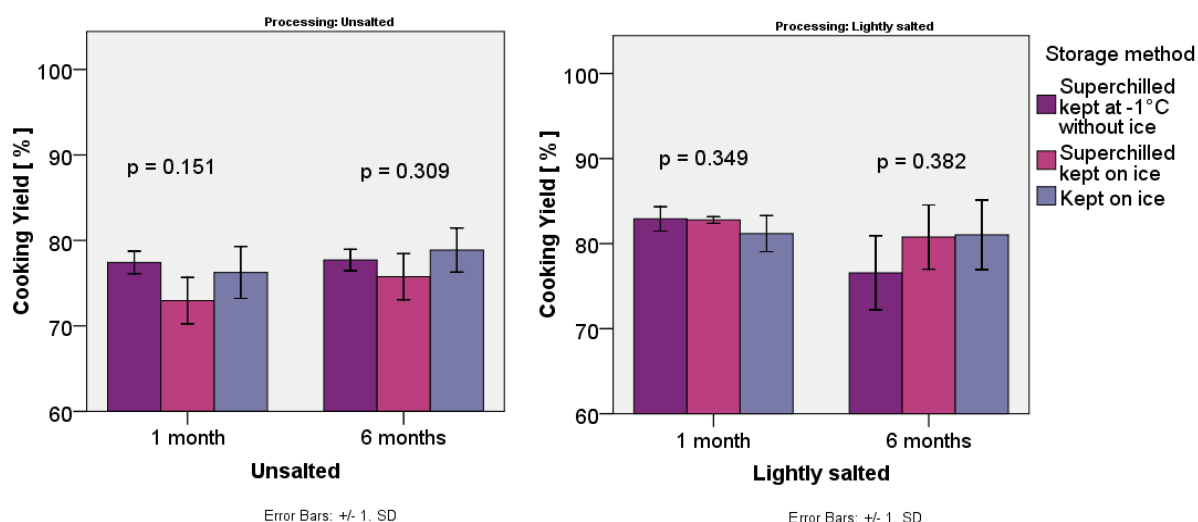
In this experiment three different storage methods on-board were examined. Groups were all bled the same way but after bleeding, gutting and washing one group was iced directly in a tub, one supercooled and then iced and one supercooled and kept at -1 °C without ice. Samples were kept under these conditions from the 4<sup>th</sup> of July, when they were caught until they were processed on the 8<sup>th</sup> of July.

Analysis of water content, protein, salt and fat content was performed (Appendix C). No significant difference was found between samples in samplings. The chemical composition of the material, when an average value is calculated for all samples in experiment regardless of groups, can be found in table 4.

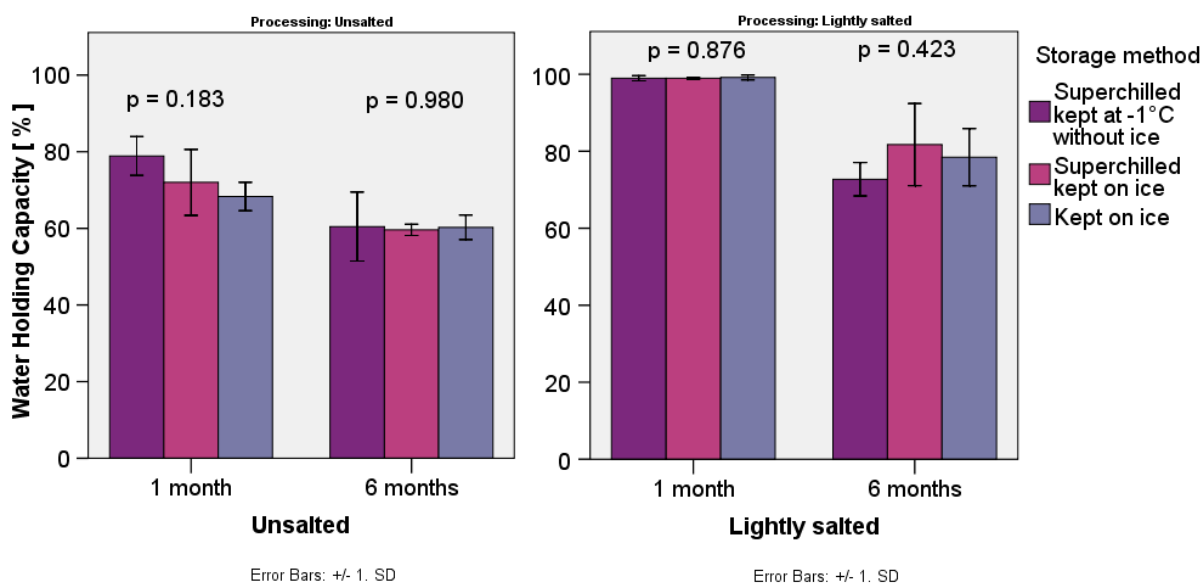
**Table 4 Chemical composition of the raw material used in the experiment where different storage conditions on-board were investigated. Samples were analyzed after 1 month of frozen storage. Table contains an average value for all samples in all groups.**

	Unsalted samples	Lightly salted samples
Water [%]	81.6±0.7	85.5±1.0
Fat [%]	0.5±0.1	0.5±0.1
Protein [%]	17.8±0.8	12.4±0.7
Salt [%]	0.3±0.1	1.6±0.1

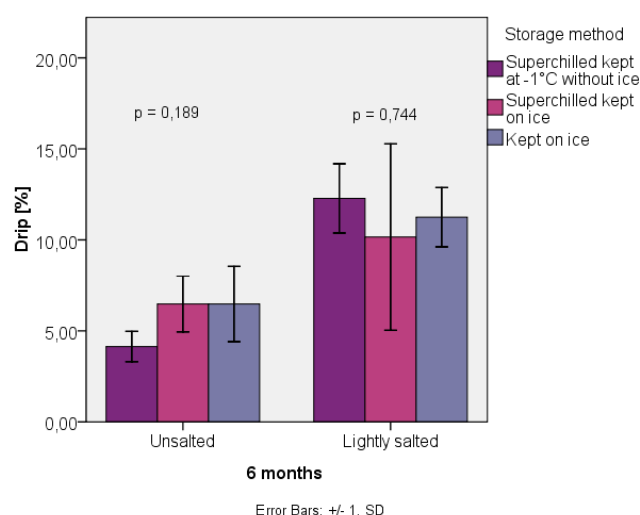
Unsalted cod stored supercooled on ice had slightly lower cooking yield than those stored with other methods ( $p>0.05$ ) at both samplings, 1 month and 6 months of storage. Results of water holding content (WHC) analysis and drip content had no clear pattern visible other than lowering of WHC ( $p>0.05$ ) and an increase in drip ( $p>0.05$ ) with prolonged storage (Figure 32, Figure 33, Figure 34).



**Figure 32** Cooking yield (%) of unsalted and lightly salted cod fillets from cod stored on-board supercooled and iced, supercooled without ice at -1 °C or only iced stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 14) (n=3).

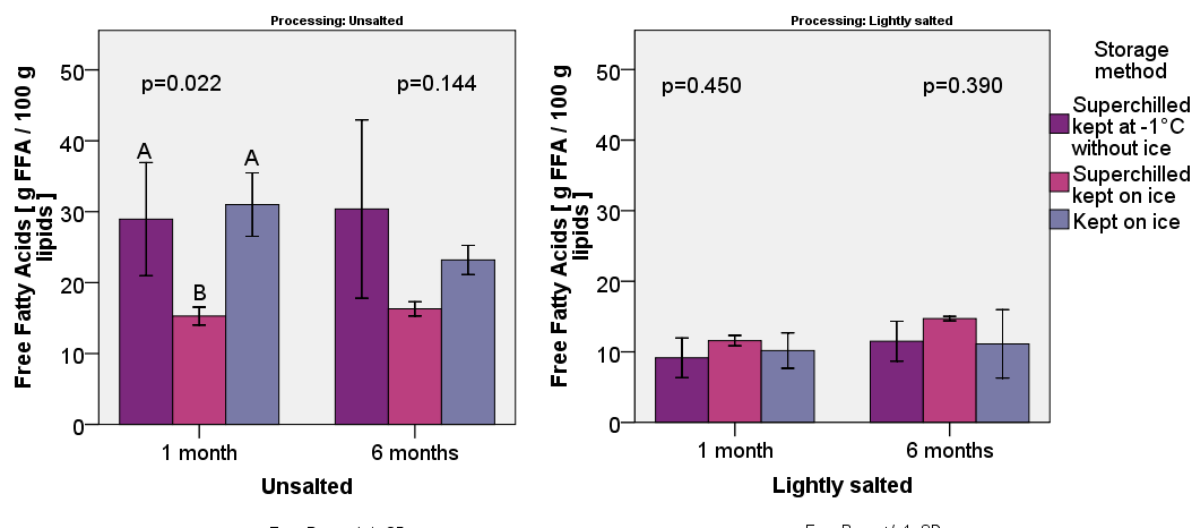


**Figure 33** Water holding capacity (%) of unsalted and lightly salted cod fillets from cod stored on-board supercooled and iced, supercooled without ice at -1 °C or only iced stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure14) (n=3).

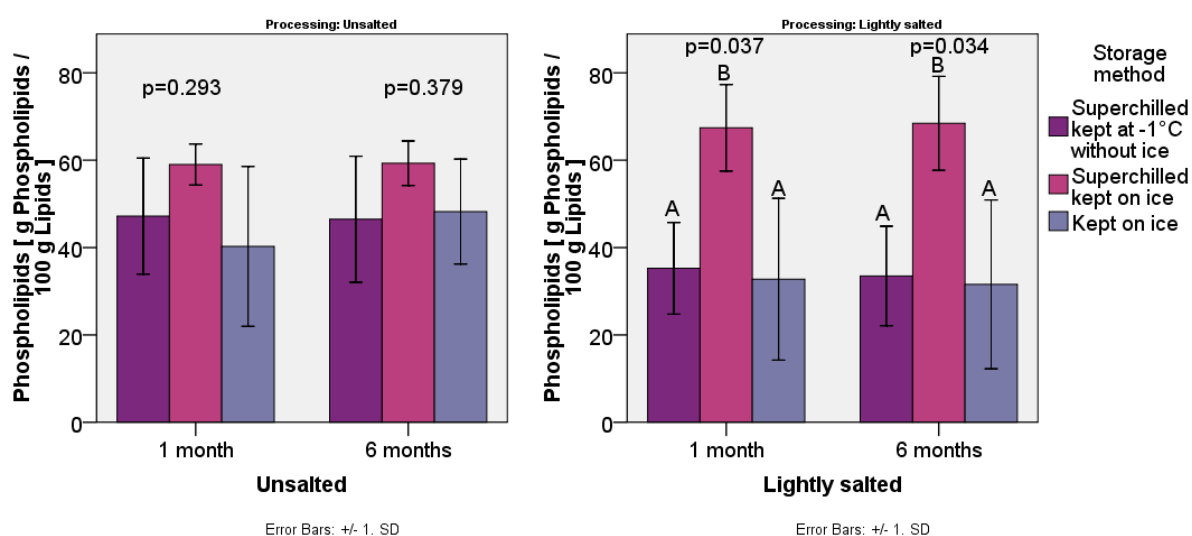


**Figure 34 Drip (%) of unsalted and lightly salted cod fillets from cod stored iced or supercooled and with or without ice before processing stored in frozen storage for 6 months at from -12 °C to -25 °C (Figure 14) (n=3).**

The results of free fatty acids (FFA) and phospholipid (PL) content analysis showed a distinctive pattern. The FFA content (Figure 35) of unsalted fillets after 1 month of frozen storage was significantly lower for groups kept supercooled on ice compared to the other two. The same pattern was visible after 6 months of storage but the difference was however not significant. The PL content (Figure 36) of unsalted fillets was higher in fillets superchilled and iced compared to the other two groups (Figure 36). The FFA of the lightly salted fillets was slightly higher for fillets supercooled and iced ( $p > 0.05$ ) and the PL higher for that group ( $p < 0.05$ ) at both sampling points. There was very little change observed both in FFA and PL content of samples during frozen storage. These groups were handled the same as other experimental groups in present project and were collected from the same haul as the samples used in the waiting time before bleeding experiment. The samples were also all stored at the same storage conditions. However these samples were caught during an unfortunate time of year with regards to the spawning cycle and that could explain (Raversu & Krzynowek 1991; Love, 2001a). Despite this the fact that the FFA content was lower and the PL higher indicates that supercooling and icing of fish on-board might be the storage method best suited to inhibit enzymatic activity and lipid hydrolysis (Olley & Lovern, 1960; Karlsdottir *et al*, 2014).



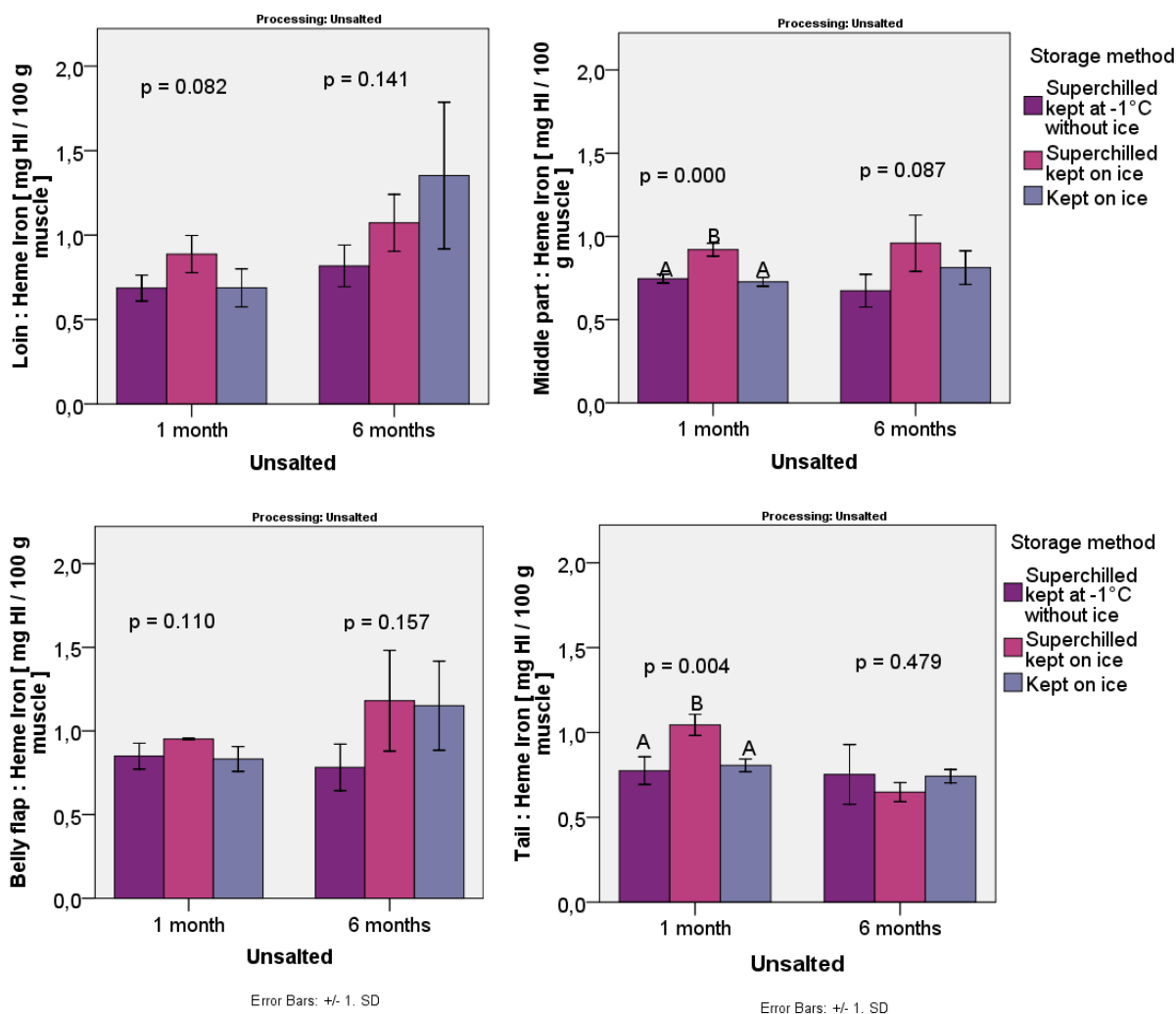
**Figure 35 Free fatty acids (g FFA/100 g lipids) in unsalted and lightly salted cod fillets from cod stored on-board supercooled and iced, supercooled without ice at -1 °C or only iced stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 144) (n=6). Columns marked with the same letter are not significantly different.**



