Sub chilling of fish

Authors: Gunnar Thordarson, Sigurjon Arason and Magnea Karlsdóttir

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Nordic Innovation
Stensberggata 25,
0170 Oslo, Norway
Phone: +47 47 61 44 00
info@nordicinnovation.org
www.nordicinnovation.org

Grieg Seafood Finnmark AS
Markedsgata 3 Postboks 1053
N-9510 Alta, Norway
Phone: +47 78 44 95 95
www.Griegseafood.no
Grieg@Grieg.no

Matis
Arnargata 2-4
400 Isafjörður, Iceland
Phone: +354 422 5110
matis@matis.is
www.matis.is

Skaginn3X
Sindragata 5
400 Ísafjörður, Iceland
Phone: +354 450 5000
albert@3xtechnology.is
www.3xtechnology.is

Fisk Seafood
Háeyri 1
550 Sauðárkrókur, Iceland
Phone: +354 455 4400
fisk@fisk.is
www.fisk.is

Iceprotein
Háeyri 1
550 Sauðárkrókur, Iceland
Phone: + 354 455 4471
iceprotein@iceprotein.is
www.protis.is

Hätälä OY
Lapinrauntiontie 3
90420 Oulu, Finland
Phone: + 358 44 351 7974
www.hatala.fi
hatala@hatala.fi

Norway Seafood A/S
Nkr. Kaigade 7
8500 Grenaa, Danmark
Phone: + 45 29 69 59 70
www.norwayseafoods.com
norway@norwayseafoods.com

Rannsóknarmiðstöð Íslands (Rannis)
Borgartúni 30, 150 Reykjavík, Iceland
Phone: + 354 515 5800
www.rannis.is
rannis@rannis.is

Project participants

Iceland

Matis
Gunnar Thordarson
Regional Manager
gunnar@matis.is

Skaginn3X
Albert Högnason
R&D manager
albert@3xtechnology.is

Iceprotein
Hólmfríður Sveinsdóttir
Managing Director
holfridur@iceprotein.is

Fisk Seafood
Gylfi Guðjónsson
Fleet Manager
gylfi@fisk.is

Norway

Grieg Seafood
Roger Pedersen
Production Manager
Roger.pedersen@Griegseafood.com

Denmark

Norway Seafood
Kim Jensen
Product Manager
denmark@norwayseafoods.com

Finland

Hätälä OY
Matti Isohatala
General Manager
Matti.isohatala@hatala.fi

Sub-Chilling of Fish

Contents

Abstract

Markmið verkefnisins var að nýta þekkingu á ofurkælingu á fiski sem þróuð hefur verið í rannsóknastofum undanfarna áratugi; iðnvæða hugmyndina og þróa aðferðir og búnað til að stýra kælingunni. Mikilvægt er að kæla hráefni niður undir frystimörk eða rétt niður fyrir það hitastig þar sem fyrstu ískristalar myndast í viðkomandi fisktegund, nægilega hratt til að stórir kristallar myndist ekki í vöðvum og valdi frumuskemmdum. Mikilvægt er að stýra kælingunni rétt og eins að viðhalda ofurkældu ástandi við geymslu og í flutningi, en sveiflur í hitastig geta valdið gæðarýrnun.

Verkefnið er mikilvægt þar sem lægra hitastig dregur úr og hægir á örverugróðri og ensímvirkni og eykur þ.a.l. geymsluþol á ferskum afurðum. Einnig hefur verið sýnt fram á að með ofurkælingu strax eftir dauða, hægir á og dregur úr samdrætti í dauðastirðnun sem bætir gæði afurða umtalsvert. Með ofurkælingu og góðri stjórn á kælikeðju má lengja líftíma ferskra afurða umtalsvert.

Rannsóknir hafa staðið yfir í tvö ár þar sem áhersla á ofurkælingu fyrir lax hefur verið í frumvinnslu í Alta í Noregi og áframvinnslu í Finnlandi og Danmörku. Rannsóknir á þorski hafa farið fram á Sauðárkróki á Íslandi, um borð í togskipi og vinnslustöð í landi.

Niðurstöður rannsókna sýna að íslaus flutningur og geymsla á ofurkældum fiski er raunhæf lausn sem dregur úr kostnaði við veiðar og vinnslu ásamt því að lækka kostnað við flutning og dregur verulega úr sótspori við framleiðslu á ferskum fiski. Ferskur lax hefur verið fluttur íslaus en ofurkældur um styttri og lengri veg og geymdur í viku fyrir vinnslu með framúrskarandi árangri. Í tengslum við verkefnið hefur ofurkæling verið notuð í stórum stíl á Sauðárkróki, þar sem togarinn Málmey SK 1 hefur landað yfir 15 þúsund tonnum undanfarin tvö ár af ofurkældum afla og þ.a.l. ekki notað ís um borð eða við geymslu fyrir framleiðslu í fiskvinnslu.

Ofurkæling; frystimörk; kristalmyndun; kælikeðja; geymsluþol; sótspor; íslaus flutningur

The project objective was to utilize knowledge of sub chilling of fish developed in laboratories for the past decades; and to industrialize the concept and to develop methods and means for centralising the process. The control of the chilling process is important, to chill raw material sufficiently without freeze out more than 20% of its water and without developing large ice crystals in the muscles. It is also important to keep storage temperature under control and stable and for the same reason temperature fluctuation can cause growth of ice crystals in the muscle.

The project is important as lower temperatures reduces and slows down microbial and enzymatic activity and can therefor increase the shelf-life of fresh fish products. Sub-chilling also slows down contraction during the rigor mortis process substantially with positive effects on the fillets quality like texture and gaping.

The project researches have been ongoing for more than two years, focusing on primary processing of sub chilled salmon in Finnmark (Norway) and secondary processing in Finland and Denmark. The research on sub chilled cod has been conducted in Saudarkrokur (Iceland) on board a wet fish trawler and in a fish processing plant.

Based on results obtained in present project it can be concluded that sub-chilling provides opportunities to use ice-free value chain for fresh fish, lowering cost of production, logistic and considerably the carbon foot-print for the final products. Fresh salmon without any external refrigerant (ice) has been transported for long distance, by trucks and airplanes, and stored for long time with acceptable results. The trawler used in this project has landed over 15 thousand tonnes of sub chilled fish for the last two years without using any ice for chilling and storage. The fish is stored in the fish plant and processed without using any ice preservation.

Sub chilling; freezing point; crystallization; value chain; shelf-life; carbon foot-print; ice-free logistic

1 Executive summary

1.1 *Objectives*

The main objective of the project was to develop a method of Sub chilling technology (Appendix 9.4 Sub chilling - Theoretical Discussion) for fish on industrial scale as well as to study the effect of the sub chilling process on quality, production and economic. The secondary objective was to investigate the effect of sub chilling of fresh fish on the logistic chain and the environmental effects of using the build in chilling to the product for preservation instead of external ice.

Sub-chilling has been studied for some time as a food preservation method but mostly on laboratory scale. However, rapid development occurs within the fisheries with industrial implementation from fishing to production. Part of this project was to implement the sub chilling method throughout the whole fresh fish value chain, i.e. from harvesting to market and to commercialize the sub chilling method to be implemented on a large scale within the fresh fish value chain. The problems were involved in managing the process of chilling and storage, where the crystals that can adversely affect the quality are most likely to form, and to chill the product sufficiently without freezing it.

The aim of this project was also to address the fluctuation of uncontrolled temperatures in fresh fish processing, storage and its logistics, from harvesting to the market. The optimal goal for this project was to promote the outcome to the fisheries business in Scandinavia and strengthen the competitiveness in the fresh food business in the world, and at the same time maximize the customer's value consuming fresh fish.

1.2 Implementation

The first phase of the project was dedicated to define and explore the border between sub-chilling and freezing for salmon and cod. The next phase was to explore the pros and cons of the sub-chilling method for quality and economic value purposes, to create better products for fresh seafood and maximize its value for the consumer.

The development of ice crystals during sub chilling and storage is the greatest challenge industrializing the concept, and its effect on quality of the product. The refrigeration requirements for sub chilling is crucial to prevent freezing and necessary to develop steering gear to use the method on industrialize scale. The knowledge on the sub chilling and its impact on quality and opportunities in increased production value and logistic have been promoted in many food shows, conferences, workshops and meetings with seafood business executives.

Matis led the project and was responsible for the scientific work. Iceprotein supported the study and, along with Matis, was responsible for experiments carried out on ground fish. Fisk Seafood provided facilities and material for the ground fish study, including the trawler Malmey SK 1 who was equipped with sub chilling equipment to use the technology on a large scale in early 2015, landing 15 thousand tonnes of fish (early 2017). Grieg Seafood provided facilities and material for the primary part of the salmon production study with close cooperation with its customers and project partners, the secondary processors Hatala YO and Norway Seafood. Skaginn 3X supported the project with its technical capacity as an equipment manufacture and expertise in technical solutions.

1.3 Deliverables

The project delivered facts on how the sub chilling method works in real live –commercialized. The idea has been introduced to the seafood business in Scandinavia, but much more is required to convince the industry to use sub chilling. The method's effect on; quality, product shelf life, production, economic and logistic has been research. Quality comparison between sub chilling products and traditional products have been done on large scale within this project. Studies on the logistic of salmon and trout were conducted to compare the sub chilling and the traditional method; using EPS boxes and tubs, transported by trucks, shipping containers and airfreight. Economic effects of using sub chilling compared with traditional product were also estimated. Salmon and trout were shipped/trucked to distant markets from Norway and Iceland without redundant ice for chilling with excellent result. This could be important for economic reasons as well as giving reason to lower carbon food-print in fresh fish logistic.

1.4 Future perspectives

Increased shelf life provides many opportunities in the market for fresh fish and provides opportunities for further development in the future. Sub-chilling does not just have economic benefits with a longer shelf life but also gives the opportunity to transport the fish in shipping container instead of airfreight, which is considerably cheaper and more environmentally friendly. This represents a saving in transportation costs, as well as a substantial reduction in the carbon footprint. Salmon has mainly been transported in single-use packaging (EPS boxes), but the sub chilling method allows the use of tubs instead of disposable packaging. By using the internal chilling of the fish instead of external ice for preservation, considerable rationalization of transport cost is gained. Approximately 10% of the overall weight in salmon transport is ice. Sub chilling makes ice redundant and reduces the strain on much of the transport chain, by air, road or by sea. The extended product shelf life introduces the possibility to ship larger volumes in containers to replace the amount of fresh whitefish exported from Iceland by air. Production of farmed salmon in Norway, the Faroe Islands and Iceland has in recent years exceeded the million tonne mark, while the domestic market for these products is estimated to be only around 36,000 tonnes. A million tonnes of salmon are shipped to other markets every year. In rough terms, it can be estimated that in the region of 200,000 tonnes of ice are shipped with this salmon. Approximately 240,000 tonnes of salmon every year are freighted by air to Asia, which means that more than 10% of it is ice, around 24,000 tonnes of total weight; equivalent to 500 Jumbo jet flights weight.

The sub chilling logistic chain will improve chilling technology and increase efficiency of the distribution chain of fresh fish around the world. It will not only improve quality of product for fresh food but also the safety of product. The traditional cold chain of fresh food from harvester to consumer have many week links with insecurity of delivering the right value for the customer, many mistakes, poor temperature control, causing reduced product quality and shortening product shelf life. The reliance of the cold chain has improved for last decade, especially by the temperature controlled shipping containers.

Introducing the idea of the sub chilling to the market, to fish-processors, with the right information and knowledge will be one of the main future target of this project! To improve supply of quality marine products. It will cost a lot of effort to change customary way of processing fish and convince the industry to change to sub chilling method. The sufficient knowledge and presentations about the sub chilling will be the icebreaker on that journey.

2 Experiments and evaluation

In theoretical terms, sub-chilling means freezing 5-30% of the total water content of the fish. Considerable amount of research has been conducted on the subject and the outcome is that super-chilling, with the right treatment, can improve the quality of the product considerably. When utilizing super-chilling, it is important to cool the product fast to reduce the risk of large ice crystals forming within the fish, which can cause damage in the cellular structure of the flesh. Smaller crystals cause less damage to the cellular structure of the flesh, whilst larger crystals can cause more damage. Crystals grow bigger when the chilling process is slower, and furthermore, instability during storage can also cause crystals to grow. Larger crystals damage the walls of the muscle cells and the product loses some of its natural juice, which makes the texture of the fish chewy and dry – the taste deteriorates and yield is reduced due to this water loss.

There is much to gain when utilizing sub-chilling as it reduces the growth of microorganisms and activity of enzymes, which in turn prolongs the lifetime of the product. Super-chilling reduces loss of water in the product during storage, increases fillet quality and results from this research on sub-chilling showed that there was a great deal to gain. Using the fish itself as a refrigerant in the sub-chilling process can improve the cold chain production of fresh fish considerably and therefore increase the quality of the fish on fresh fish markets. The main problem that arose was how to manage the production on an industrial scale, which proved to be more complicated than in the controlled environment of the laboratory.

Figure 1. Temperature in fillet from sub chilled salmon.

During our research the aim was to freeze only 10-20% of the water content of the fish, to reduce the risk of freeze damage due to ice crystals.

Many experiments were executed within the project to compare sub chilled products with traditional products, considering many factors, including growth of microorganism, physicochemical properties, sensory/fillet evaluation and on production matters.

2.1 Bleeding process

Samples were taken of sub chilled and traditional cod on-board a trawler (#6) to test different bleeding process at different temperature (Sveinsdóttir, 2016). Bleeding cod in three different temperatures were tested; -1 °C, +2 °C and +6 °C.

The results from analysis of free fatty acids (FFA) suggested that bleeding of cod at sub-zero temperature could be beneficial. However, images taken of the samples during processing as well as results of colour analysis indicate that it could affect the colour of the fillets, mainly the belly flap of the fillets but not the loin part (Figure 2). As soon as the fillets had been injected and brined, the difference between the groups was minimal and all spoilage was slowed down. However, there were indicators that the lower temperature, mainly -1 °C, may not be beneficial with regard to overall quality. Further experiments would have to be performed in order to determine the viability of this method with more extensive chemical analysis and sensory evaluation. The effect of different temperatures of bleeding medium on fillet quality were negligible for fresh fillets. Less variability in FFA content was observed in muscle of cod bled at -1 °C. 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 recommended to bleed white fish, such as cod, at sub-zero temperatures without performing further experiments. Chilling could be suitable when temperature at summertime in Iceland is high, 10 – 12 °C, to use the bleeding time for chilling. Chilling the fish to around 5-6 °C in the chilling process on-board the trawler seems to be more suitable.

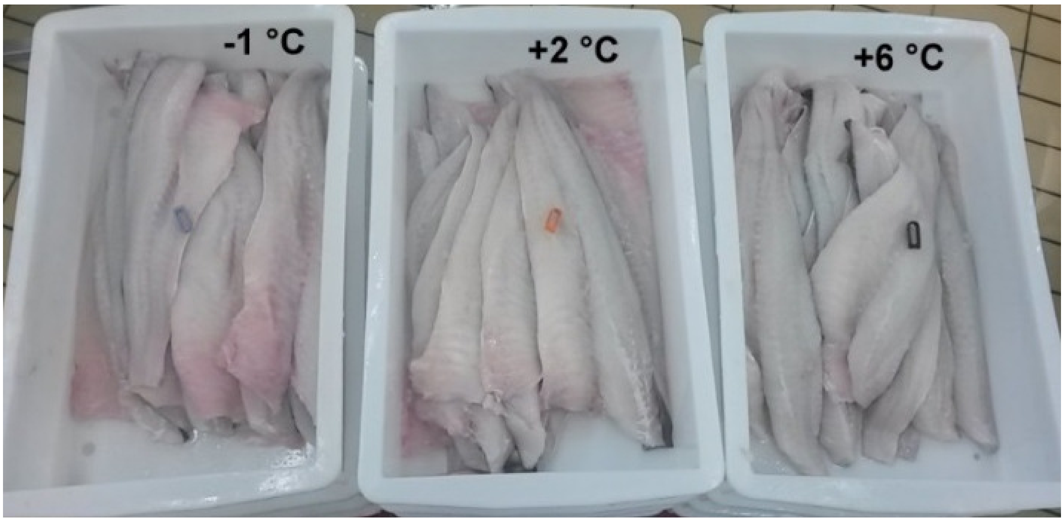


Figure 2. Samples from Malmey, four days after catching, bled at three different temperature; from left, -1 °C, +2 °C and right +6 °C (Photo by S. Eliasson).

2.2 Rigor mortis

The effect of sub chilling on rigor mortis was studied and compared with traditional chilling with ice (Thordarson, Hognason, & Gudjonsson, 2016). Promotional material was prepared to enlighten the fishery industry on the importance of managing the process of rigor mortis for product quality. A study was conducted on cod and salmon, including seasonality effect on rigor mortis for cod.