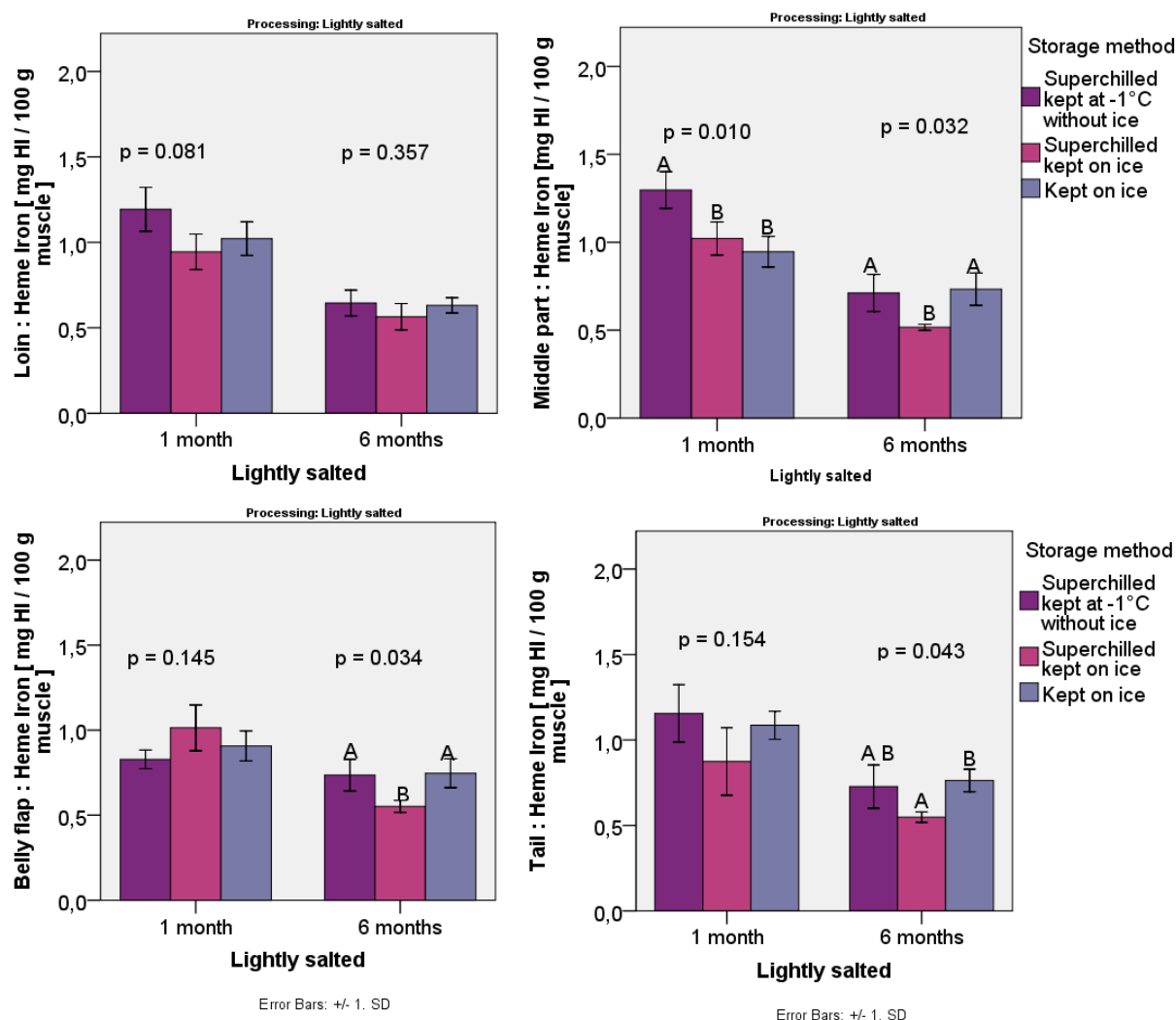
**Figure 36 Phospholipids (g phospholipids/ 100 g lipids) in unsalted and lightly salted cod fillets from cod stored on-board supercooled and iced, supercooled without ice at -1 °C or only iced stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 144) (n=6). Columns marked with the same letter are not significantly different.**

In order to investigate possibly how residual blood is spread within the different parts of the fillets heme iron was measured in middle part, loin, tail and belly flap of the fillets in this experiment (Figure 37. Figure 38). For unsalted fillets after 1 month of frozen storage a pattern of higher heme iron content in samples kept supercooled on ice compared to samples iced directly out of bleeding chamber of rotex system and the difference was significant in the middle part and the tail. The pattern

was however not present after 6 months of frozen storage. No significant changes in heme iron content was detected with prolonged frozen storage of these samples. Moreover, no significant difference was observed between sample groups of lightly salted after 1 month of frozen storage. Analysis after 6 months showed that the amount of heme iron was higher in samples supercooled and kept without ice and iced fish. The difference was significant in the middle part, tail and belly flap.

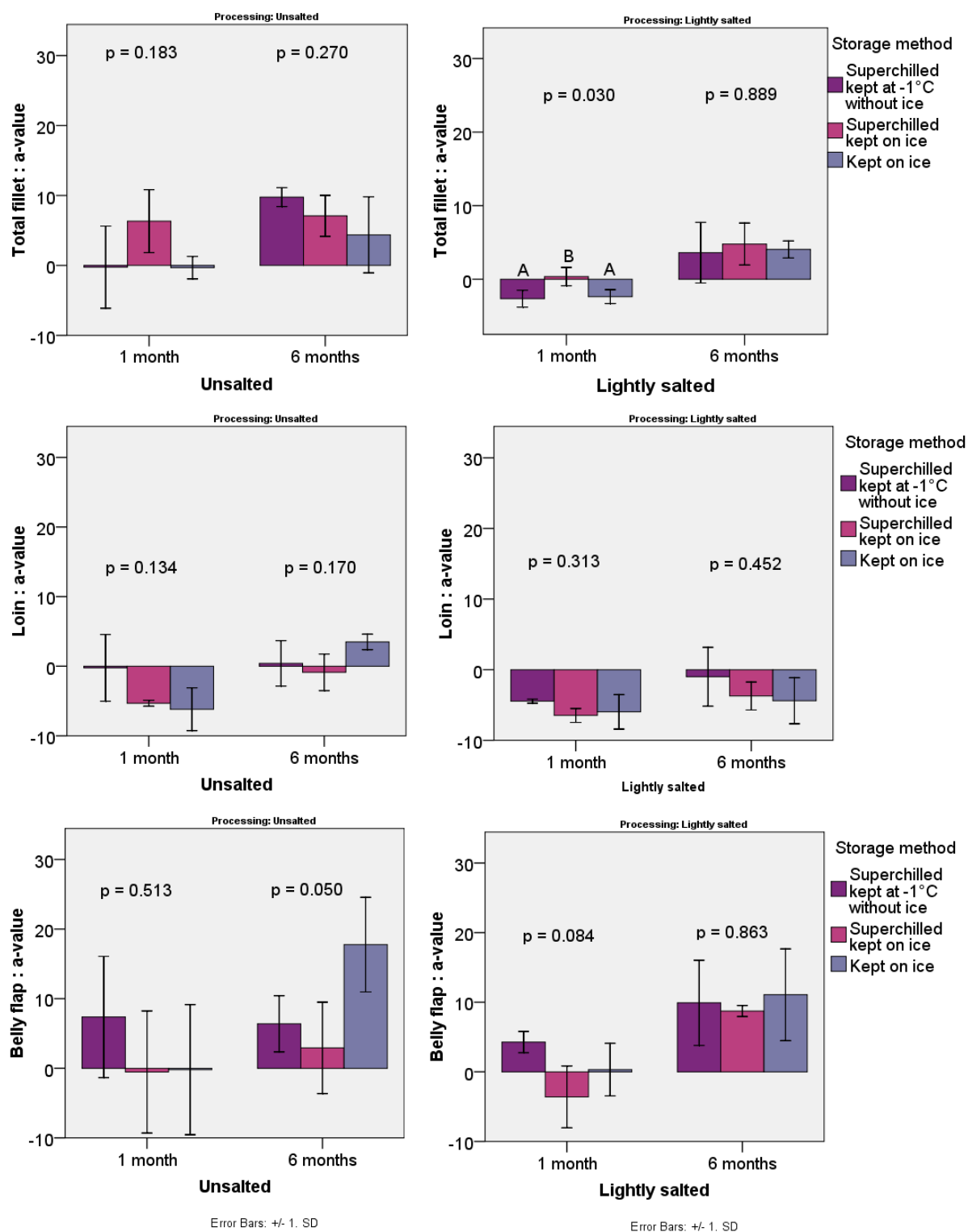


**Figure 37 Heme iron content (mg heme iron/100 g muscle) of unsalted cod fillets from cod stored on-board supercooled and iced, supercooled without ice at -1 °C or only iced stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 14) (n=3).**



**Figure 38 Heme iron content (mg heme iron/100 g muscle) of lightly salted cod fillets from cod stored on-board supercooled and iced, supercooled without ice at -1 °C or only iced stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 144) (n=6). Columns marked with the same letter are not significantly different.**

The surface color of the collected fillets was recorded according to the CIA L\*a\*b (Appendix C). The redness or a-value indicated presence of red to blue color where increasing positive values indicate red color darkening and a decreasing negative values indicate a darkening blue color. Results from analysis of a-value show no visible pattern (Figure 39). A significant difference was present in the belly flap of the unsalted fillets after 6 months of storage, where the fillets from iced cod had a higher redness value then of those supercooled and iced but not those kept at -1 °C without ice. When fillets were analyzed whole lightly salted fillets were significantly different after 1 month of storage, the fillets supercooled and iced into tubs had in this case the highest redness value ( $p > 0.05$ ).



**Figure 39** The a-values from lightly salted and unsalted fillets whole and loin and belly flap individually (n=3), cod fillets from cod stored on-board supercooled and iced, supercooled without ice at -1 °C or only iced stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 °C (Figure 144) (n=3). Columns marked with the same letter are not significantly different.

Present results indicate that cod supercooled and kept on ice on-board would be of higher quality than those stored without ice or directly iced after bleeding. That the fast cooling in the rotex down to -1 °C is better than cooling on ice and that storage on ice is better than storage without ice at -1°C. The rapid cooling slows down f.ex.growth of spoilage bacteria, enzymatic activity and rigor mortis explaining why it would be the more suitable cooling method. The storage with or without ice has however not been researched as extensively. It is possible that the reason iced fish was of higher quality was because the storage on ice provides an environment in which the fish is continuously surrounded by water as the ice melts providing a bearer for residual blood to leave the fillets during storage.

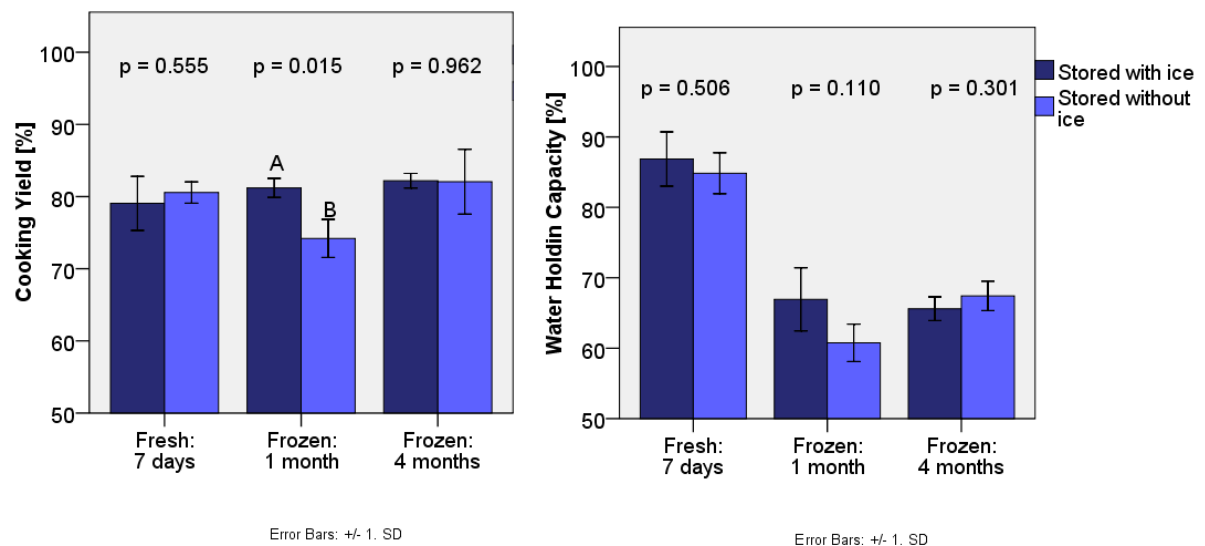
#### 4.4 Effect of iceless storage of large fillets on fillet quality: March 2016

In this experiment the effect of the supercooling and storage on Málmey was compared to cod bled and iced directly after bleeding and gutting. For this experiment large fillets were used, +1000 g. Samples were kept in this manner for 4 days before being processed. Part of the samples were frozen to be analyzed after 1 month and 4 months of storage the rest stored at 0 – 2 °C and analyzed 7 days from catching. Analysis of chemical composition was performed (Appendix E). The chemical composition of the material, when an average value is calculated for all samples in experiment regardless of groups, can be found in table 5.

**Table 5 Chemical composition of the raw material (+ 1000 g fillets) used in the experiment regarding different storage conditions on-board. Samples were analyzed after 7 days of chilled storage (fresh). An average value for all samples in all groups were combined.**

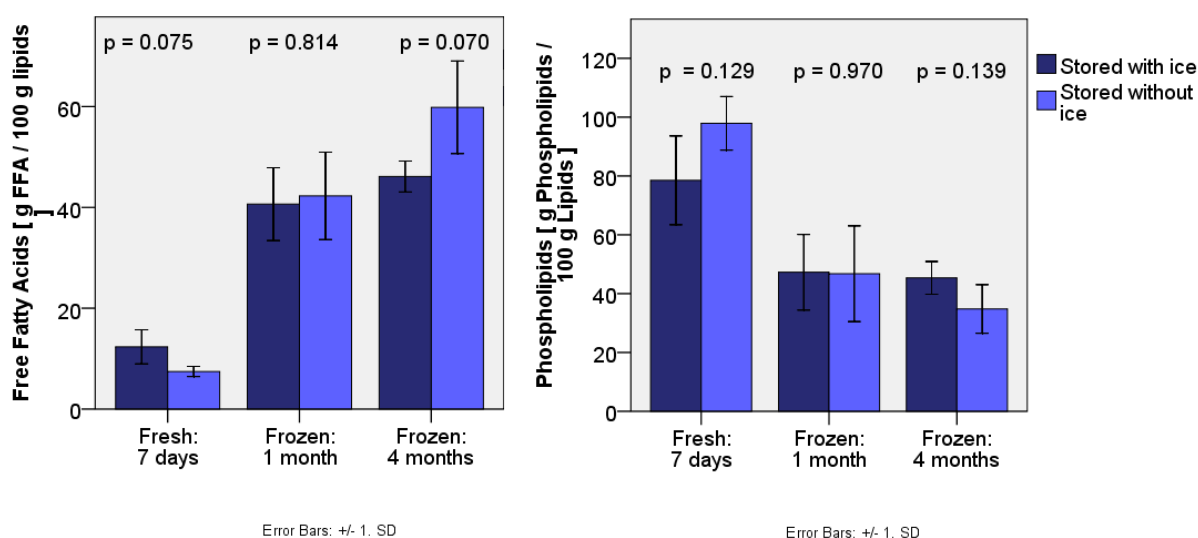
Samples, stored for 7 days	
Water [%]	82.4±0.3
Fat [%]	0.7±0.1
Protein [%]	16.8±0.5
Salt [%]	0.2±0.0

The results of analysis of cooking yield and water holding capacity (WHC) showed no clear pattern (Figure 40). There was a significant difference between cooking yield of groups after 1 month of frozen storage where cod stored on ice had higher cooking yield. However, no clear difference between the groups was observed throughout the frozen storage period, indicating that the storage methods on-board do not play a large role in deciding the water binding ability of the fillets (Careche *et al.*, 1998; Careche *et al.*, 1999; Chichester & Stewart, 1981).



**Figure 40 Cooking yield (%) and water holding capacity (%) of cod fillets (+1000 g) stored iced or stored supercooled without ice at -1 °C on-board the trawler, analyzed 7 days old unfrozen and stored frozen from 1 month at -25 °C up to 4 months at a range from -12 °C to -25 °C (Figure 19) (n=3). Columns marked with the same letter are not significantly different.**

The results showed that prolonged frozen storage affects the amount of both free fatty acid (FFA) and phospholipid (PL) content (Figure 41). When FFA increased the PL decreased. The difference between groups was not significant in any of the sampling points. At the beginning of the storage, when samples were 7 days old and unfrozen, samples stored without ice on-board have higher PL and lower FFA content indicating lower enzymatic activity. However during the frozen storage the difference evened out after 1 month of storage. After 4 months of frozen storage fillets stored on-board without ice contained slightly higher FFA and less PL than the fillets stored on ice on-board the trawler. Present results suggest that even though supercooled fillets kept at -1 °C without ice appeared to be of higher quality in the beginning of the storage period the quality deterioration was more rapid in those fillets than those iced into tubs directly after being bled in bleeding rotex chamber. Results were however not statistically significant so further research is needed.