The results indicate a large difference in the contraction process depending on whether the fish is sub-chilled or if traditional cooling is used. Substantial difference was observed between the two groups for both species with more intense contraction by the rigor process on traditional fillets compared with sub chilled fillets. The process was filmed for six days to cover the rigor from start to finish (pre-rigor – rigor stage – post rigor). Cod and salmon of both types were filleted pre-rigor on one side but the other one was left on the vertebrae, through the rigor process. The second fillet was then cut off post-rigor and both fillets photographed to see the difference. Good results were achieved with filming and photographing; the result for the film were stored on YouTube (Thordarson, Hognason, & Gudjonsson, YouTube, 2016) (Thordarson, Hognason, & Gudjonsson, YouTube, 2016). Figure 3 shows the contraction on a fillet cut of before rigor and photographed on top of the fish after the end of the process.

Figure 3. Fillet cut off before rigor process and laid on the original cod after rigor mortis contraction

The conclusion of the study indicates that sub-chilling, which reduces the contraction and consequently the tension between muscle and backbone in the process, and can therefore have a considerate effect on fillet quality with less gaping and a firmer product (Figures 4 and 5).

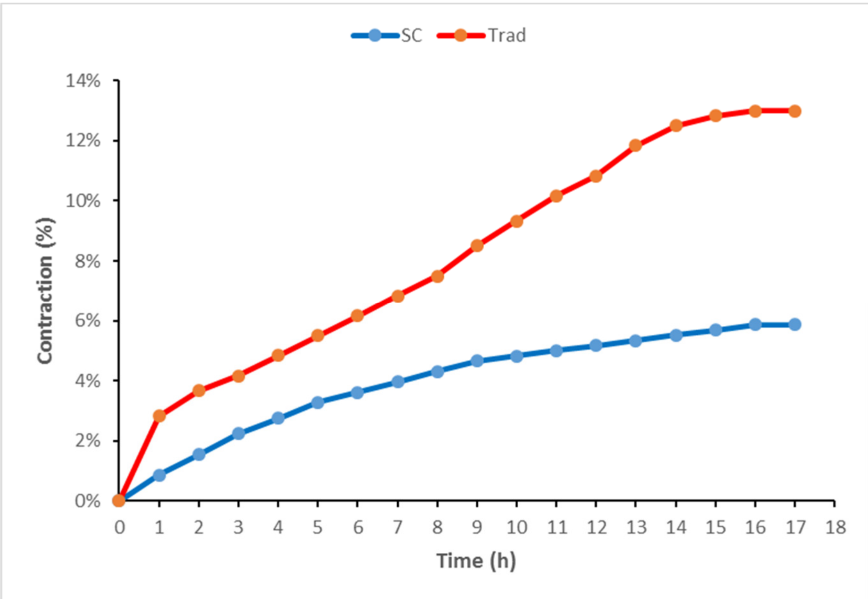


Figure 4. Timing of the rigor process (pre-rigor to rigor stage) of sub chilled (SC) and traditional chilled (Trad) salmon (n=1).

In pre-rigor state the ice crystals form within the cells regardless of sub chilling rate. In this stage, a large amount of water is on the inside and outside of the muscle cells. In a low sub chilling rate, there seem to be larger ice crystals than in a high sub chilling rate, which is extremely important factor for reducing damage caused by ice crystals to the muscle cells. Therefore, the chilling of the fish must be carried out as quickly as possible to limit the size of the crystals being formed.

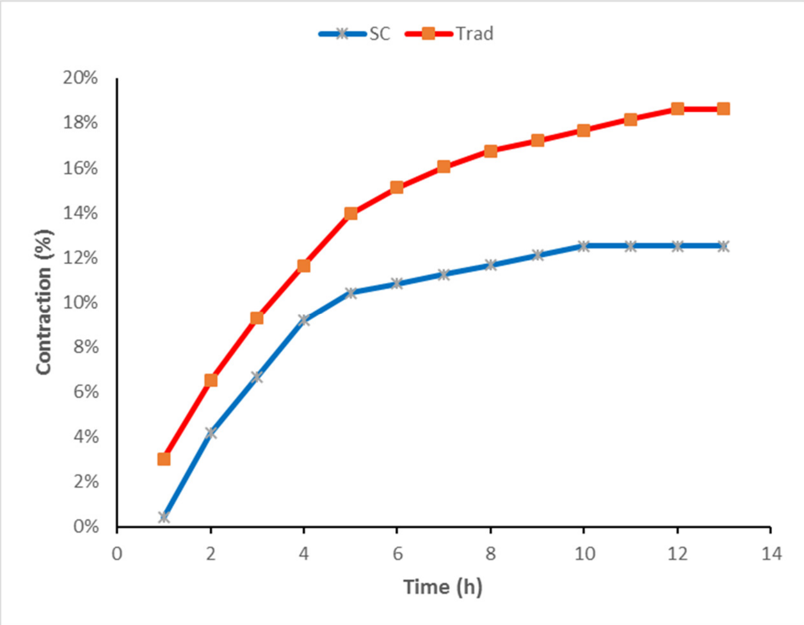


Figure 5. **Timing of rigor process (pre-rigor to rigor stage) of sub chilled (SC) and traditional chilled (Trad) cod (n = 1).**

2.3 Salmon

Experiments which were executed within the project were from different locations, heading to different destination, using different transport mode and different packaging (Table 1). In most cases, a comparison was made between sub chilled salmon and traditional processed salmon, but in some cases, only one group was available. Temperature within the product as well as the ambient temperature were logged to understand the logistic chain better and to evaluate the effect of ambient temperature on the product for both groups.

Table 1. List of chilling/logistic experiments within the project.

Exp. #	Primary producer location	2nd Producer location	Transport mode	Time/ hours	Date	Sub Chilled (kg)	Traditional (kg)
1	Finnmark Norway	Finland	Truck	9	January 2015	500	15,000
2					March 2015	500	15,000
3					May 2015	500	15,000
4					July 2015	7,000	7,000
5					Nov 2016	4,000	7,000
6	Finn mark Norway	Japan	Truck/ Airfreight	96	Feb 2015	80	80
7	Finnmark Norway	Italy	Truck	36	April 2015		15,000
8	Finnmark Norway	Iceland	Airfreight; Alta-Oslo-Keflavik	24	June 2015	40	40
9	Finnmark Norway	Denmark	Truck	48	April 2015	500	20,000
10					October 2015	500	15,000

11					Nov 2015	4,000	10,000
12	Faroe Island	Reykjavik	Airfreight	24	June 2015	30	30
13	Bildudalur	San Francisco USA	Truck/ Airfreight	36	Nov 2016	40	500
14	Finnmark Norway	Japan	Simulation	150	February 2016	20	
15	Finnmark Norway	Japan	Truck to Oslo/ Copenhagen Airfreight Tokyo	100	Nov 2016	240	
16	Finnmark Norway	Dubai	Truck/ Airfreight	100	Des 2016	80	
17	Bildudalur	Reykjavik	Truck	10	Oct 2015	40	40
18	Isafjordur	Slupsk Polland	Sea container/ truck	264	March 2017	700	700
19	Isafjordur	Slupsk Polland	Sea container/ truck	216	March 2017	700	700

2.3.1 Chilling process

Salmon was sub chilled at Grieg Seafood in Alta and sent to secondary processors in Finland and Denmark to analyse the product quality and compare with traditional products. Salmon was also sub chilled to be sent to customers in Italy, Dubai, Tokyo, San Francisco and Iceland for quality inspection and comparison with traditional products. Two large tests were sent to project partners, secondary processors, Hätälä Finland and Norway Seafood in Denmark. These samples contained almost eight tonnes of sub chilled fish in a large screw conveyer cooler as well as traditionally chilled fish for comparison. Multiple samples were sent by truck or aeroplane to different destinations, both with sub chilled salmon and traditionally handled product, monitoring product and ambient temperature.

Traditional and sub chilled salmon was trucked in 660 litres tubs, each with 300 kg, from Grieg Seafood in Alta to target destinations in Finland and Denmark. Sub chilled salmon was transported without ice but the traditional one contained 50 kg of ice in each tub.

The chilling process for 6 kg salmon, measured at different parts and depths in the flesh; in middle of the loin, 15 millimetres deep and also in the tail, is shown presented on Figure 6. The red line on the figure demonstrates the temperature of the brine used for the chilling, starting at -1 °C and decreased to -3 °C for around 80 minutes.