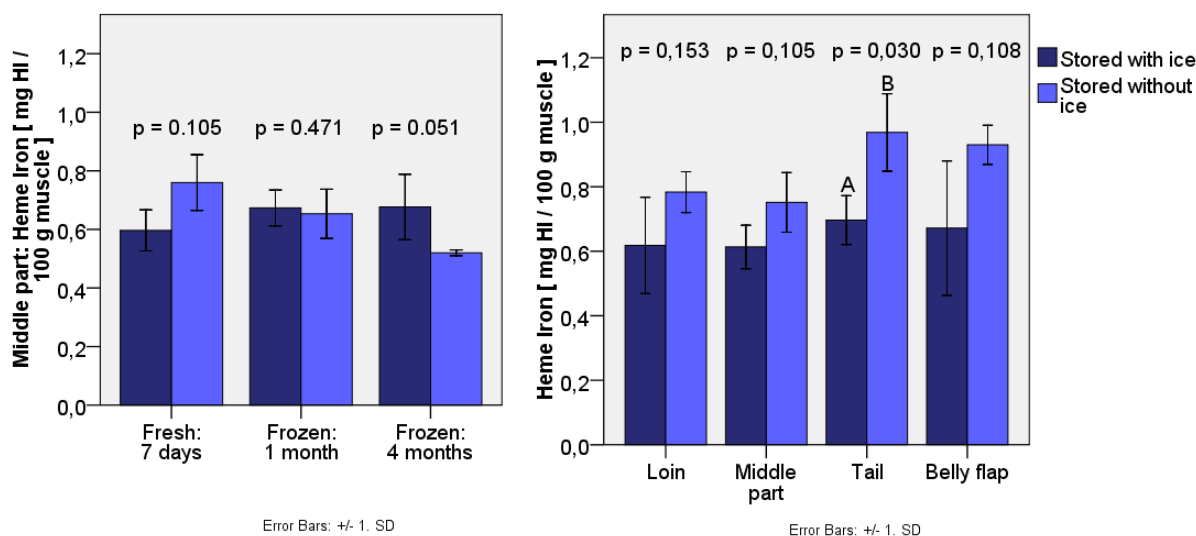


**Figure 41 Free fatty acid (g FFA/100 g lipids) and phospholipid (g phospholipids/100 g lipids) content of cod fillets (+1000 g) stored iced or stored supercooled without ice at -1 °C on-board trawler, analyzed 7 days old unfrozen and stored frozen from 1 month at -25°C up to 4 months at a range from -12 °C to -25 °C (Figure 19) (n=6).**

The figure on the left (Figure 42) shows the difference between heme iron content of different parts of the fillets, measured in samples 7 days from catching, unfrozen. On the right heme iron content in samples was measured in the middle part of fillets throughout the frozen storage period.

When investigating difference between heme iron content of different parts of the fillet more heme iron was found in samples stored without ice on-board, the difference between the groups is significant in the tail but not the other fillet parts. Heme iron content is also slightly higher in the belly flap and tail of samples stored without ice at -1 °C on-board ( $p > 0.05$ ).

Changes of heme iron content during frozen storage period shows a similar pattern as the PL content, even though there was not a significant difference between the groups. The group stored on-board without ice initially had a higher heme iron content but after 1 month of frozen storage the difference evened out. After 4 months of storage the heme iron content of the group stored without ice had lowered.



**Figure 42 Heme iron content (mg heme iron / 100 g muscle) of cod fillets (+1000 g) stored iced or stored supercooled without ice at -1 °C on-board trawler, analyzed 7 days old unfrozen and stored frozen from 1 month at -25°C up to 4 months at a range from -12 °C to -25 °C (Figure 19) (n=6). Columns marked with the same letter are not significantly different.**

There was no clear, statistically significant, difference between the two groups investigated in present experiment. There was however a trend present in the results of FFA, PL and heme indicating which group was more vulnerable towards quality deterioration. Mainly, when the fish, analyzed unfrozen 7 days from catching, stored with ice on-board had higher FFA and lower PL indicating more enzymatic activity in those samples. After 1 month of frozen storage the difference between groups evens out. At the end of frozen storage period, after 4 months, cod stored on-board without ice at -1 °C had higher FFA content and lower PL and heme iron. These results indicate that even though samples supercooled and stored without ice at -1 °C on-board seem to be of higher quality at the beginning of the storage period they are more vulnerable to quality deterioration. In order to conclude definitively on which method would yield longer shelf life further research is needed.

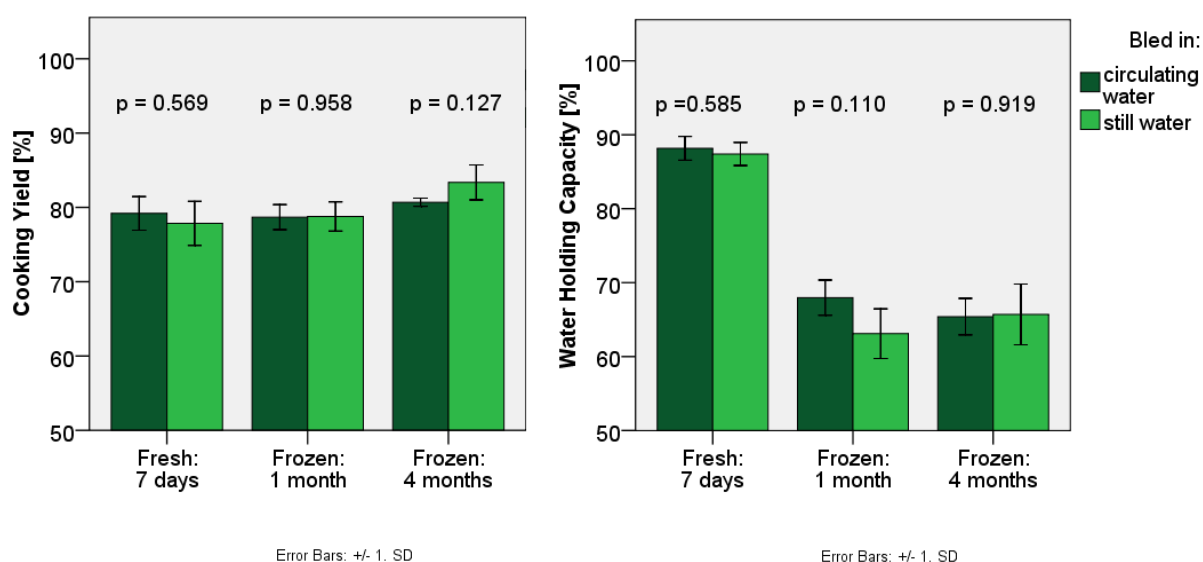
#### 4.5 Effect of circulation of bleeding medium on fillet quality: March 2016

In this experiment the effect of circulating the medium in the bleeding tanks was examined. The two groups were treated the same in all way with the exception that one was bled in still water and the other in circulating water. Both were bled at sea temperature, 5 °C. Analysis of chemical composition was performed (Appendix F). The chemical composition of the material, when an average value is calculated for all samples in experiment regardless of groups, can be found in table 6.

**Table 6 Chemical composition of the raw material used in the experiment where samples bled in still and circulating bleeding medium were compared. Samples analyzed after 7 days of storage (fresh). An average value for all samples in all groups was combined.**

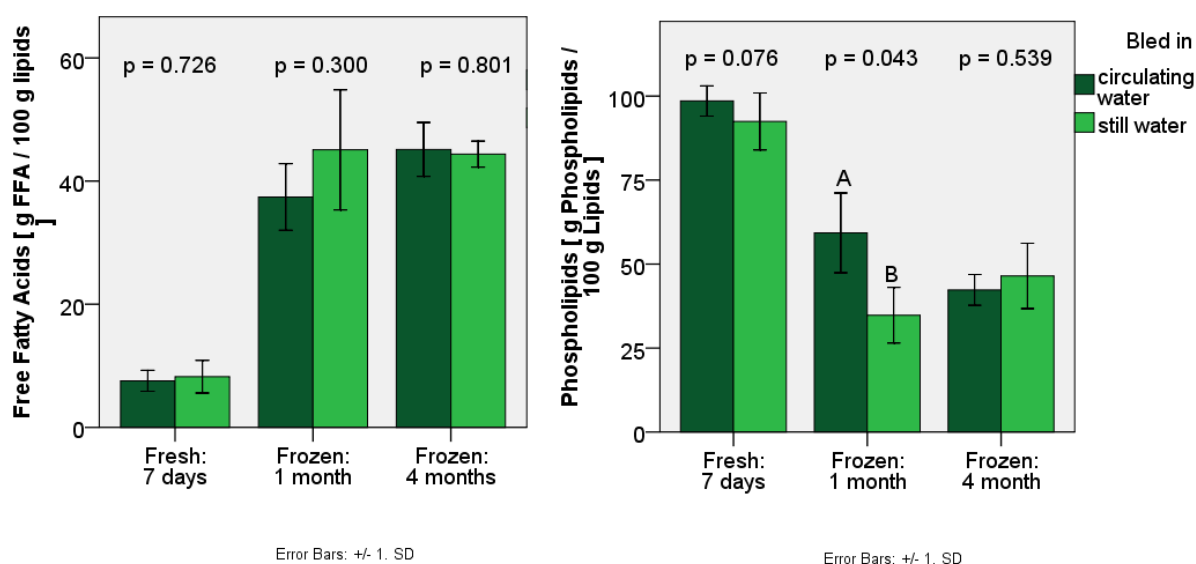
Samples, stored for 7 days	
Water [%]	81.5±0.2
Fat [%]	0.7±0.1
Protein [%]	17.1±0.2
Salt [%]	0.3±0.1

Analysis of cooking yield and water holding capacity (WHC) was performed (Figure 43). No significant difference was observed between the groups, indicating that whether cod is bled in still or circulating water does not play a large role in deciding the water binding ability of fillets (Careche *et al.*, 1998; Careche *et al.*, 1999; Chichester & Stewart, 1981).



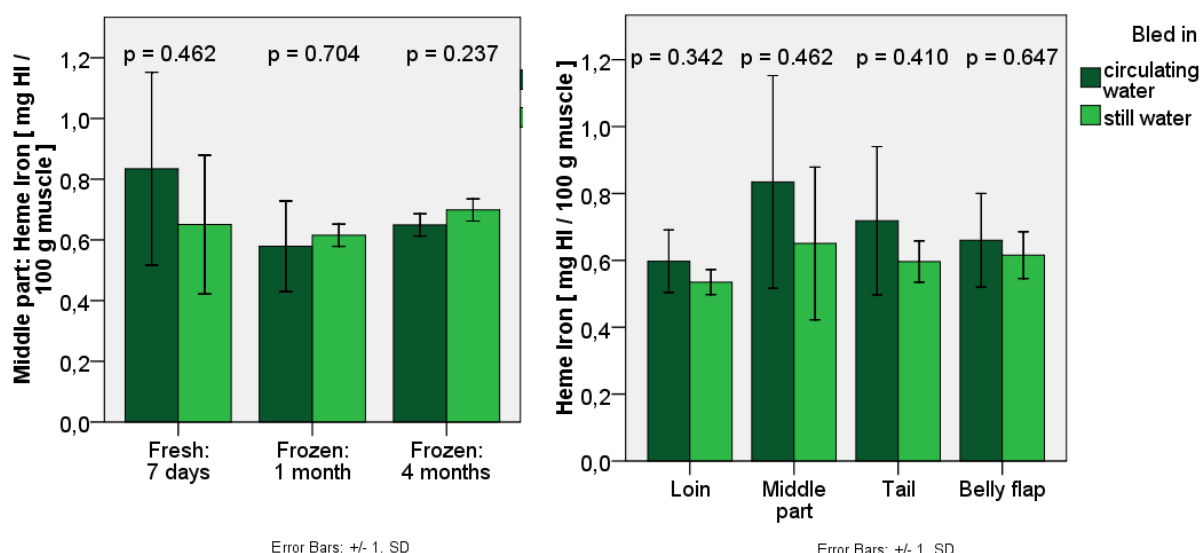
**Figure 43 Cooking yield (%) and water holding capacity (%) of cod bled in still or circulating water, analyzed 7 days old unfrozen and stored frozen from 1 month at -25 °C up to 4 months at a range from -12 °C to -25 °C (Figure 19) (n=3).**

The free fatty acid (FFA) and phospholipid (PL) content of samples revealed no difference between the groups of fresh samples (Figure 44). Difference becomes apparent after 1 month of frozen storage, where the difference was significant in PL content but not FFA content. At this time FFA of samples bled in circulating water was lower than of those bled in still water and PL higher. After 4 months of frozen storage the groups had similar values again.



**Figure 44 Free fatty acid (g FFA/100 g lipids) and phospholipids (g phospholipids/100 g lipids) content of cod bled in still or circulating water, analyzed 7 days old unfrozen and stored frozen from 1 month at -25°C up to 4 months at a range from -12 °C to -25 °C (Figure 19) (n=6). Columns marked with the same letter are not significantly different.**

Heme iron was measured in middle part throughout the storage but in loin, tail and belly flap in 7 days old samples, unfrozen (Figure 45). There was no significant difference between sample groups. Results of analysis of different parts of fillets show higher heme iron content in samples bled in circulating water ( $p > 0.05$ ). When investigating effects of prolonged storage on the heme iron content a similar trend emerges as seen in results of PL content in results of samples analyzed after 7 days of storage, unfrozen, and after 4 months of frozen storage.



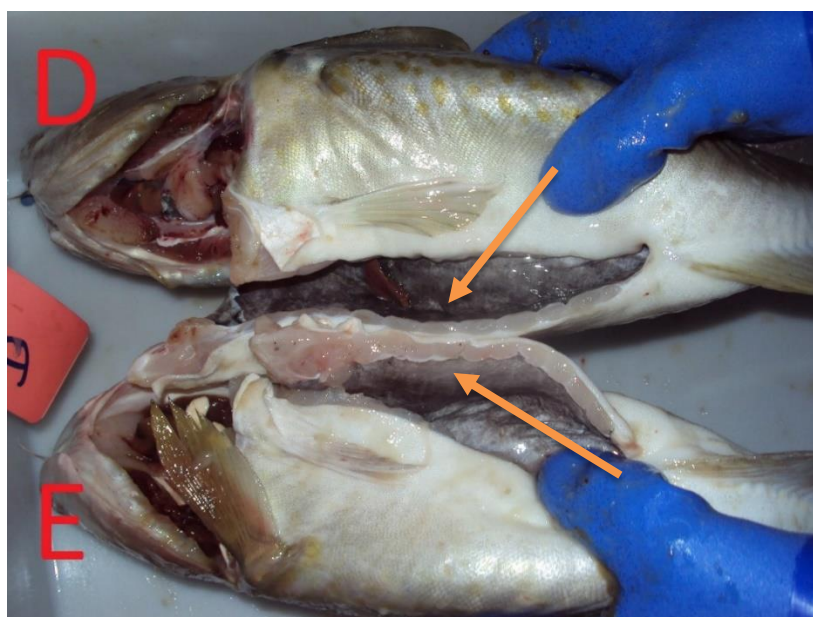
**Figure 45 Heme iron content (mg heme iron/100 g muscle) of cod bled in still or circulating water, analyzed 7 days old unfrozen and stored frozen from 1 month at -25°C up to 4 months at a range from -12 °C to -25 °C (Figure 19) (n=6).**

From analysis of PL and FFA content (Figure 44) the main pattern visible is that possibly the samples bled in still water were deteriorating faster. However after 4 months of frozen storage at extreme temperatures and fluctuations the groups evened out.

Figure 46 shows there was a clear difference between the appearance of the samples bled in circulating or still water. On the left the samples bled in circulating water had bright red gills after 4 days of storage while the samples bled in still water had brown gills. Figure 47 shows the belly flap of the samples, it points to the same conclusion, that cod bled in circulating water had whiter belly flap than those bled in still water. Therefore, even though results of chemical and physical analysis are not significant they, as well as the photos, do indicate that it might be better to bleed cod in circulating water rather than still.



**Figure 46 Images taken, before filleting, of the gills of samples. On the left are samples bled in circulating water and on the right bled in still water. Photos were taken at the same time. (Photos by S. Eliasson)**



**Figure 47** Photos of samples taken before processing of samples. Shows difference between color of belly flap. The sample marked D is bled in circulating water and E in still water. (Photo S. Eliasson)

#### **4.6 Effects of different water flow during bleeding on fillet quality: March 2016**

In this experiment the effect of different circulation of bleeding medium and how quickly bleeding medium was replaced on the bleeding efficiency of cod were examined. This was performed by placing samples in different rotex system on-board Málmeý, all with different pumps and injection frequency and amount, therefore different rate of bleeding medium replacement (Table 7). The rotex system design ensures that bleeding medium is replaced continuously throughout the bleeding.