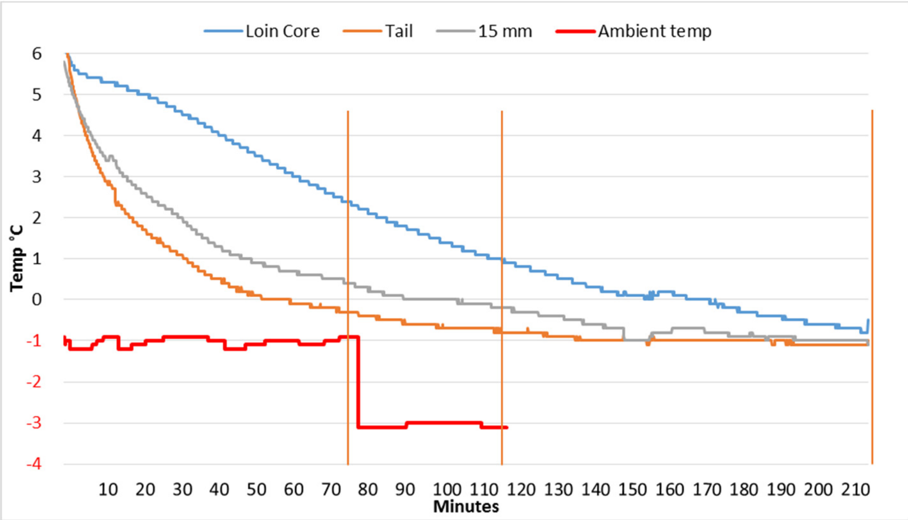


Figure 6. Chilling process for sub chilled salmon (n=1); core, tail and 15 mm depth as well as ambient (brine) temperature. The zone between the vertical lines represents the critical point of chilling; first the lowering the brine temperature from -1 to -3 °C, the second one the end of sub chilling and the third one the point when temperature throughout the salmon reach equilibrium

It is important to realise that fish as a constantly changing material throughout its lifespan, and each species has its own seasonal variation. This changes the relative fat, protein and water content of the fish, impacting the thermal constants affecting chilling. These variations between species can also be regionally determined as well as the feed of the fish has a large impact on the composition. Directly impacting the cooling process is the sea temperature as well as contributing to small variations in consistency of the muscles.

Currently, Matis's research specialist Sigurdur Orn and master´s student is working on building forecast models for the cooling processes of cod and salmon cooperation with this project. These models will be able to predict the temperature fluctuations in the fish based on the relative fat, protein and water content of the muscle and therefore generate a useful framework for small coastal vessels as well as large ice fish trawlers. Furthermore, the results will be used to re-iterate an ice calculation tool currently used by many fishermen operating and aquaculture companies.

2.3.2 Microbiological analysis

Many tests were made on bacteria growth in salmon fillets and sub chilled products compared with traditional products. Tests were made of sub chilled salmon after slaughtering but stored at traditional storage temperature after processing (4-5 days) to compare the effect of using sub chilling only at the primary part of the value chain. These tests were made in October 2015 and can be seen in Fig. 2 and 3 are used from that test. Other results from microbial counts are in appendix 9.6 and 97.

Results from experiment #11 performed on sub chilled salmon fillets and compared with traditional products can be seen in Figure 7. The sub chilled salmon was kept at -1 °C but the traditional one in 1 °C cold storage. After 18 days of storage, the traditional fillets were spoiled reaching a count of 7.6 log cfu/g while the sub chilled fillets contained 6.2 log cfu/g at the same time and reached 7.4 at day 25. The SC + trad experimental group presented on Figure 7 was a product sub chilled after slaughtering and kept at -1 °C until processing four days later, and kept after that in 1 °C storage. The sub chilled salmon kept at 1 °C after processing had 7.4 log cfu/g on day 18 and was already spoiled at that time.

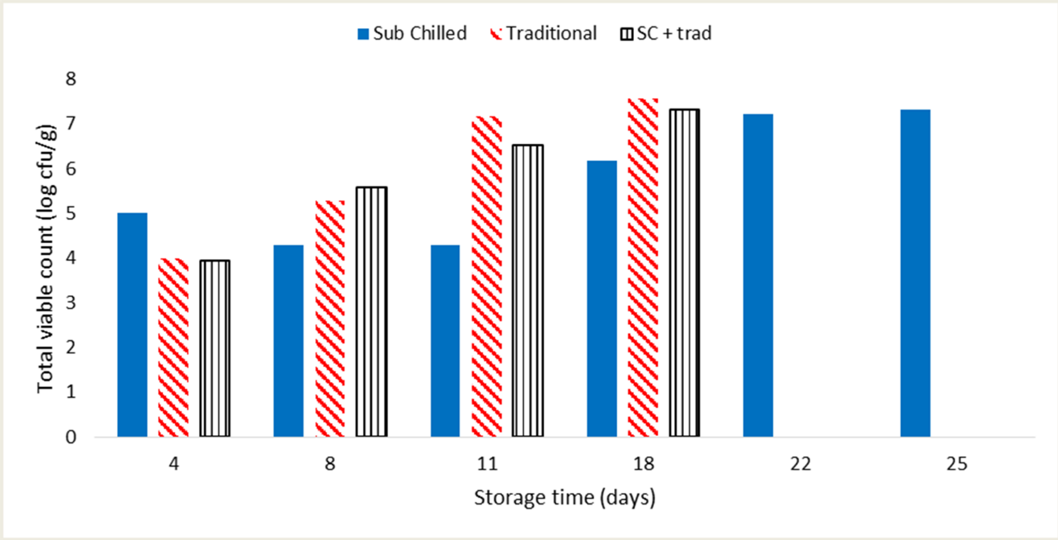


Figure 7. Total viable count (log cfu/g) of sub-chilled and traditional chilled salmon fillets from experiment #11, stored for up to 25 days from slaughtering (n = 1). Sub-chilled fillets were kept at around -1 °C the whole time, while traditional fillets were chilled with ice and kept at 1 °C the whole time. The SC + trad fillets were sub chilled after slaughtering but stored at 1 °C.

The H_2S producing bacteria count showed similar result as the total viable count. On day 25, the sub chilled product had 6.4 log cfu/g, the traditional product 8.0 log cfu/g and the SC + Trad product 7.8 log cfu/g (Figure 8).

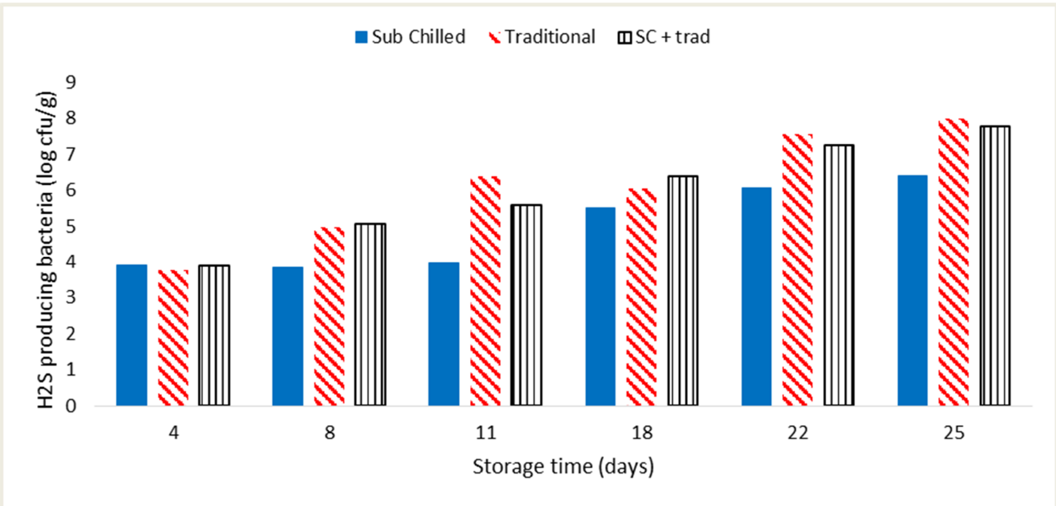


Figure 8. Experiment # 11, H_2S producing bacteria (log cfu/g) present in salmon fillets from experiment #11, stored for 25 days from slaughtering (n = 1). Sub chilled fillets were kept at -1 °C the whole time, traditional fillets at 1 °C the whole time but SC + Trad fillets were sub chilled before processing and stored at 1 °C during the storage period.

2.3.3 Physicochemical properties

2.3.3.1 Chemical composition

There was a significant difference in fat and water content in different parts of salmon fillets (Figure 9) which can then affect cooking yield and water holding capacity among other things. Water and fat content in fillets are important factors regarding optimization of the chilling process, and can vary between different part of the salmon fillets (loin, middle part and tail). The chilling process should be optimized with regard to the part with the highest water content since it has lower freezing point than part with lower water content.

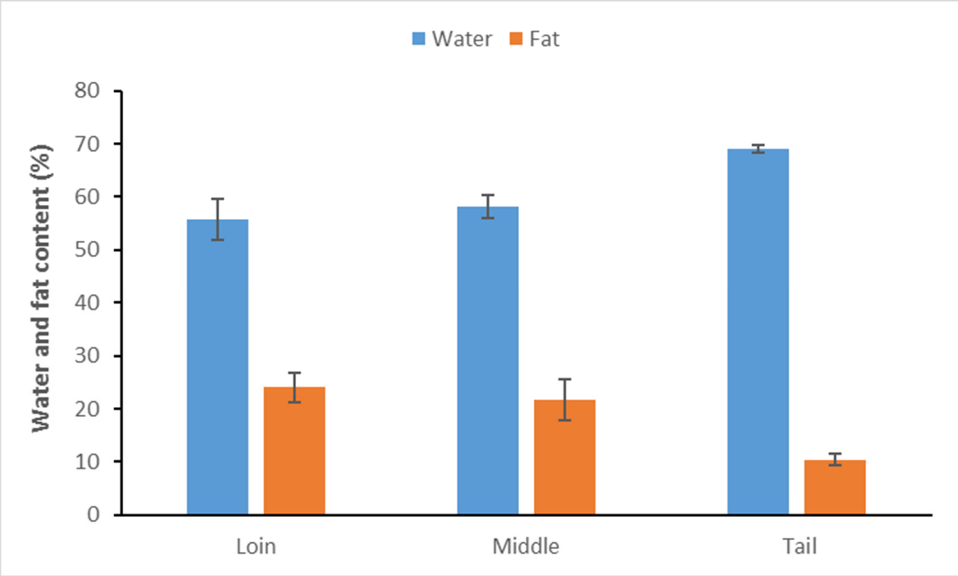


Figure 9. **Fat and water content (%) in different part of salmon fillet; loin, middle and tail (n=2; Mean±SD).**

Without adequate care, the tail could already be frozen while the core temperature in the loins has not yet reached the target temperature. Due to these circumstances, obtaining the same temperature in the tail as in the loins of a whole salmon while sub-chilling is almost impossible. However, with the right time spent and with the correct temperature in the cooling medium, this process can be sufficient and without the raw material sustaining any notable damage.

Given the particular combination of a salmon muscle, the respective amount of water, fat and free fatty acid content (FFA) varies distinctly within the fish. The loins contained around 50-60% water but then it rises constantly throughout the fish until it reaches roughly 70% in the tail. The same can be said for the FFA content but the fat bound between muscle layers decreases from loins to tail, inversely to the water and FFA. Due to these variations, it can be difficult to properly chill the salmon. On average, the initial freezing point is at around -1.5 °C like mentioned in appendix 9.5, but given the lack of water in the loins as well as their thickness compared to the tail, sub-chilling demands that special care must be taken when chilling whole salmon (Figure 10).

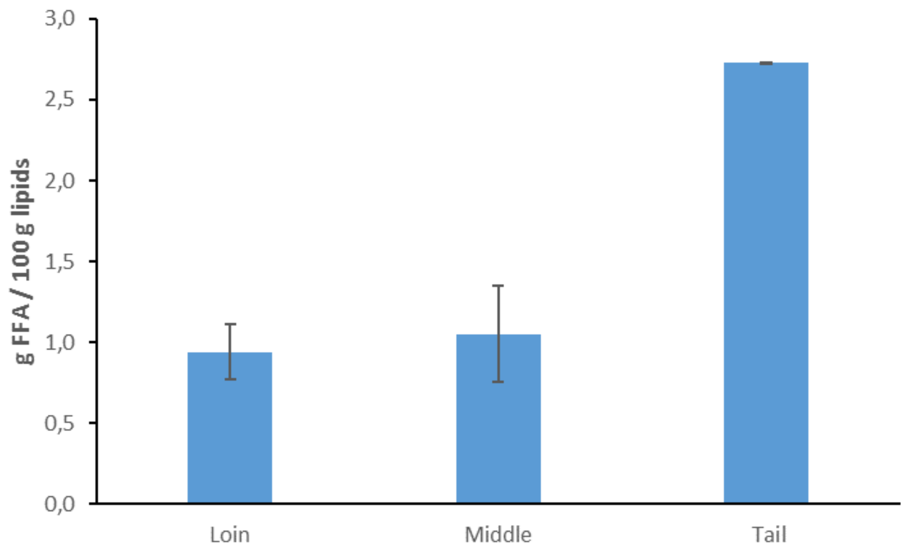


Figure 10. Free fatty acid content (g FFA/100 g lipids) in different part of salmon fillet; loin, middle and tail (n=2; Mean±SD).

In this project, several studies were conducted on the salinity in sub chilled products and compared with traditional salmon. Natural salinity in a salmon is around 0.1% and that will be doubled to 0.2% by using brine for sub chilling a whole fish. The highest measurement in sub chilled salmon was around 0.25%, which is far below any standard of salinity for fresh fish.

2.3.3.2 Water holding capacity (WHC)

Much of the muscle water is entrapped in cell structure and therefore any changes in the cell can influence the ability of the muscle to retain its natural water, especially through the process of rigor mortis and can therefore cause some drip-loss. Measuring the water holding capacity is a key factor to measure cellular damage from crystallisation build up and causing drip-loss.

Tests of WHC were conducted to explore the effects of sub chilling on the muscle quality. In general, no differences between sub chilled products and traditional products were observed suggesting that minimum ice crystal formation during the sub chilling process and subsequent storage.

2.3.3.3 Cooking yield

Cooking yield is an important quality parameter, not only because of business integrity as this is the final product weight for the consumer, but holding the natural juice within the fish is also important for texture and the final nutritional value of the food. Measuring the cooking yield and comparing sub chilled product with traditional one is important to estimate any damage afflicted by crystal structure within the muscle cell. Measuring this during storage time is also important where the sub-chilling process could be successful but with fluctuation during storage the build-up of large crystal can be accelerated. The outcome of several experiments showed no statistical difference between the experimental groups.

2.3.4 Sensory/fillet evaluation

Eight tonnes of 4-5 kg salmon were sub chilled at Simanes and trucked to Hätälä in a refrigerated truck along with a large order of traditional salmon from the same lot. The salmon from both groups was packed in 35 litres EPS boxes and 660 litres Saeplast tubs. The sub-chilled salmon was without ice but the traditional processed salmon was iced normally; 5 kg in each of the EPS boxes.

Figure 11. Salmon fillets from the same lot of production after four days of storage; sub chilled on right and traditional on left

On day three, four and five from slaughtering, 30 fillets samples were taken of each group from the production line at a secondary production facility, resulting in total of 90 fillets from each group, for quality inspection. An expert in salmon quality[1] tested the fillets using the FHF (appendix 9.1.3) method to compare quality differences between the two groups, sub chilled and traditional. The whole process was filmed for later examination. The same person followed samples sent by airfreight to Tokyo and took 36 samples of sub chilled salmon for the same test, seven days post-slaughtering. Figure 12 summarize the outcome of all quality samples taken in this study.

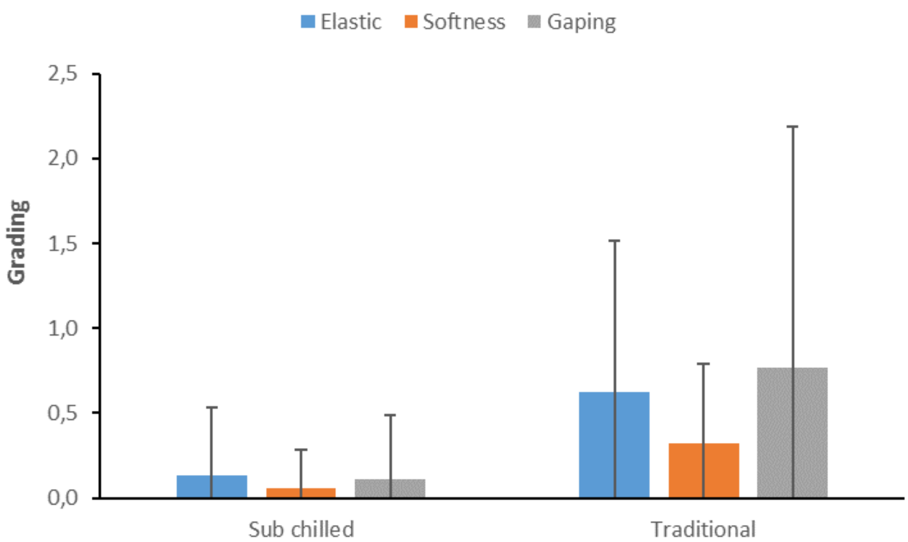


Figure 12. Quality inspection of sub chilled and traditional chilled salmon fillets collected in experiment #3 and #15, using the Norwegian quality standard FHF (appendix 9.1.3). The columns are average scores of 90 fillets from the same slaughtering that were tested, from both experimental groups, at different time and location

[1] Quality Manager of Grieg Seafood in Alta

comparing salmon. Lower numbers represent higher quality. The scale for elastic and softness is 0-2 and 0-5 for gaping, lower number better quality

2.3.5 Production

Sub chilled salmon is stiffer than traditional raw material (Figure 12) and it must be considered when setting of production machines. Therefore, some yield comparisons were made between the groups, sub chilled and traditional, after heading and after filleting and trimming. The difference between the two experimental groups were not notable but the sub chilled products tended to have around 1 – 2% better yield compared to the traditional products. Firmer raw material can give advantage compared to soft tissue, especially in a muscle with a high fat content. Sub chilling before processing can thereby give more effective cutting and uniform filleting and giving 1-2% better yield and hence reduce labour considerably.