**Table 7** Groups investigated in the experiment on different water flow during bleeding on fillet quality, March 2016.

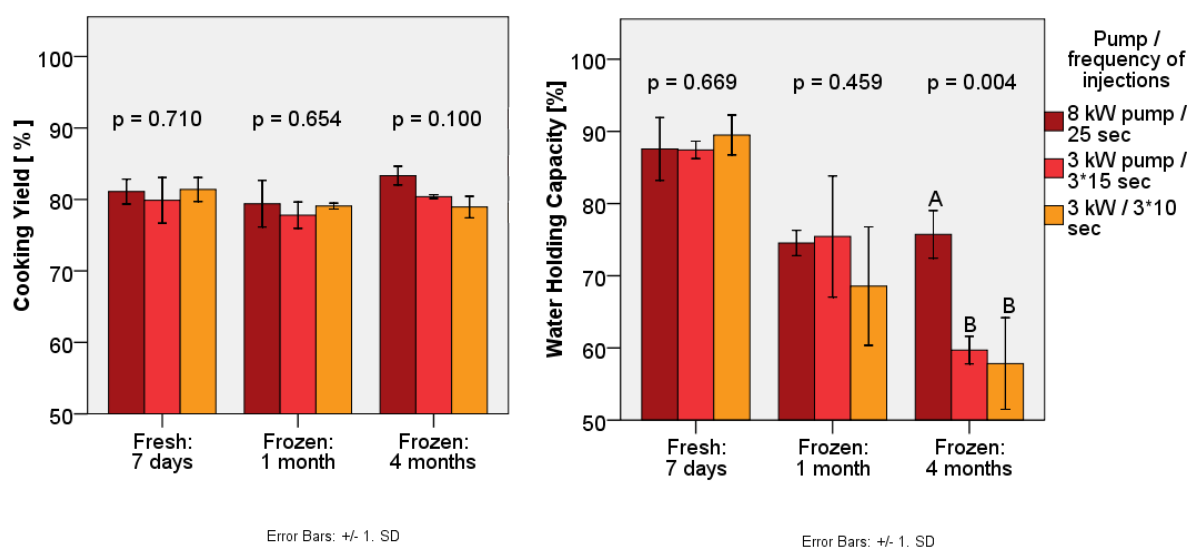
Placement of rotex	Pump	Frequency of injection, every:	Whole tank replaced every:
Port side	8 kW	25 sec	30 mins
Middle	3 kW	15 sec	12 mins
Starboard side	3 kW	10 sec	18 mins

In appendix G results from analysis of chemical composition can be found, that is water, protein, salt and fat content. The chemical composition of the material, when an average value is calculated for all samples in experiment regardless of groups, can be found in table 8.

**Table 8 Chemical composition of the raw material used in the experiment designed to investigate the effects of different circulation and replacement rate of bleeding medium during bleeding on fillet quality. Samples were analyzed after 7 days of storage (fresh). An average value for all samples in all groups were combined.**

Samples, stored for 7 days	
Water [%]	81.7±0.4
Fat [%]	0.8±0.1
Protein [%]	17.0±0.4
Salt [%]	0.3±0.1

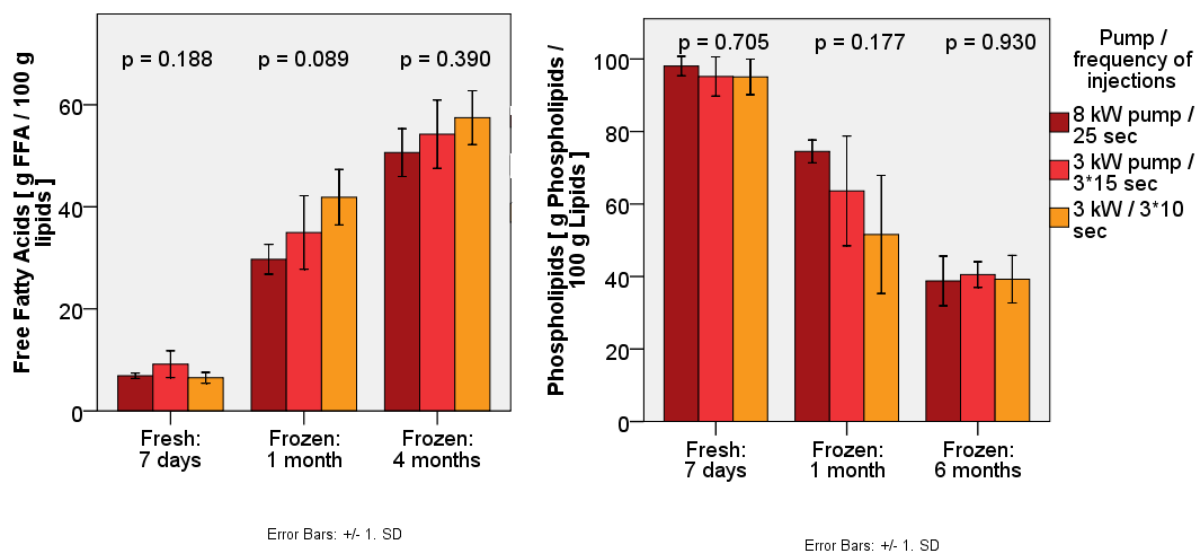
In figure 48 results from analysis of cooking yield and water holding capacity (WHC) are shown. There was no difference apparent between the three groups with the exception of the analysis of WHC after 4 months of frozen storage. In that case the cooking yield is highest for samples that went through rotex on port side, where circulation of bleeding medium is the most and replacement of medium the least ( $p < 0.05$ ). That indicates that those factors affect the water binding ability of the fillets.



**Figure 48 Cooking yield (%) and water holding capacity (%) of cod bled with different bleeding medium flow, analyzed 7 days old unfrozen and stored frozen from 1 month at -25 °C up to 4 months at a range from -12 °C to -25 °C (Figure 19) (n=3). Columns marked with the same letter are not significantly different.**

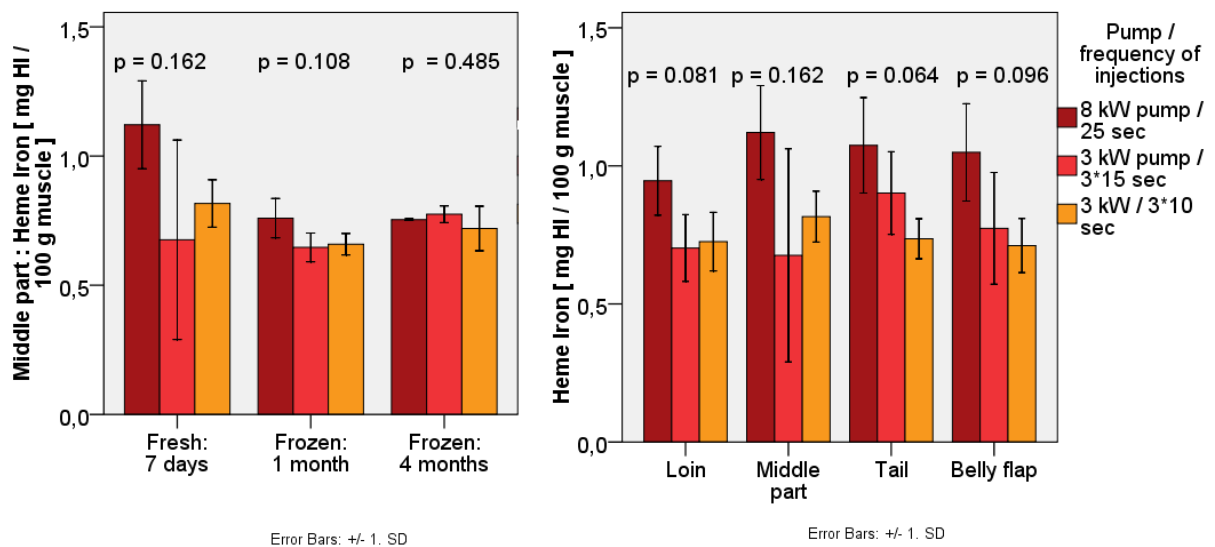
The results of the free fatty acids (FFA) and phospholipid (PL) content showed again that prolonged frozen storage affects the amount of both FFA and PL. Despite that the results show no significant difference between the experimental groups. Some pattern was observed indicating that the FFA increased at different rates within the muscle depending on the group. The results indicated that fish bled in rotex on the port side of Málmey, with the largest pump but slowest replacement of bleeding medium formed FFA slower than those of the other two groups ( $p > 0.05$ ). The same pattern was visible in PL results, where cod bled in port side ritex had higher PL content ( $p > 0.05$ ). This indicates that as

long as the ratio between fish and bleeding medium, or bleeding medium and blood, does not exceed that of the port side rotex the power of the pump within the rotex, and therefore the circulation of the water, has more effect on bleeding efficiency and quality of the fillets. In order to find the ideal ratio between blood and bleeding medium during bleeding and power of the circulation of the medium further research is needed.



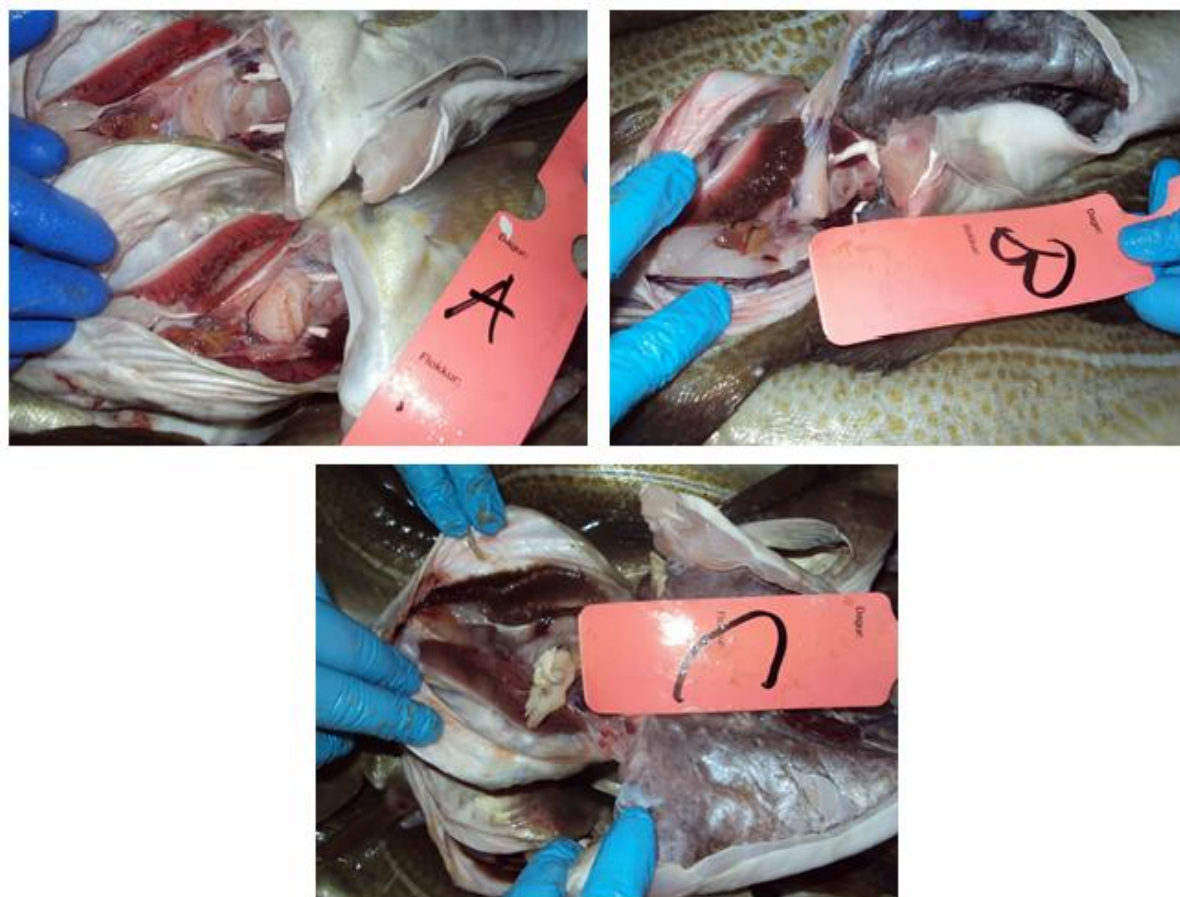
**Figure 49 Free fatty acid (g FFA /100 g lipids) and phospholipid (g phospholipids/100 g lipids) content of cod bled with different bleeding medium flow, analyzed 7 days old unfrozen and stored frozen from 1 month at -25°C up to 4 months at a range from -12 °C to -25 °C (Figure 1919) (n=6).**

Heme iron content of samples is highest in all parts of the fillets in 7 day old, unfrozen, samples bled in port side rotex ( $p>0.05$ ) compared to those bled in middle or starboard rotex (Figure r8). It did, however, change with prolonged frozen storage and after 4 months of storage heme iron content of middle part of fillets had evened out (Figure 50).



**Figure 50 Heme iron content (mg heme iron/100 g muscle) of cod bled with different bleeding medium flow, analyzed 7 days old unfrozen and stored frozen from 1 month at -25°C up to 4 months at a range from -12 °C to -25 °C (Figure 19) (n=6).**

Photos taken of the gills of samples before processing, 4<sup>th</sup> day after bleeding, showed that cod bled in rotex on port side, where the circulating pump is the most powerful but replacement of bleeding medium the slowest (marked A) had much redder gills indicating better bleeding and over all state of the samples (Figure 51).



**Figure 51 The gills of the samples before processing. Sample marked A was bled in the port side rotex, B in the middle rotex and C in the starboard rotex. (Photo S. Elíasson)**

Correlations analysis was performed on the results obtained from this experiment. The results were split up and the correlation coefficient and corresponding p-value can be found in appendix G. When the correlation coefficient was positive the pair of variables tend to increase or decrease together. If the coefficient was negative the one of the variables tends to increase while the other decreases. Correlation was only found when comparing results of heme iron analysis and bleeding in different rotex systems (Table 9). The results show that correlation was found between which rotex was used during bleeding and heme iron content of the belly flap and tail of samples, 7 days from bleeding and unfrozen. The correlation was negative, showing that a decrease in circulation led to an increase in heme iron content in these parts of the fillets.

**Table 9 Pearson's correlation between measured variables of samples of rotex experiment March 2016. \*\*Correlation is significant at the 0.01 level. \*Correlation is significant at the 0.05 level. N.a.: Analysis was only performed at beginning of storage therefore no data was available for the effect of storage.**

	Rotex	Prolonged Storage	HI Loin	HI Middle	HI Tail	HI Belly flap
<b>Rotex</b>	1					
<b>Prolonged Storage</b>	1	1				
<b>HI Loin</b>	-0.077	N.a.	1			
<b>HI Middle</b>	-0.096	-0.171	0.001**	1		
<b>HI Tail</b>	-0.014	N.a.	0.012*	0.088	1	
<b>HI Belly flap</b>	-0.039	N.a.	0.001**	0.001**	0.002**	1

According to present results of the chemical parameters and the visual evaluation of the gills (Figure 51), it can be concluded that the cod bled in rotex on the port side was of higher quality compared to cod bled in the other rotex systems. Therefore that the circulation of bleeding medium has a larger effect on the quality of the fillets than replacement of medium, up to the ratio in the port side rotex.



## 5 Conclusions

As previously stated, this project includes two major trials on-board modern wet fish trawler. There are results from experiments performed in July 2015 with the aim to examine the effects of storage method on-board the trawler as well as the bleeding medium temperature to explore if it is beneficial to start the sub-cooling process in the bleeding tank of the rotex system. Then experiments were performed in March 2016 set up to compare the different rotex systems on-board Málmeý, exploring the effects of circulation and rate of replacement of the bleeding medium.

The first thing that was apparent when analyzing results of experiments performed in July 2015 was the difference between unsalted and lightly salted samples. When looking for factors indicating a difference between groups they were seldom apparent in lightly salted samples. The salting method clearly affected the rate at which the samples spoiled as well as how apparent difference between groups was. This indicates that in order to see if these factors investigated in present project effect the quality of lightly salted fillets dramatically a more extensive study would need to be performed, f. ex. with longer storage than in this case.

In July 2015 experiment on different waiting time before bleeding was performed. It was in order to see if the different storage on-board Málmeý affected the importance of rapid bleeding of cod directly after hauling. As discussed in chapter 4.2 the results from analysis of samples in waiting time experiment were inconclusive due to error in sampling. They are available in appendix but were not discussed specifically in chapter 4.2. The experiment would need to be repeated differently in order to get the information needed to answer the questions raised. In the last few years at least two similar experiments have been performed, one in Iceland and the other one in Norway. Both concluded that waiting time before bleeding is a significant factor in ensuring quality of fish, especially white fish (Olsen *et al.*, 2014; Eliasson, 2013). However neither of them was performed on catch subjected to storage without ice at -1 °C, similar to the handling on Málmeý so it is not possible to conclude about the effects of this storage method on the importance of rapidly bleeding the catch.