2.4 *Cod*

Most of the studies dealing with cod were made on board Fisk Seafood´s wet fish trawler, Malmey SK 1, and in the company's processing plant in Saudarkrokur. For all samples, comparisons were performed between sub chilled fish from Malmey and traditional fish prepared on-board the same trawler.

Table 2. List of experiments with cod carried out within the project.

Exp #	Primary producer	2nd Producer	Transport mode	Distance (km)/	Time/ hours	Date	Sub Chilled (kg)	Traditional (kg)
1	Saudarkrokur	Reykjavik	Truck	400	5	Dec 2015	50	50
2	Saudarkrokur	Reykjavik	Truck	400	5	March 2016	50	50
3	Trawler	Saudar-krokur	Fork lift	50	1	April 2016	600	600
4	Trawler	Saudar-krokur	Fork lift	50	1	Oct 2016	600	600
5	Trawler	Saudar-krokur	Fork lift	50	1	Nov 2016	600	600
6	Saudarkrokur	Reykjavik	Trucking	400	5	March 2016	200	200

The temperature logging on-board the trawler is presented on Figure 13 to summarize the chilling process of three different sizes of cod, including the ambient temperature (the brine).

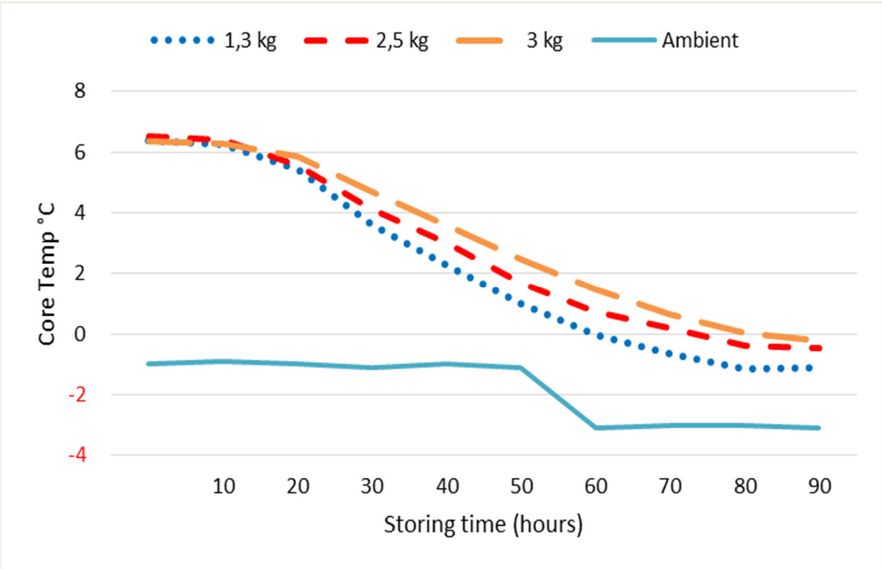


Figure 13. Temperature profiles of three different size of cod during the sub chilling process on-board Malmey SK 1 including the ambient temperature (n=1).

Fish for both groups were collected from the same catch of similar size; sub chilled in Rotex tanks and traditionally by ice. Both groups were stored in 660 litres tubs. The two samples were considered identical except for the chilling method.

Quality evaluation was made at the fish plant in Saudarkrokur and/or at Matis lab in Reykjavik. Microbiological and chemical analysis were performed at Matis, Reykjavik. Figure 14 shows the temperature profile from landing, throughout storing in chilled room, during processing and pre-cooling after packing in EPS boxes.

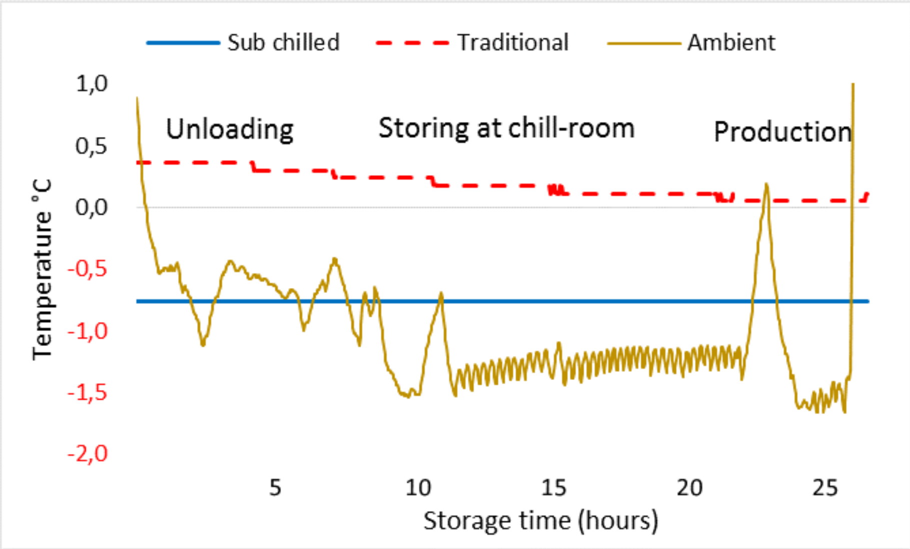


Figure 14. Experiment 3, temperature profile of sub chilled and traditional chilled cod from landing from trawler until processing in fishing plant. Ambient temperature is also included (n=1).

Two studies were conducted on cod, processed by the two methods, sub chilled and traditional. Several measurements were conducted including microbiological and physicochemical properties, sensory and fillet evaluation and yield. The analysis were performed on four sub groups (except for the yield) as follow:

1. Sub chilled after catching and through processing and storing at -0.8 °C.
2. Sub chilled after catching but stored at 0 °C after processing and during storage (SC + Trad).
3. Traditionally chilled with ice after catching but sub chilled after processing and during storage
4. at -0.8 °C (Trad + SC).
5. Traditional chilled with ice after catching and stored at 0 °C after processing and during storage.

2.4.1 The chilling process

On-board Malmey, the sub chilling has been carried out for almost two years in three large screw conveyers, as seen on Figure 15. Each conveyor has three sections. The first one (red) is for the bleeding, the next one handles pre-chilling (light blue) and at the end is the sub-chilling conveyor (dark blue). In each conveyor, it is possible to control speed and temperature during the process for different sizes and species of fish. The aim is to chill the fish until it reaches -0.5 °C core temperature and equilibrium at close to -0.7 to -1 °C. The fresh fish trawler has landed around 20 thousand tonnes of sub chilled catch for the past two years.

The screw conveyors are fully automated to sub chill the catch with accuracy. Larger fish needs longer time in the brine, automatically blended by seawater and salt, than smaller so different speed is used in each screw conveyer to handle different sizes. Fish can also have different seasonal proximate content and loins have more fat than tail and less water content. There is also a difference between species and all this can affect the chilling process.

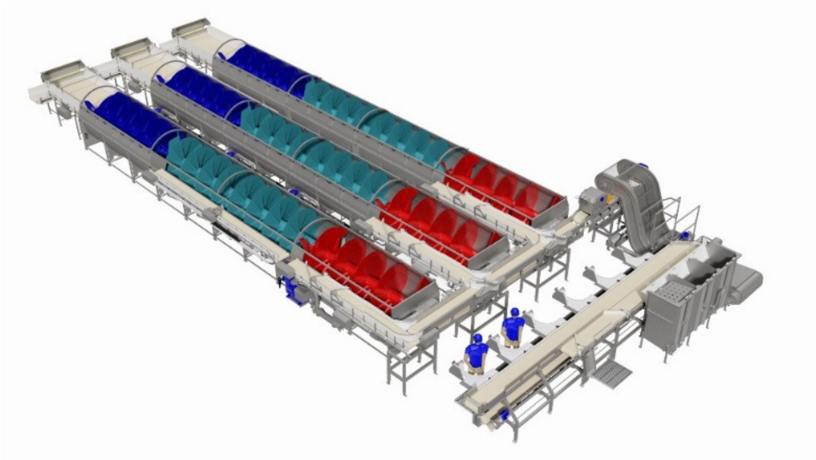


Figure 15. Screw conveyor from Skaginn3x used for sub chilling on-board trawlers. Red is bleeding, light blue chilling and dark blue sub chilling sections.