The effect of different temperatures of bleeding medium on fillet quality were negligible for unsalted fillets. Less variability in FFA content and slightly higher PL was observed in muscle of cod bled at -1 °C ( $p>0.05$ ). However when appearance of fillets is taken into consideration the group bled at -1 °C is clearly redder than the other two. Based on these results it would not be feasible to bleed white fish, such as cod, at subzero temperatures without performing further experiments. The main reason for this conclusion is due the fact that color and overall appearance are very important quality attribute of whitefish fillets with regard to consumer preferences. It would be interesting to perform further experiments with other fish species. When comparing cod bled at sea temperature and at 2 °C, there seems to be minor difference between them based on the factors evaluated in present study. Only a slight difference between groups could be detected in results of color analysis, where group bled at 2 °C seemed to be slightly redder than group bled at sea temperature ( $p>0.05$ ). Therefore the results of this experiment indicate that when bleeding cod in this type of rotex system, the best results are obtained by bleeding in sea water rather than starting the cooling during bleeding.

The storage method experiment performed in July 2015 overlapped with one of the March 2016 experiments. The results from the July 2015 experiment showed that the best storage method on-board after bleeding, gutting and cooling was supercooled cod kept on ice. There was a significant difference in FFA content between the groups, with the lowest amount observed in the supercooled and iced cod after 1 month of frozen storage. The March 2016 experiment was similar to this one and investigated the difference between large fillets kept on ice without pre-cooling or supercooled and stored iceless at subzero temperature. The results indicated that even though the supercooled cod was of better initial quality with lower FFA ( $p>0.05$ ) it deteriorated more rapidly. After 4 months of frozen storage, the fillets from cod stored on ice contained less FFA and more PL ( $p>0.05$ ). The same pattern was found for heme iron content of the same samples. Since the results from the March 2016 experiment were not statistically significant they can only give an idea of what the difference is between the groups, another experiment performed with more samples would need to be performed in order to get more definitive result.

The last two experiments, performed in March 2016, were both created in order to answer a question related to the conditions of bleeding tanks on-board Málmeý. The first was set up to see the difference between a cod bled in still water and a cod bled in circulating. The results were not conclusively showing which method ensures better bleeding. After 1 month of frozen storage, fillets from cod bled in still water had however a higher FFA content ( $p>0.05$ ), but after 4 months of store, the results for both groups were similar. Visual observation of the gills and the belly flap of the cod bled in circulating water indicated better quality compared to the cod bled in still water. It had lighter belly flap and red gills. That indicates that keeping the water circulating during bleeding should prompt better bleeding under these conditions.

Having established that circulating the bleeding water prompts better bleeding the last question unanswered was which factor had more effect on ensures the best bleeding. The rotex systems on-board Málmeý are three and all have pumps circulating bleeding medium as well as continuous replacement of medium through timed injections into chamber. The rotex on the port side with a powerful 8 kW pump replacement of bleeding medium every 30 mins, on the starboard side with a 3 kW pump and replacement of bleeding medium every 18 min and middle rotex with a similar 3 kW pump and replacement every 12 min. When looking at the results a clear pattern was visible. The FFA content increased fastest in cod bled in the rotex system on starboard side and slowest in cod bled in rotex system on port side. The results indicated that a more efficient bleeding was obtained by using the rotex system on the port side. Mainly, the rotex system with the strongest pump (8 kW), and therefore the most movement of the water, and the slowest replacement of water. These results indicated that the frequency of water replacement within the bleeding tank, as long as it is not less than 30 min, does not have as much effect on bleeding as how strongly the water within the tank is circulated.

## 6 Future perspective

There are a lot of possibilities in research of on-board handling of fish. In order to better understand even improve the processes studied in this project further research is needed.

With regards to storage methods on-board supercooling and icing seemed most effective. However trying to eliminate the ice from the storage would be a step towards lowering the carbon footprint of the fishing industry and is therefore something worth striving for. In this study the idea that possibly the reason supercooled samples stored at -1 °C without ice proved to spoil quicker than those iced might be that when ice is not present there is a very limited amount of water present in the tubs. The water might enhance the quality of the fish in some way, by washing away residual blood for example. Therefore installing a device that creates a liquid mist in the air coating the fish with water during the on-board storage and keeping it wet might slow down this spoilage.

The results of this study indicated that when working with white fish it might not be feasible to start the cooling process in the bleeding tubs due to the effects it had on fillets color. The chemical factors analyzed in order to evaluate the samples spoilage were however not affected. Indicating that this method might possibly be better suited for other fish species not affected as dramatically by changes in color of flesh.

In order to evaluate the amount of blood still present in muscle in this study the objective was to measure both heme iron and non-heme iron content. The method used to measure non-heme iron was first developed for use on meat, not fish, and has in most cases been used on fattier fish such as mackerel. It is therefore clear that a better method to evaluate the amount of blood and iron present in muscle of lean fish is needed. Before further experiments in this field are performed finding and testing possible methods would be feasible.



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## Appendix A

This appendix contains results of analysis performed on samples bled in bleeding medium at different temperatures. That is, chemical composition, results of correlation calculation and other results not presented in chapter 4.1.

**Table 10 Chemical composition of unsalted samples from experiment investigating effects of cooling bleeding medium on quality of fillets.**

Bled at:	Water [%]		Fat [%]		Protein [%]	Salt [%]
	1 month	6 months	1 month	6 months	1 month	1 month
6 °C	80.7±0.6	80.4±0.4	0.6±0.1	0.6±0.1	18.6±0.2	0.3±0.06
2 °C	81.3±0.3	81.1±0.3	0.6±0.0	0.6±0.1	18.0±0.5	0.3±0.06
-1 °C	81.6±0.4	80.8±0.5	0.5±0.0	0.7±0.0	17.4±0.4	0.3±0.00

**Table 11 Chemical composition of lightly salted samples from experiment investigating effects of cooling bleeding medium on quality of fillets.**

Bled at:	Water [%]		Fat [%]		Protein [%]	Salt [%]
	1 month	6 months	1 month	6 months	1 month	1 month
6 °C	85.8±0.2	80.7±0.6	0.5±0.1	0.5±0.1	12.1±0.3	1.5±0.2
2 °C	85.2±0.4	81.3±0.3	0.4±0.1	0.5±0.0	13.0±0.5	1.6±0.0
-1 °C	85.7±0.6	81.6±0.4	0.3±0.1	0.4±0.1	12.4±0.6	1.6±0.2

**Table 12 Results of L\*a\*b\* color analysis of unsalted and lightly salted samples analyzed after 1 month of frozen storage. Samples from experiment investigating effects of cooling bleeding medium on quality of fillets.**

Bled at:	6 °C		2 °C		-1 °C	
	Unsalted	Lightly salted	Unsalted	Lightly salted	Unsalted	Lightly salted
L-value	90.1±0.9	92.8±2.1	90.7±0.9	89.7±3.7	90.6±0.9	93.8±1.4
b-value	31.9±0.9	32.4±1.2	35.8±2.5	30.6±1.9	33.1±2.0	31.2±0.5
Whiteness	66.4±0.4	66.7±1.3	62.8±2.0	66.8±0.3	65.0±1.2	68.1±0.5

**Table 13 Results of L\*a\*b\* color analysis of unsalted and lightly salted samples analyzed after 6 month of frozen storage. Samples from experiment investigating effects of cooling bleeding medium on quality of fillets.**

<b>Bled at:</b>	6 °C		2 °C		-1 °C	
	Unsalted	Lightly salted	Unsalted	Lightly salted	Unsalted	Lightly salted
L-value	85.0±1.7	89.2±2.2	82.3±3.1	86.2±2.5	82.2±1.8	85.3±3.9
b-value	36.0±2.7	36.6±1.7	36.8±3.6	32.8±2.8	33.7±0.5	34.1±3.3
Whiteness	60.4±1.8	61.8±1.1	58.0±1.7	64.1±1.7	60.6±1.2	63.0±2.4

**Table 14 Pearson's correlation between measured variables of lightly salted samples from experiment investigating effects of cooling bleeding medium on quality of filletts. Pearson Correlation value shows whether correlation is positive or negative. N value represents the amount of values used in calculations.**

		Temperature of bleeding medium	Duration of frozen storage	Cooking yield	WHC	Color: a-value	FFA	PL	HI
Temperature of bleeding medium	Pearson Correlation Sig. (2-tailed) N	1 18							
Duration of frozen storage	Pearson Correlation Sig. (2-tailed) N	,000 1,000 18	1 18						
Cooking yield	Pearson Correlation Sig. (2-tailed) N	,063 ,804 18	-,043 ,865 18	1 18					
WHC	Pearson Correlation Sig. (2-tailed) N	,140 ,579 18	-,855** ,000 18	,081 ,749 18	1 18				
Color: a -value	Pearson Correlation Sig. (2-tailed) N	,212 ,398 18	,749** ,000 18	-,023 ,927 18	-,570* ,014 18	1 18			
FFA	Pearson Correlation Sig. (2-tailed) N	-,209 ,406 18	,772** ,000 18	-,207 ,409 18	-,829** ,000 18	,485* ,041 18	1 18		
PL	Pearson Correlation Sig. (2-tailed) N	,023 ,928 18	-,876** ,000 18	,066 ,795 18	,859** ,000 18	-,596** ,009 18	-,881** ,000 18	1 18	
HI	Pearson Correlation Sig. (2-tailed) N	,139 ,582 18	-,762** ,000 18	,161 ,522 18	,818** ,000 18	-,474* ,047 18	-,902** ,000 18	,921** ,000 18	1 18

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\* . Correlation is significant at the 0.05 level (2-tailed).

a. Processing = Unsalted

**Table 15 Pearson's correlation between measured variables of lightly salted samples from experiment investigating effects of cooling bleeding medium on quality of fillets. Pearson Correlation value shows whether correlation is positive or negative. N value represents the amount of values used in calculations.**

		Temperature of bleeding medium	Duration of frozen storage	Cooking yield	WHC	Color: a-value	FFA	PL	HI
Temperature of bleeding medium	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	18							
Duration of frozen storage	Pearson Correlation	,000	1						
	Sig. (2-tailed)	1,000							
	N	18	18						
Cooking yield	Pearson Correlation	,131	-,130	1					
	Sig. (2-tailed)	,604	,607						
	N	18	18	18					
WHC	Pearson Correlation	-,089	-,812**	,366	1				
	Sig. (2-tailed)	,724	,000	,135					
	N	18	18	18	18				
Color: a-value	Pearson Correlation	-,008	,241	-,336	-,244	1			
	Sig. (2-tailed)	,975	,336	,173	,329				
	N	18	18	18	18	18			
FFA	Pearson Correlation	,117	,921**	-,164	-,841**	,361	1		
	Sig. (2-tailed)	,644	,000	,516	,000	,141			
	N	18	18	18	18	18	18		
PL	Pearson Correlation	-,226	-,424	,076	,547	,290	-,385	1	
	Sig. (2-tailed)	,367	,080	,766	,019	,244	,115		
	N	18	18	18	18	18	18	18	
HI	Pearson Correlation	-,147	-,829**	,139	,680**	-,260	-,822**	,465	1
	Sig. (2-tailed)	,561	,000	,582	,002	,298	,000	,052	
	N	18	18	18	18	18	18	18	18

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\* . Correlation is significant at the 0.05 level (2-tailed).

a. Processing = Lightly salted

## Appendix B

This appendix contains results of analysis performed in samples from experiment (July 2015) investigating effect of waiting time before bleeding on fillet quality. That is, chemical composition, results of correlation calculation and other results not presented in chapter 4.2.

**Table 16 Chemical composition of unsalted samples from experiment investigating effects of waiting time before bleeding on quality of fillets stored on-board trawler at -1 °C without ice.**

Waiting time	Water [%]		Fat [%]		Protein [%]		Salt [%]
	1 month	6 months	1 month	6 months	1 month		1 month
0 min	86.3±0.6		84.3±0.6	0.3±0.1	0.4±0.1	11.7±0.6	1.8±0.2
30 min	85.8±0.8		85.2±0.4	0.4±0.1	0.4±0.1	12.4±1.0	1.5±0.1
60 min	85.4±0.4		84.9±0.6	0.4±0.0	0.4±0.0	12.7±0.6	1.6±0.0
90 min	85.8±0.9		85.5±0.5	0.4±0.1	0.4±0.0	12.0±1.0	1.6±0.1

**Table 17 Chemical composition of lightly salted samples from experiment investigating effects of waiting time before bleeding on quality of fillets stored on-board trawler at -1 °C without ice.**

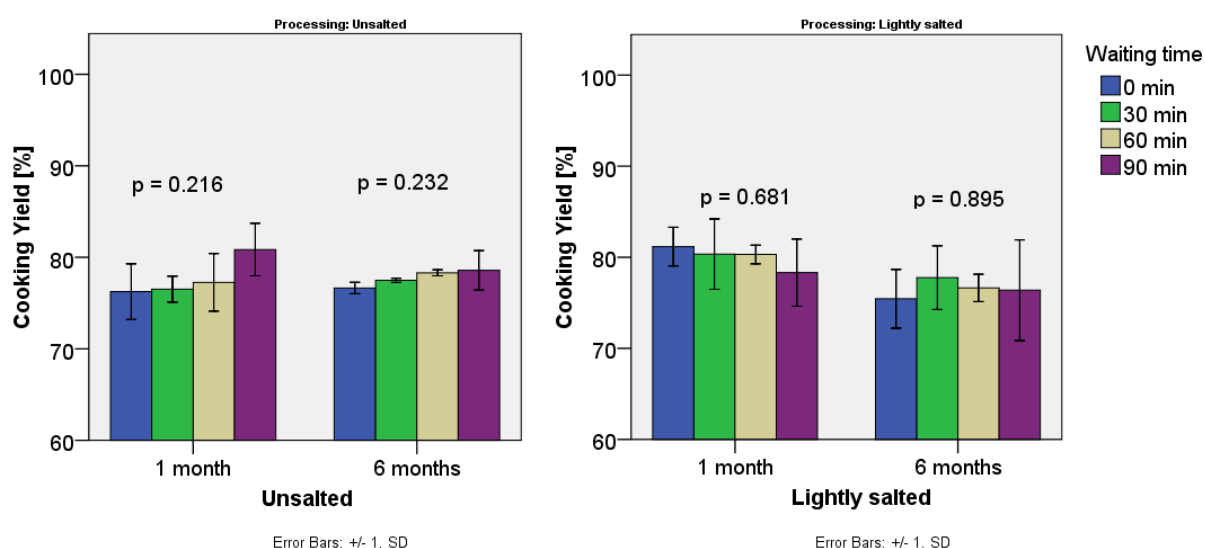
Waiting time	Water [%]		Fat [%]		Protein [%]		Salt [%]
	1 month	6 months	1 month	6 months	1 month		1 month
0 min	81.7±0.9	80.2±0.8	0.5±0.0	0.5±0.1	17.7±0.5		0.3±0.0
30 min	82.0±0.4	80.5±0.2	0.5±0.0	0.6±0.1	18.1±0.3		0.3±0.1
60 min	81.2±0.3	80.2±0.2	0.6±0.0	0.6±0.1	<b>18.0±0.2</b>		0.3±0.0
90 min	80.9±0.4	80.2±0.5	0.6±0.0	0.6±0.1	19.5±0.4		0.2±0.1

**Table 18 Results of L\*a\*b\* color analysis of unsalted and lightly salted samples analyzed after 1 month of frozen storage. Samples from experiment investigating effects of waiting time before bleeding on quality of fillets stored on-board trawler at -1 °C without ice.**