2.4.2 Microbiological analysis

The total viable count throughout the 16 days of storage is presented on Figure 16. The results indicated that sub chilling before processing, three days from catching, has minimum effect on the bacterial load. This process is during the period of rigor mortis and the degradation of the fish has therefore not started. There was no significant difference between the sub chilled group and the Trad + SC group as well as between the SC + Trad and the traditional group. It could therefore be concluded that sub chilling during storage after processing was more important to avoid bacterial growth than sub chilling directly after catching before processing.

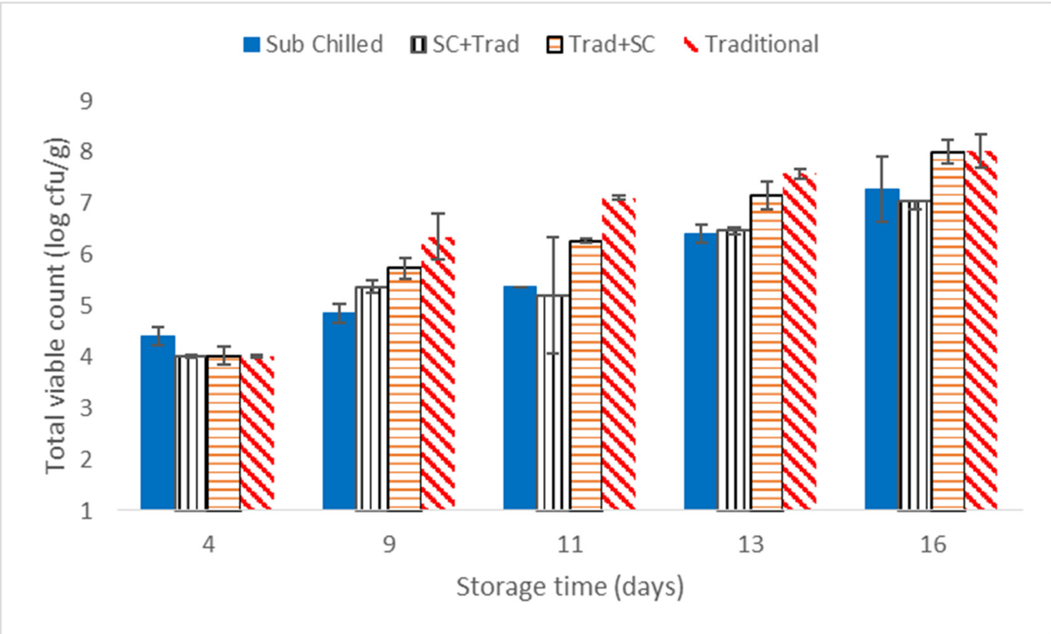


Figure 16. Total viable count (log cfu/g) during 16 days of storage of four different experimental groups within experiment #2: Sub chilled after catching and throughout processing and storing; sub chilled after catching and stored at 0 °C (SC + Trad); traditional chilled with ice after catching and sub-chilled during storage (Trad + SC); and traditional chilled after catching and throughout processing and storing. (n=2; Mean±SD).

There was a correlation between better chilling and H_2S producing bacteria growth, where sub chilled storage tended to have lower bacterial growth, but the difference between the experimental groups was however not significant after 16 days of storage (Figure 17).

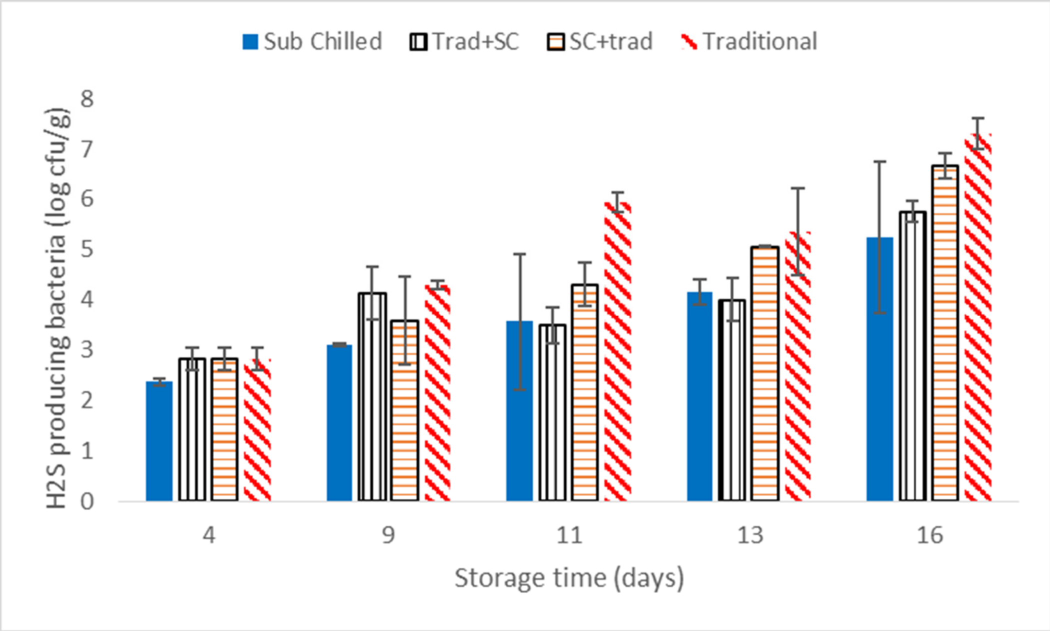


Figure 17. H_2S producing bacteria (log cfu/g) during 16 days of storage of four different experimental groups within experiment #2: Sub chilled after catching and throughout processing and storing; sub chilled after catching and stored at 0 °C (SC + Trad); traditional chilled with ice after catching and sub-chilled during storage (Trad + SC); and traditional chilled after catching and throughout processing and storing. (n=2; Mean±SD).

2.4.3 Physicochemical properties

2.4.3.1 Water and salt content

The water content was measured in sub chilled and traditional cod throughout the storage time. The water content of the initial raw material was around 82%. No changes were observed throughout the storage period as well as between the experimental groups. More detailed results can be viewed in Appendix II.

The salinity of the sub chilled cod muscle was measured and compared with the traditionally one. The salinity in both groups was 0.2% and no notable difference during storage time or between groups was detected. More detailed results can be viewed in Appendix II.

2.4.3.2 Total volatile basic nitrogen (TVB-N)

Substantial differences in total volatile basic nitrogen (TVB-N) content were observed between the experimental groups after 13 and 16 days of storage (Figure 18). The sub chilled product with traditional storage were equal to the traditional group, but the sub chilled group and the traditional group with sub chilled storage (Trad + SC) showed similar and significantly lower values compared to the other two experimental groups.

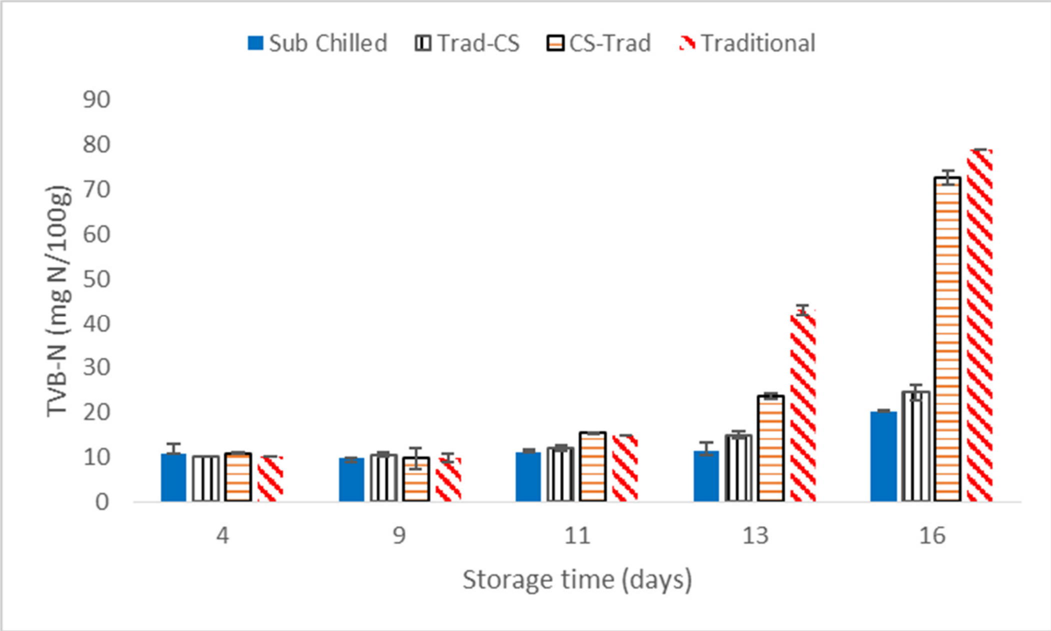


Figure 18. TVB-N content (mg N/100 g muscle) during 16 days of storage of four different experimental groups within experiment #2: Sub chilled after catching and throughout processing and storing; sub chilled after catching and stored at 0 °C (SC + Trad); traditional chilled with ice after catching and sub-chilled during storage (Trad + SC); and traditional chilled after catching and throughout processing and storing. (n=2; Mean±SD).