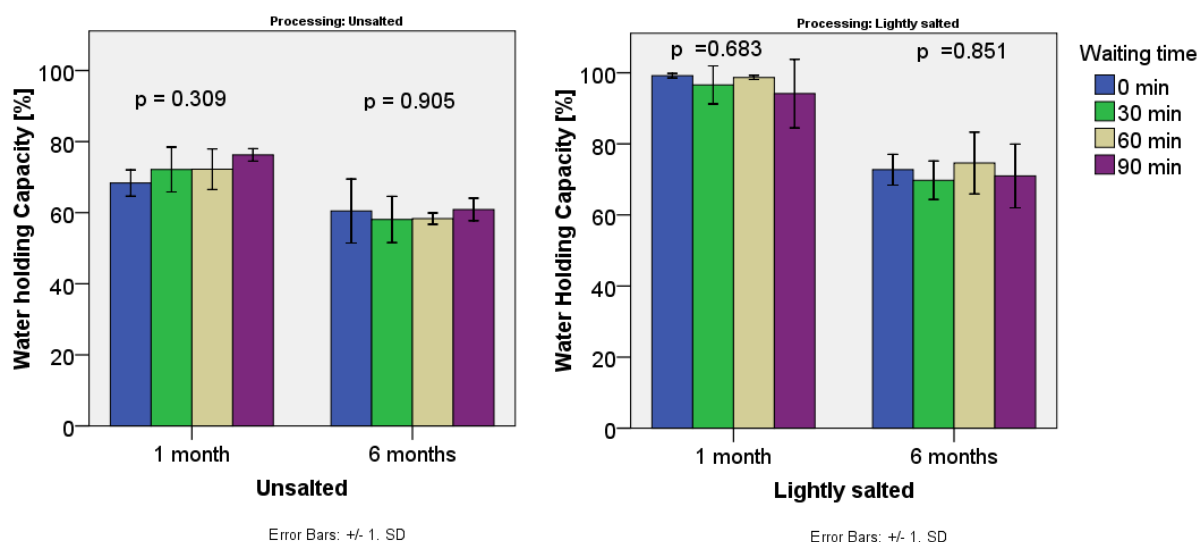
		L-value	a-value	b-value	Whiteness
0 min	Unsalted	92.2±3.4	-0.2±5.9	33.7±0.3	64.9±1.0
	Lightly salted	93.9±0.7	-2.6±1.1	31.8±0.4	67.5±0.5
30 min	Unsalted	89.0±0.8	5.4±1.5	31.3±0.8	66.3±0.3
	Lightly salted	92.6±1.6	-1.0±2.1	32.3±2.8	66.8±2.8
60 min	Unsalted	92.0±0.8	3.0±1.7	30.7±2.5	67.7±2.1
	Lightly salted	88.4±1.9	5.5±2.4	29.2±0.8	68.0±1.6
90 min	Unsalted	88.4±2.7	9.1±5.2	30.2±2.8	65.5±0.3
	Lightly salted	91.3±2.9	0.6±4.2	31.2±1.0	67.5±1.7

**Table 19 Table 18 Results of L\*a\*b\* color analysis of unsalted and lightly salted samples analyzed after 1 month of frozen storage. Samples from experiment investigating effects of waiting time before bleeding on quality of fillets stored on-board trawler at -1 °C without ice.**

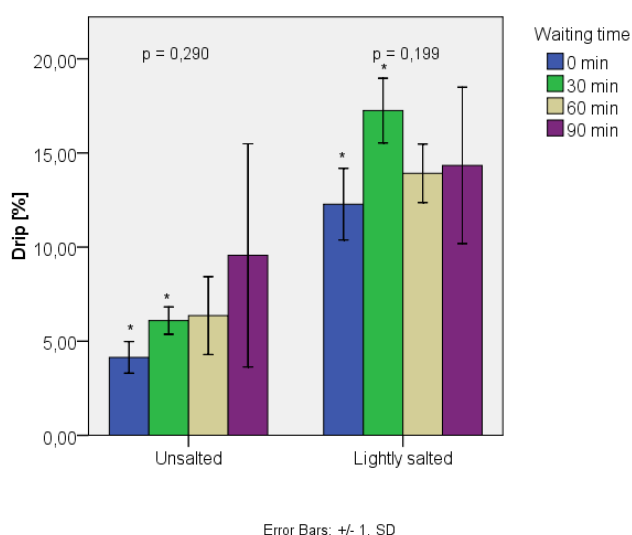
		L-value	a-value	b-value	Whiteness
0 min	Unsalted	83.0±1.1	9.8±1.4	37.0±1.0	58.1±0.5
	Lightly salted	87.6±2.3	3.6±4.1	32.6±1.7	64.8±2.7
30 min	Unsalted	83.1±2.1	12.0±3.8	33.7±0.7	59.9±2.1
	Lightly salted	91.0±1.2	0.0±2.2	33.7±2.8	65.6±4.0
60 min	Unsalted	77.7±2.5	12.1±0.6	36.5±2.6	56.6±0.7
	Lightly salted	85.2±1.8	3.9±1.5	31.3±1.7	65.5±2.0
90 min	Unsalted	83.6±3.0	9.6±3.8	37.4±1.8	57.7±1.9
	Lightly salted	87.0±2.4	5.7±0.9	30.1±0.9	66.8±1.1



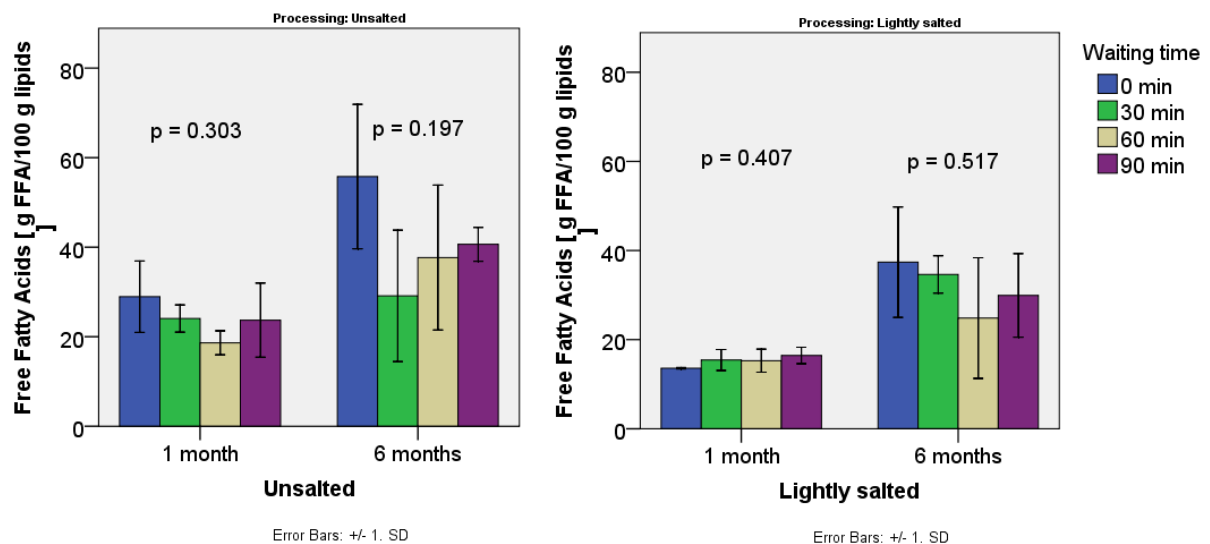
**Figure 52 Cooking yield (%) of unsalted and lightly salted cod fillets from experiment on effects of waiting time before bleeding on quality of fillets. Stored in frozen storage from 1 month up to 6 months (n=3).**



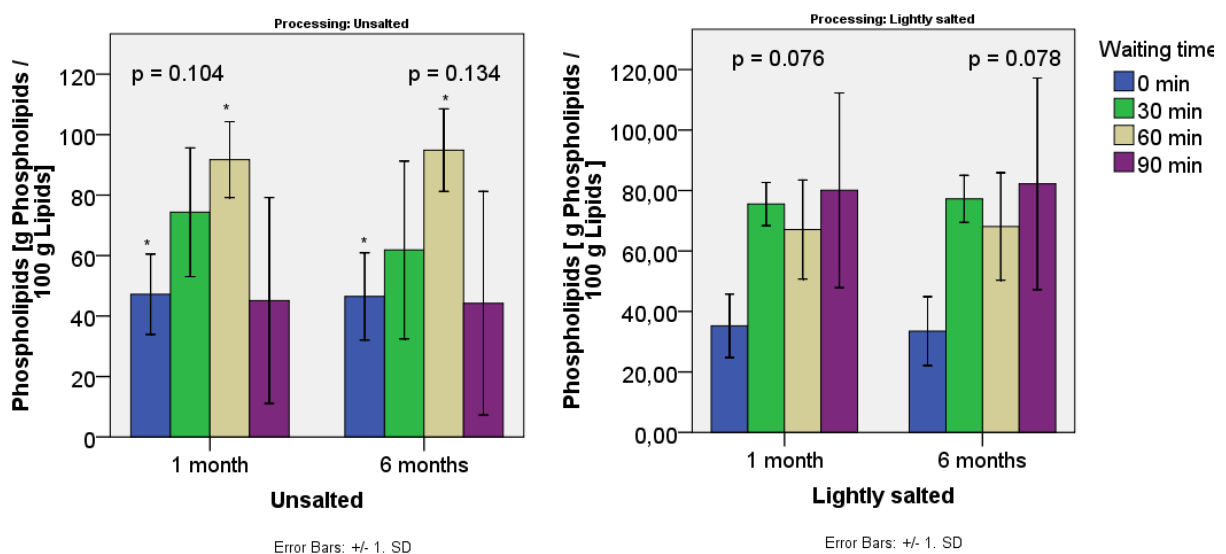
**Figure 53** Water holding capacity (%) of unsalted and lightly salted cod fillets from experiment on effects of waiting time before bleeding on quality of fillets. Stored in frozen storage from 1 month up to 6 months (n=3).



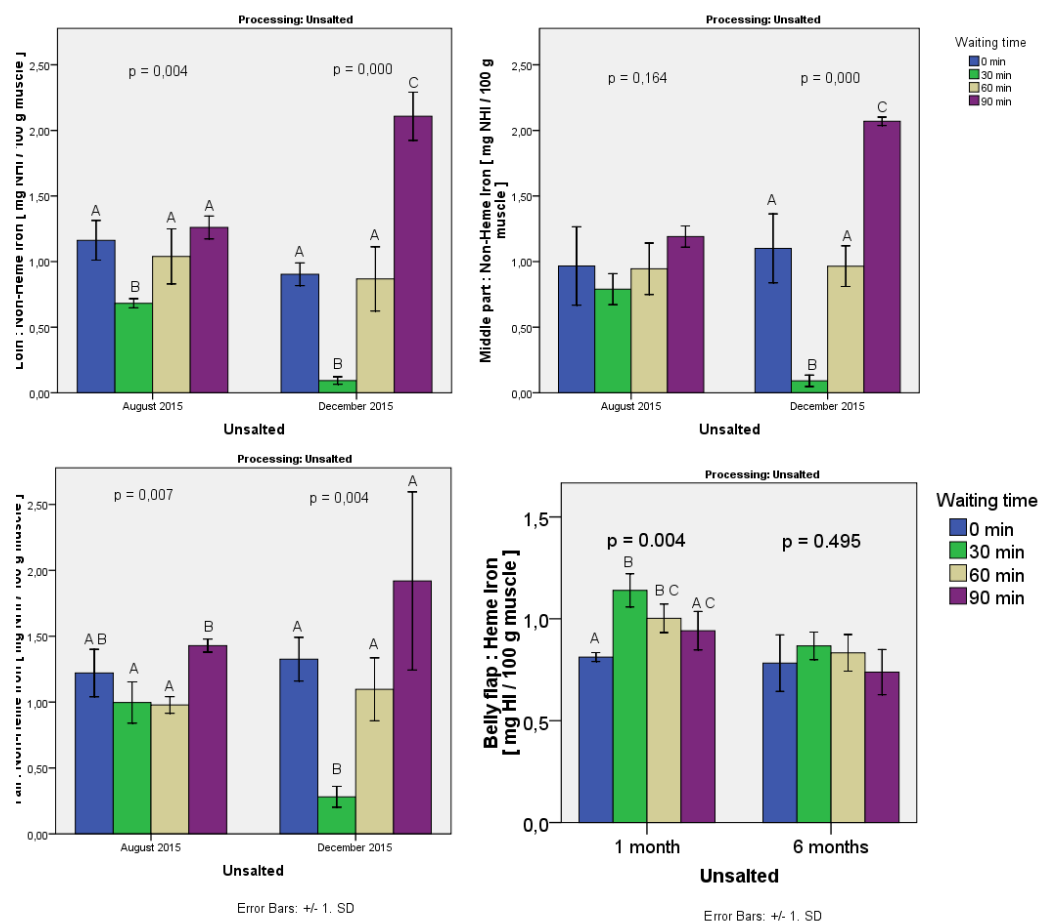
**Figure 54** Drip (%) of unsalted and lightly salted after 6 months of frozen storage, cod fillets were from experiment on effects of waiting time before bleeding on quality of fillets \*Columns marked were not found to be statistically different with ANOVA analysis but when T-test was performed  $p < 0.05$ .



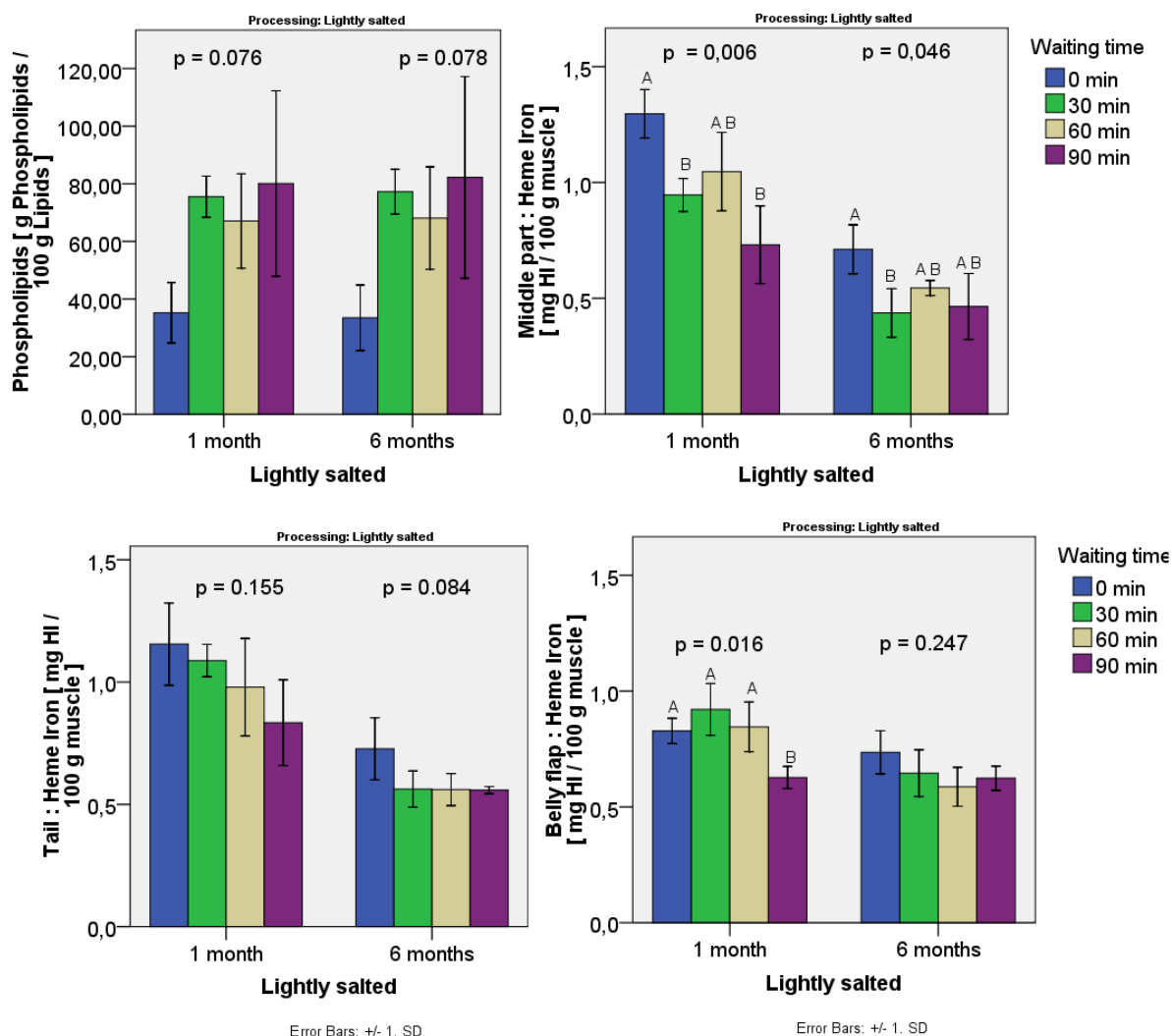
**Figure 55 Free fatty acids (g FFA/100 g lipids) in unsalted and lightly salted cod fillets from cod bled at different time, with various different waiting time before bleeding. Samples were stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 (Figure 14) (n=6).**



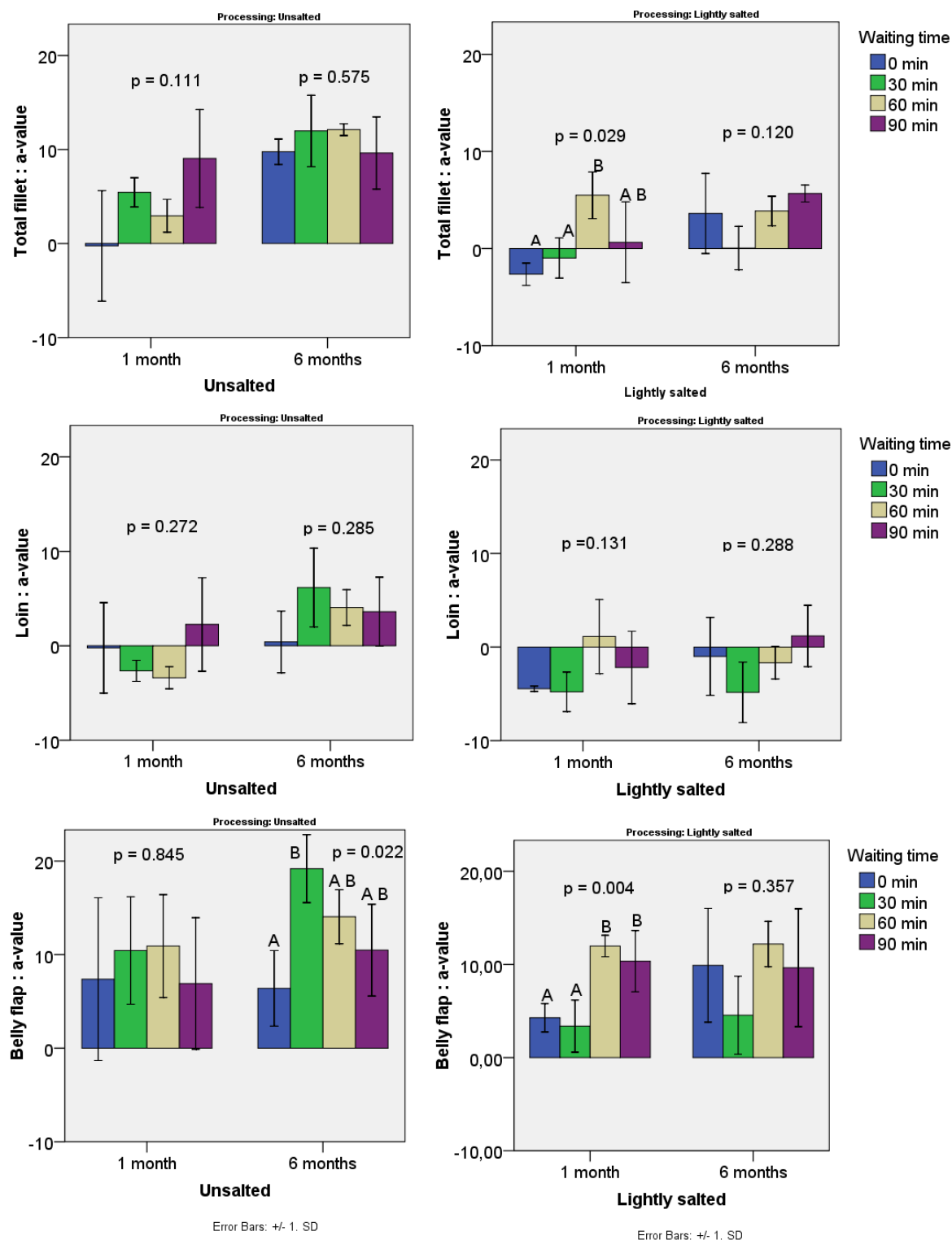
**Figure 56 Free fatty acids (g FFA/100 g lipids) in unsalted and lightly salted cod fillets from cod bled at different time, with various different waiting time before bleeding. Samples were stored in frozen storage for 1 month at -25 °C or up to 6 months at a range from -12 °C to -25 (Figure 14) (n=6).**



**Figure 57** Results from analysis of heme iron of unsalted samples experiment where effects of waiting time before bleeding on the quality of fillets (n=6). Where a significant difference is between groups ( $p < 0.05$ ) columns are marked with letters. Columns marked with the same letter are not significantly different.



**Figure 58** Results from analysis of heme iron of lightly salted samples experiment where effects of waiting time before bleeding on the quality of fillets (n=6). Where a significant difference is between groups ( $p < 0.05$ ) columns are marked with letters. Columns marked with the same letter are not significantly different.



**Figure 59** The redness values, a-values, from lightly salted and unsalted fillets whole and loin and belly flap individually stored for 1 month at  $-25^{\circ}\text{C}$  or up to 6 months at a range from  $-12^{\circ}\text{C}$  to  $-25^{\circ}\text{C}$  (Figure 14) ( $n=3$ ). \*Columns marked were not found to be statistically different with ANOVA analysis but when T-test was performed  $p < 0.05$ . At the time of sample collection sea temperature was  $6^{\circ}\text{C}$ .

**Table 20 Pearson's correlation between measured variables of unsalted samples from experiment investigating effects of waiting time before bleeding on quality of fillets stored on-board trawler at -1 °C without ice. Pearson Correlation value shows whether correlation is positive or negative. N value represents the amount of values used in calculations.**

		Waiting time before bleeding	Duration of frozen storage	Cooking yield	WHC	FFA	HI	PL
Waiting time before bleeding	Pearson Correlation Sig. (2-tailed) N	1  24						
Duration of frozen storage	Pearson Correlation Sig. (2-tailed) N	,000 1,000 24	1  24					
Cooking yield	Pearson Correlation Sig. (2-tailed) N	,536** ,007 24	,008 ,970 24	1  24				
WHC	Pearson Correlation Sig. (2-tailed) N	,177 ,407 24	-,797** ,000 24	,063 ,770 24	1  24			
FFA	Pearson Correlation Sig. (2-tailed) N	-,230 ,281 24	,600** ,002 24	-,134 ,531 24	-,475* ,019 24	1  24		
HI	Pearson Correlation Sig. (2-tailed) N	,198 ,353 24	,472 ,020 24	,070 ,745 24	-,373 ,072 24	,147 ,492 24	1  24	
PL	Pearson Correlation Sig. (2-tailed) N	,085 ,701 23	-,033 ,881 23	-,176 ,421 23	,010 ,965 23	-,199 ,363 23	,159 ,468 23	1  23

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\* . Correlation is significant at the 0.05 level (2-tailed).

a. Processing = Unsalted

**Table 21 Pearson's correlation between measured variables of unsalted samples from experiment investigating effects of waiting time before bleeding on quality of fillets stored on-board trawler at -1 °C without ice. Pearson Correlation value shows whether correlation is positive or negative. N value represents the amount of values used in calculations.**

		Waiting time before bleeding	Duration of frozen storage	Cooking yield	WHC	FFA	HI	PL
Waiting time before bleeding	Pearson Correlation	1						
	Sig. (2-tailed)							
	N	24						
Duration of frozen storage	Pearson Correlation	,000	1					
	Sig. (2-tailed)	1,000						
	N	24	24					
Cooking yield	Pearson Correlation	-,115	-,516**	1				
	Sig. (2-tailed)	,594	,010					
	N	24	24	24				
WHC	Pearson Correlation	-,055	-,915**	,416*	1			
	Sig. (2-tailed)	,799	,000	,043				
	N	24	24	24	24			
FFA	Pearson Correlation	-,122	,759**	-,490*	-,757**	1		
	Sig. (2-tailed)	,570	,000	,015	,000			
	N	24	24	24	24	24		
HI	Pearson Correlation	-,261	-,873**	,528**	,884**	-,694**	1	
	Sig. (2-tailed)	,218	,000	,008	,000	,000		
	N	24	24	24	24	24	24	
pl	Pearson Correlation	,600**	,016	,186	-,108	-,082	-,151	1
	Sig. (2-tailed)	,002	,942	,383	,615	,704	,482	
	N	24	24	24	24	24	24	24

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\* . Correlation is significant at the 0.05 level (2-tailed).

a. Processing = Lightly salted



## Appendix C

This appendix contains results of analysis performed in samples from experiment (July 2015) investigating effect of different storage methods on-board, after bleeding and gutting, on fillet quality. That is, chemical composition, results of correlation calculation and other results not presented in chapter 4.3.

**Table 22 Chemical composition of unsalted samples from experiment investigating effects of different storage methods on-board trawler on fillets quality.**

Storage method	Water [%]		Fat [%]		Protein [%]	Salt [%]
	1 month	6 months	1 month	6 months	1 month	1 month
Supercooled and iced	80.9±0.2	80.2±0.8	0.5±0.0	0.5±0.1	18.4±0.4	0.3±0.1
Iced	82.1±0.5	81.1±0.6	0.6±0.0	0.6±0.1	17.2±0.9	0.2±0.1
Supercooled kept at -1°C	81.7±0.9	81.3±0.4	0.5±0.0	0.7±0.1	17.7±0.5	0.3±0.0

**Table 23 Chemical composition of lightly salted samples from experiment investigating effects of different storage methods on-board trawler on fillets quality.**

Storage method	Water [%]		Fat [%]		Protein [%]	Salt [%]
	1 month	6 months	1 month	6 months	1 month	1 month
Supercooled and iced	84.6±1.0	84.8±0.6	0.3±0.1	0.4±0.1	13.0±0.6	1.6±0.1
Iced	85.6±0.2	84.4±0.5	0.4±0.1	0.5±0.1	12.5±0.4	1.6±0.1
Supercooled kept at -1°C	86.3±0.6	84.4±0.4	0.3±0.1	0.5±0.1	11.7±0.6	1.8±0.1

**of different storage methods on-board trawler on fillets quality.**

**Table 24 Results of L\*a\*b\* color analysis of unsalted and lightly salted samples analyzed after 1 month of frozen storage. Samples from experiment investigating effects of different storage methods on-board trawler on fillets quality.**

Storage	Supercooled and iced		Iced		Supercooled kept at -1 °C	
	Unsalted	Lightly salted	Unsalted	Lightly salted	Unsalted	Lightly salted
L-value	92.2±3.4	93.9±0.7	89.5±2.4	91.7±1.4	93.2±0.9	94.1±0.9
b-value	33.7±0.3	31.8±0.4	33.0±1.8	31.2±1.2	32.3±1.1	32.7±0.7
Whiteness	64.9±1.0	67.5±0.5	64.2±0.9	67.7±1.3	66.8±0.9	66.6±0.7

**different storage methods on-board trawler on fillets quality.**

**Table 25 Results of L\*a\*b\* color analysis of unsalted and lightly salted samples analyzed after 6 month of frozen storage. Samples from experiment investigating effects of different storage methods on-board trawler on fillets quality.**

Storage	Supercooled and iced		Iced		Supercooled kept at -1 °C	
	Unsalted	Lightly salted	Unsalted	Lightly salted	Unsalted	Lightly salted
L-value	83.0±1.1	87.6±2.3	86.6±2.5	83.5±1.2	89.3±2.3	87.8±1.8
b-value	37.0±1.0	32.6±1.7	38.3±3.2	33.5±0.9	37.9±3.5	32.4±0.6
Whiteness	58.1±0.5	64.8±2.7	58.4±1.7	62.3±1.7	59.7±1.8	65.0±0.8

**Table 26 Pearson's correlation between measured variables of unsalted samples from experiment investigating effects of waiting time before bleeding on quality of fillets stored on-board trawler at -1 °C without ice. Pearson Correlation value shows whether correlation is positive or negative. N value represents the amount of values used in calculations.**

		Storage method	Duration of frozen storage	Cooking yield	WHC	Drip	Color: a-value	FFA	HI	PL
Storage method	Pearson Correlation	1								
	Sig.(2-tailed)									
	N	18								
Length of frozen storage	Pearson Correlation	.000	1							
	Sig.(2-tailed)	1.000								
	N	18	18							
Cooking yield	Pearson Correlation	-.001	.352	1						
	Sig.(2-tailed)	.996	.151							
	N	18	18	18						
WHC	Pearson Correlation	-.254	.750 <sup>a</sup>	-.155	1					
	Sig.(2-tailed)	.309	.000	.540						
	N	18	18	18	18					
Drip	Pearson Correlation	.565	. <sup>a</sup>	-.262	-.122	1				
	Sig.(2-tailed)	.113	.000	.496	.755					
	N	9	9	9	9	9				
Color: a-value	Pearson Correlation	-.224	.518 <sup>*</sup>	-.053	-.318	-.393	1			
	Sig.(2-tailed)	.371	.028	.834	.199	.295				
	N	18	18	18	18	9	18			
FFA	Pearson Correlation	-.126	-.107	.347	.101	-.251	-.177	1		
	Sig.(2-tailed)	.619	.672	.159	.689	.514	.482			
	N	18	18	18	18	9	18	18		
HI	Pearson Correlation	.192	.067	-.154	-.151	.340	.039	-.624 <sup>**</sup>	1	
	Sig.(2-tailed)	.446	.791	.540	.550	.371	.878	.006		
	N	18	18	18	18	9	18	18	18	
PL	Pearson Correlation	-.086	.103	-.372	.036	-.159	.341	-.755 <sup>**</sup>	.219	1
	Sig.(2-tailed)	.733	.684	.128	.886	.683	.166	.000	.384	
	N	18	18	18	18	9	18	18	18	18

<sup>\*\*</sup>. Correlation is significant at the 0.01 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

<sup>\*</sup>. Correlation is significant at the 0.05 level (2-tailed).

b. Processing = Unsalted

**Table 27 Pearson's correlation between measured variables of unsalted samples from experiment investigating effects of waiting time before bleeding on quality of fillets stored on-board trawler at -1 °C without ice. Pearson Correlation value shows whether correlation is positive or negative. N value represents the amount of values used in calculations.**

		Storage method	Duration of frozen storage	Cooking yield	WHC	Drip	Color: a-value	FFA	HI	PL
Storage method	Pearson Correlation	1								
	Sig(2-tailed)									
	N	18								
Duration of frozen storage	Pearson Correlation	.000	1							
	Sig. (2-tailed)	1.000								
	N	18	18							
Cooking yield	Pearson Correlation	.171	-.433	1						
	Sig. (2-tailed)	.499	.073							
	N	18	18	18						
WHC	Pearson Correlation	.102	-.897**	.683**	1					
	Sig. (2-tailed)	.688	.000	.002						
	N	18	18	18	18					
Drip	Pearson Correlation	-.149	<sup>a</sup>	-.103	-.499	1				
	Sig. (2-tailed)	.703	.000	.793	.172					
	N	9	9	9	9	9				
Color: a-value	Pearson Correlation	.041	.806**	-.216	-.641**	-.575	1			
	Sig. (2-tailed)	.871	.000	.388	.004	.105				
	N	18	18	18	18	9	18			
FFA	Pearson Correlation	.047	.377	-.429	-.397	-.267	.441	1		
	Sig. (2-tailed)	.852	.124	.076	.102	.488	.067			
	N	18	18	18	18	9	18	18		
HI	Pearson Correlation	-.257	-.830**	.440	.731**	.215	-.750**	-.648**	1	
	Sig. (2-tailed)	.304	.000	.068	.001	.578	.000	.004		
	N	18	18	18	18	9	18	18	18	
PL	Pearson Correlation	-.045	-.016	-.043	.053	-.503	.209	.665**	-.268	1
	Sig. (2-tailed)	.858	.950	.865	.833	.167	.406	.003	.282	
	N	18	18	18	18	9	18	18	18	18

\*\* . Correlation is significant at the 0.01 level (2-tailed).

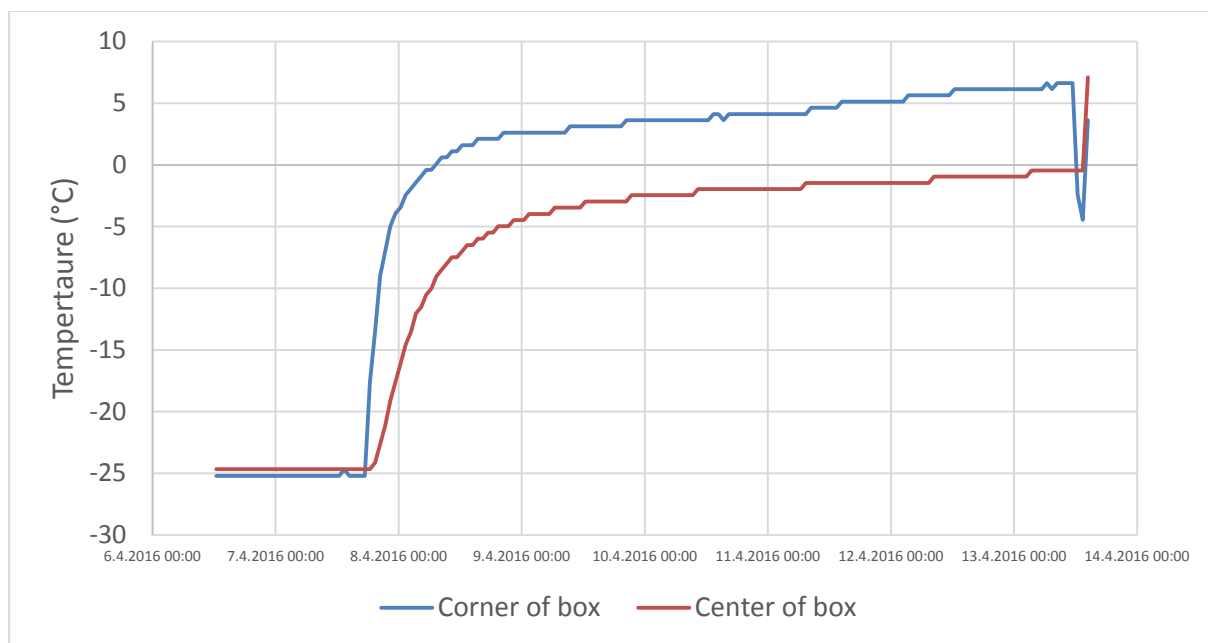
a. Cannot be computed because at least one of the variables is constant.

b. Processing = Lightly salted



## Appendix D

During storage of samples (March 2016) at -25 °C a malfunction occurred. Appendix D contains data from temperature logger stored inside the samples packaging. All samples from March 2016 experiment were affected.



**Figure 60 Results from temperature loggers. During storage of samples, March 2016 experiment, frozen storage malfunctioned. Temperature loggers were placed inside the middle of a box as well in the corner.**





## Appendix E

This appendix contains results of analysis performed in samples from experiment (March 2016) investigating effect of different storage methods on-board, after bleeding and gutting, on fillet quality of large fillets, +1000 g. That is, chemical composition, results of correlation calculation and other results not presented in chapter 4.4.

Storage method on-board		Water [%]	Fat [%]	Protein [%]	Salt [%]
With ice	7 days	82.2±0.3	0.6±0.1	17.1±0.4	0.2±0.0
	1 month	81.8±0.4	0.6±0.1	-	-
	4 months	79.3±0.4	0.8±0.1	-	-
Without ice (-1°C)	7 days	82.6±0.3	0.7±0.0	16.6±0.5	0.2±0.0
	1 month	81.8±0.6	0.5±0.1	-	-
	4 months	79.6±0.5	0.7±0.1	-	-

**Table 28 Pearson's correlation between measured variables of unsalted samples from experiment investigating effects of storage with or without ice of large, + 1000 g, fillets. Pearson Correlation value shows whether correlation is positive or negative. N value represents the amount of values used in calculations.**

		1000+ g sample storage	Duration of frozen storage	Cooking yield	WHC	PL	HI	FFA
1000+ g sample storage	Pearson Correlation	1						
	Sig. (2-tailed)							
	N	18						
Duration of frozen storage	Pearson Correlation	.000	1					
	Sig. (2-tailed)	1.000						
	N	18	18					
Cooking yield	Pearson Correlation	-.264	.266	1				
	Sig. (2-tailed)	.290	.287					
	N	18	18	18				
WHC	Pearson Correlation	-.103	-.766**	.128	1			
	Sig. (2-tailed)	.684	.000	.614				
	N	18	18	18	18			
PL	Pearson Correlation	.057	-.812**	-.056	.804**	1		
	Sig. (2-tailed)	.821	.000	.825	.000			
	N	18	18	18	18	18		
HI	Pearson Correlation	.246	-.049	-.558*	-.269	.062	1	
	Sig. (2-tailed)	.358	.856	.025	.314	.820		
	N	16	16	16	16	16	16	
FFA	Pearson Correlation	.090	.908**	.185	-.824**	-.951**	-.041	1
	Sig. (2-tailed)	.722	.000	.463	.000	.000	.881	
	N	18	18	18	18	18	16	18

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\* . Correlation is significant at the 0.05 level (2-tailed).



## Appendix F

This appendix contains results of analysis performed in samples from experiment (March 2016) investigating effect of circulation of bleeding medium on fillet quality. That is, chemical composition, results of correlation calculation and other results not presented in chapter 4.5.

**Table 29 Chemical composition of samples from experiment investigating effects of circulation of bleeding medium on quality of fillets.**

Bled in:		Water [%]	Fat [%]	Protein [%]	Salt [%]
Still bleeding medium	7 days	81.6±0.2	0.7±0.0	17.1±0.3	0.3±0.0
	1 month	82.0±0.7	0.7±0.1	-	-
	4 months	79.0±0.4	0.8±0.1	-	-
Circulating bleeding medium	7 days	81.4±0.2	0.7±0.1	17.1±0.1	0.3±0.0
	1 month	82.5±0.4	0.7±0.1	-	-
	4 months	78.3±0.2	0.8±0.1	-	-

**Table 30 Pearson's correlation between measured variables of samples from experiment investigating effects circulation of bleeding medium on fillet quality. Pearson Correlation value shows whether correlation is positive or negative. N value represents the amount of values used in calculations.**

		Circulation of bleeding medium	Duration of frozen storage	Cooking Yield	WHC	HI	PL	FFA
Circulation of bleeding medium	Pearson Correlation	1						
	Sig. (2-tailed)							
	N	18						
Duration of frozen storage	Pearson Correlation	.000	1					
	Sig. (2-tailed)	1.000						
	N	18	18					
Cooking yield	Pearson Correlation	.095	.571*	1				
	Sig. (2-tailed)	.709	.013					
	N	18	18	18				
WHC	Pearson Correlation	-.082	-.839**	-.276	1			
	Sig. (2-tailed)	.746	.000	.268				
	N	18	18	18	18			
HI	Pearson Correlation	-.112	-.170	.206	.307	1		
	Sig. (2-tailed)	.669	.515	.429	.231			
	N	17	17	17	17	17		
PL	Pearson Correlation	-.172	-.817**	-.319	.919**	.295	1	
	Sig. (2-tailed)	.494	.000	.197	.000	.250		
	N	18	18	18	18	17	18	
FFA	Pearson Correlation	.073	.871**	.374	-.924**	-.301	-.968**	1
	Sig. (2-tailed)	.773	.000	.126	.000	.240	.000	
	N	18	18	18	18	17	18	18

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\*. Correlation is significant at the 0.01 level (2-tailed).

**Table 31 Results of correlation calculations comparing results of heme iron measurements of individual parts of fillets. Samples in experiment investigating effects of circulation of bleeding media on fillet quality. Pearson Correlation value shows whether correlation is positive or negative. N value represents the amount of values used in calculations.**

		Circulation of bleeding medium	Duration of frozen storage	HI: Loin	HI: Middle part	HI: Tail	HI: Belly flap
Circulation of bleeding medium	Pearson Correlation	1					
	Sig. (2-tailed)						
	N	18					
Duration of frozen storage	Pearson Correlation	.000	1				
	Sig. (2-tailed)	1.000					
	N	18	18				
HI: Loin	Pearson Correlation	-.474	. <sup>a</sup>	1			
	Sig. (2-tailed)	.342	.000				
	N	6	6	6			
HI: Middle part	Pearson Correlation	-.112	-.170	.944 <sup>**</sup>	1		
	Sig. (2-tailed)	.669	.515	.005			
	N	17	17	6	17		
HI: Tail	Pearson Correlation	-.417	. <sup>a</sup>	.894 <sup>*</sup>	.762	1	
	Sig. (2-tailed)	.410	.000	.016	.078		
	N	6	6	6	6	6	
HI: Belly flap	Pearson Correlation	-.240	. <sup>a</sup>	.189	.410	-.243	1
	Sig. (2-tailed)	.647	.000	.720	.419	.642	
	N	6	6	6	6	6	6

a. Cannot be computed because at least one of the variables is constant.

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\* . Correlation is significant at the 0.05 level (2-tailed).



## Appendix G

This appendix contains results of analysis performed in samples from experiment (March 2016) investigating effect of circulation and rate of replacement of bleeding medium on fillet quality. That is, chemical composition, results of correlation calculation and other results not presented in chapter 4.6.

**Table 32 Chemical composition of samples from experiment investigating effects of circulation and the rate at which bleeding medium is replaced during bleeding on quality of fillets.**

<b>Bled in:</b>		Water [%]	Fat [%]	Protein [%]	Salt [%]
Port rotex	7 days	81.8±0.4	0.8±0.1	16.9±0.1	0.3±0.0
	1 month	81.4±0.4	0.8±0.1	-	-
	4 months	76.6±0.6	0.95±0.1	-	-
Middle rotex	7 days	81.9±0.6	0.7±0.0	16.7±0.5	0.2±0.0
	1 month	81.7±0.3	0.8±0.1	-	-
	4 months	79.8±0.7	0.70.1	-	-
Starboard rotex	7 days	81.3±0.3	0.7±0.1	17.4±0.1	0.3±0.1
	1 month	82.0±0.7	0.6±0.1	-	-
	4 months	80.3±0.6	0.6±0.1	-	-

**Table 33 Pearson's correlation between measured variables of samples from experiment investigating effects circulation and rate of replacement of bleeding medium during bleeding on fillet quality. Pearson Correlation value shows whether correlation is positive or negative. N value represents the amount of values used in calculations.**

		Rotex system	Duration of frozen storage	Cooking yield	WHC	FFA	HI	PL
Rotex system	Pearson Correlation	1						
	Sig. (2-tailed)							
	N	27						
Duration of frozen storage	Pearson Correlation	,000	1					
	Sig. (2-tailed)	1,000						
	N	27	27					
Cooking yield	Pearson Correlation	-,271	,018	1				
	Sig. (2-tailed)	,172	,929					
	N	27	27	27				
WHC	Pearson Correlation	-,254	-,825**	,239	1			
	Sig. (2-tailed)	,201	,000	,230				
	N	27	27	27	27			
FFA	Pearson Correlation	,128	,960**	-,070	-,850**	1		
	Sig. (2-tailed)	,525	,000	,730	,000			
	N	27	27	27	27	27		
HI	Pearson Correlation	-,327	-,271	,394	,264	-,371	1	
	Sig. (2-tailed)	,096	,171	,042	,183	,057		
	N	27	27	27	27	27	27	
PL	Pearson Correlation	-,139	-,929**	,033	,807**	-,975**	,389	1
	Sig. (2-tailed)	,488	,000	,872	,000	,000	,045	
	N	27	27	27	27	27	27	27

\*\* . Correlation is significant at the 0.01 level (2-tailed).

\* . Correlation is significant at the 0.05 level (2-tailed).

**Table 34 Results of correlation calculations comparing results of heme iron measurements of individual parts of fillets. Samples in experiment investigating effects of circulation of bleeding media rate at which it is replaced during bleeding on fillet quality. Pearson Correlation value shows whether correlation is positive or negative. N value represents the amount of values used in calculations**

		Rotex system	Duration of frozen storage	HI: Loin	HI: Middle part	HI: Tail	HI: Belly flap
Rotex system	Pearson Correlation	1					
	Sig. (2-tailed)						
	N	27					
Duration of frozen storage	Pearson Correlation	,000	1				
	Sig. (2-tailed)	1,000					
	N	27	27				
HI: Loin	Pearson Correlation	-,617 <sup>a</sup>		1			
	Sig. (2-tailed)	,077	,000				
	N	9	9	9			
HI: Middle part	Pearson Correlation	-,327	-,271	,891 <sup>**</sup>	1		
	Sig. (2-tailed)	,096	,171	,001			
	N	27	27	9	27		
HI: Tail	Pearson Correlation	-,774 <sup>a</sup>		,785 <sup>*</sup>	,599	1	
	Sig. (2-tailed)	,014	,000	,012	,088		
	N	9	9	9	9	9	
HI: Belly flap	Pearson Correlation	-,692 <sup>a</sup>		,911 <sup>**</sup>	,898 <sup>**</sup>	,871 <sup>**</sup>	1
	Sig. (2-tailed)	,039	,000	,001	,001	,002	
	N	9	9	9	9	9	9

\*. Correlation is significant at the 0.05 level (2-tailed).

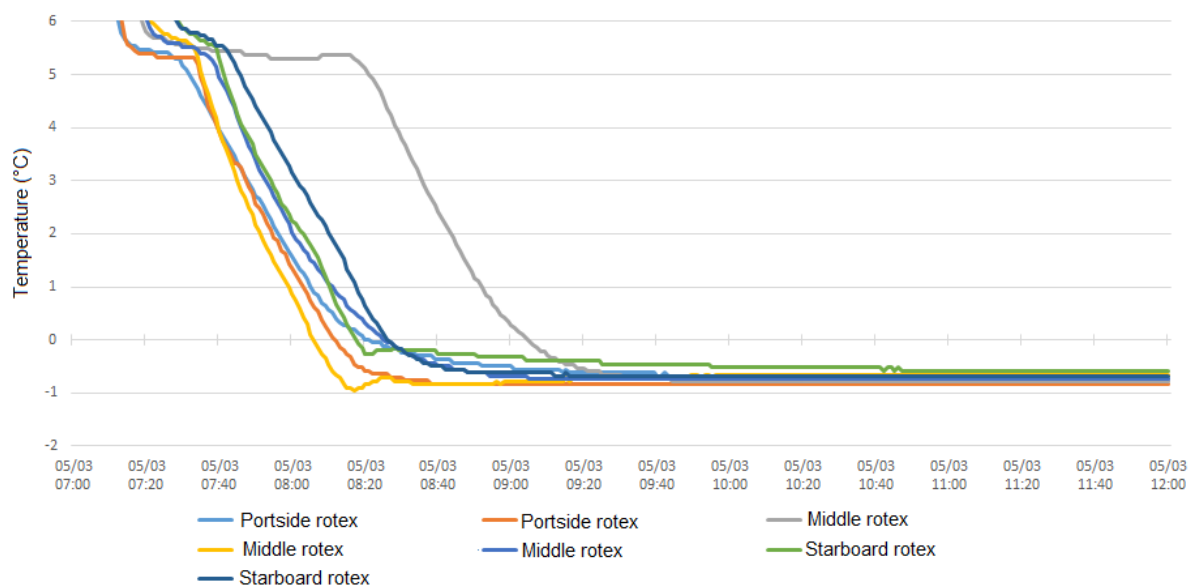
a. Cannot be computed because at least one of the variables is constant.

\*\* . Correlation is significant at the 0.01 level (2-tailed).

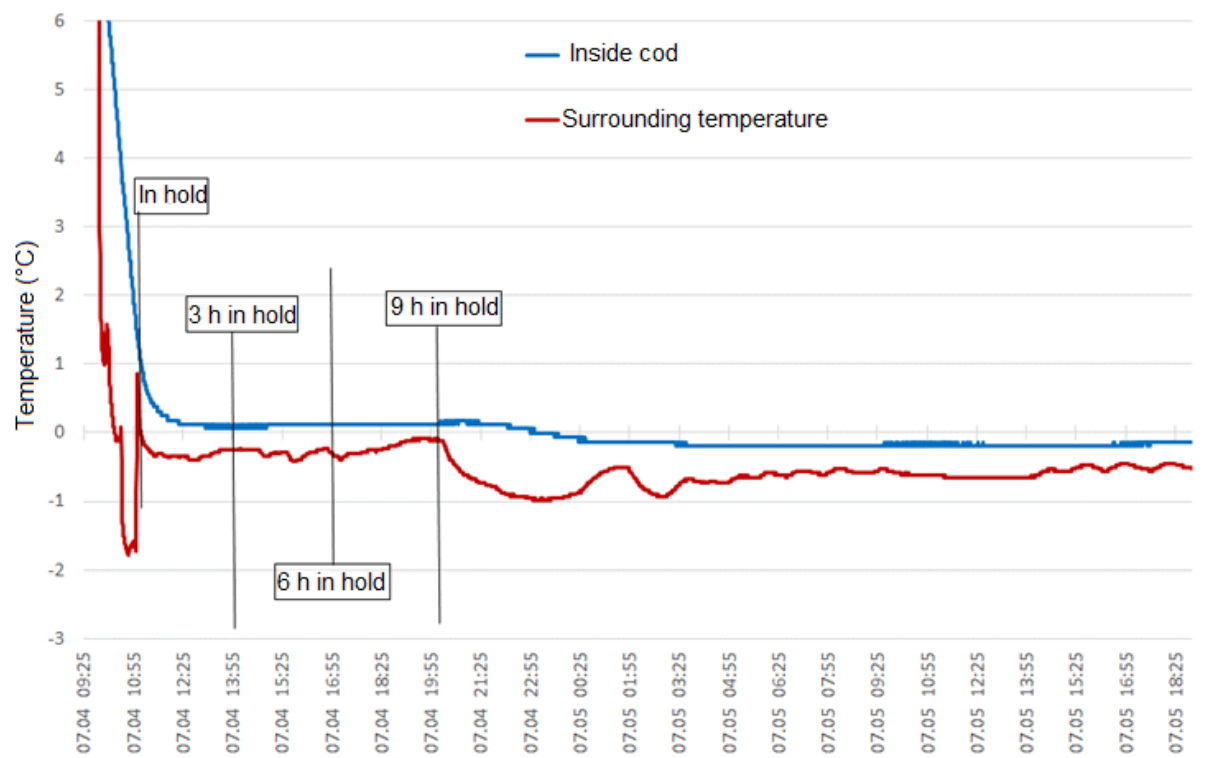


## Appendix H

This appendix contains data from temperature loggers used to check temperature in rotex system and storage on-board Málmeý.



**Figure 61 Data from temperature loggers placed inside cod (<2 kg) going through rotex systems on-board Málmeý during March 2016 sampling.**



**Figure 62** Data from temperature logger inside fish hold on-board Málmeý, both surrounding temperature and temperature inside fish during storage.