The TVB-N content is a useful quality index of fresh fish and are one of the most widely used chemical indicators of marine fish spoilage (Zhong-Yi *et al*, 2010). TVB-N includes the measurement of trimethylamine which is produced by spoilage bacteria, ammonia which is formed by bacterial deamination of proteins and other volatile basic nitrogenous compounds associated with seafood spoilage. The concentration of TVB-N in freshly caught fish is typically reported to vary between 5 and 20 mg N/100 g (Muhammet & Sevim, 2007). Gulsun *et al* (2009) proposed that the quality classification of fish and fish products regarding TVB-N values would be accordingly: ‘‘high quality” up to 25 mg N/100 g, ‘‘good quality” up to 30 mg N/100 g, ‘‘limit of acceptability” up to 35 mg N/100 g, and ‘‘spoilt” above 35 mg N/100 g (Amegovu *et al*, 2012; EU, 2008; Gulsun *et al*, 2009; Huss, 1995).

2.4.3.3 Water Holding Capacity (WHC)

There were no notable differences in WHC between groups nor during the storage time (Figure 19). This was essential due to the fact that one of the main threats by using sub chilling instead of traditional chilling is the build-up of ice crystals within the muscle, which can lead to increased drip loss and impacting the product quality. Not seeing any notable difference between traditionally chilled products and sub chilled products with respect to WHC therefore strengthens the idea of sub chilling the products.

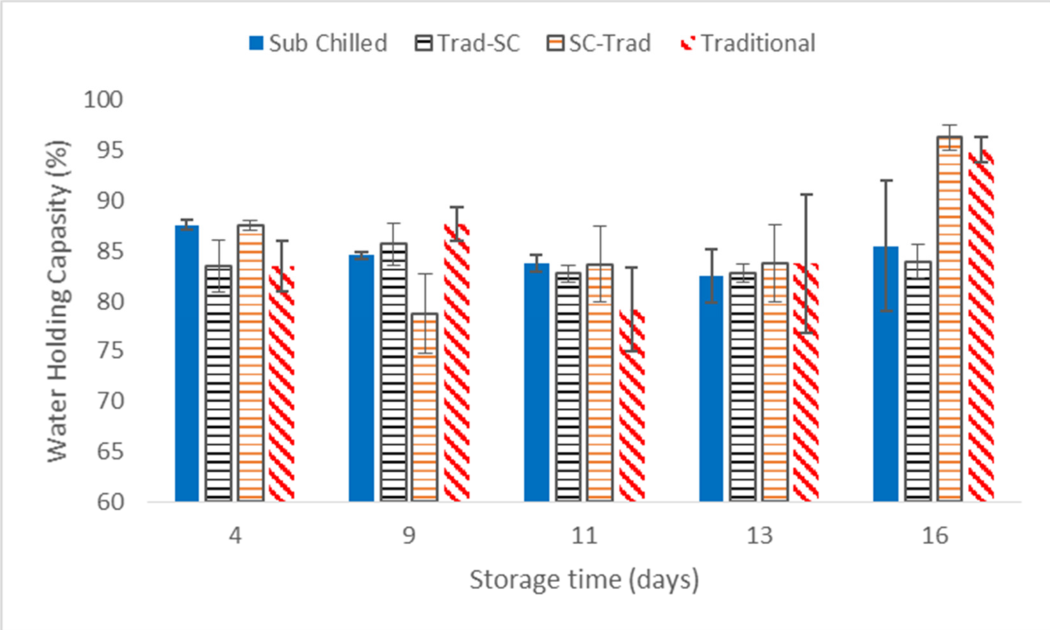


Figure 19. **Water holding capacity (WHC; %) during 16 days of storage of four different experimental groups within experiment #2: Sub chilled after catching and throughout processing and storing; sub chilled after catching and stored at 0 °C (SC + Trad); traditional chilled with ice after catching and sub-chilled during storage (Trad + SC); and traditional chilled after catching and throughout processing and storing. (n=2; Mean±SD).**

2.4.3.4 Cooking yield

The cooking yield is an important quality attribute since it represents the final yield for the customer, to maximise the quality and integrity of the final product. The cooking yield tended to be slightly higher for the traditional products but the difference between experimental groups was however not significant (Appendix II).

2.4.4 Sensory/fillet evaluation

2.4.4.1 Fillet Quality

Two fillet tests (experiments # 3 and 4) were conducted to evaluated quality of the two groups of cod, sub chilled and traditional, using texture, colour and gaping as quality attributes. Figure 20 shows the result of the former test, where by applying the sub chilling resulted in significantly higher quality compared to the traditional processed fillets. Description of the evaluation scheme used can be viewed in Appendix 9.1.3.

The former test was made in April 2016 but the second test in October same year. There was no notable difference between the two groups in the October test.

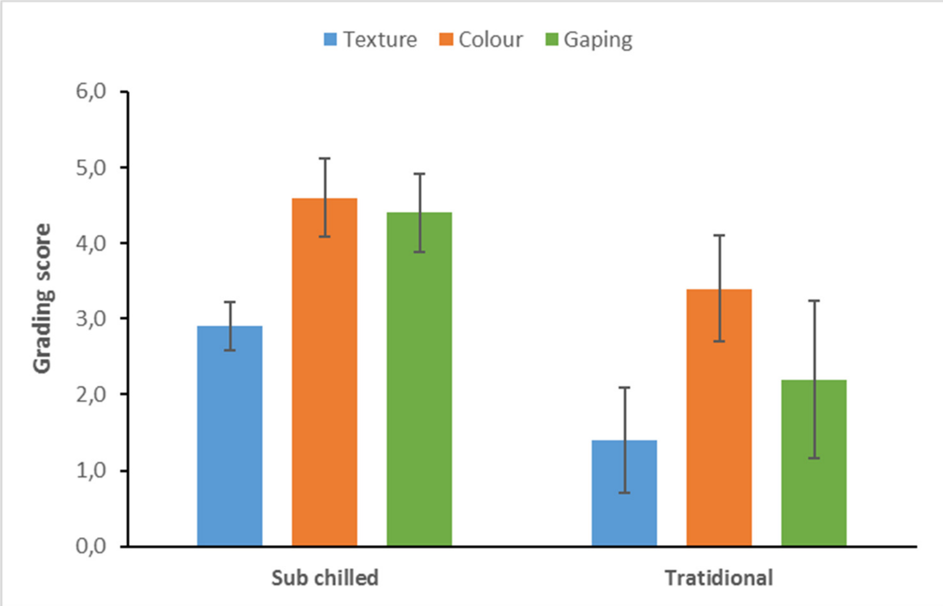


Figure 20. Fillet quality evaluation (texture, colour and gaping) of cod fillets from raw material sub chilled and traditionally chilled after catching and before processing (n=10; Mean±SD). Higher number represent better quality.

2.4.4.2 Torry freshness score

Sensory evaluations were performed to estimate the shelf-life of the fresh cod fillets by using the Torry freshness scheme (Figure 21). Quality deterioration of fish is first characterised by the initial loss of the fresh fish flavour (sweet, sea weedy) which is followed by the development of a neutral odour/flavour (i.e. the end of freshness period: Torry score = 7), leading to the detection of off-odours/flavours (Torry score = 5). The two horizontal lines on the graph represent excellent quality (7 in Torry score) and minimum score a fresh fish product can have 5 on Torry score to be fit for human consumption. The results were in accordance with the microbiological analysis, with sub chilled timing out just before day 16 along with fillet that were traditional chilled after catching and sub chilled after processing and during storage (Trad + SC). The traditional product along with products from sub chilled fish after catching and sub chilled after processing and during (SC + Trad) are dating out on day 12.

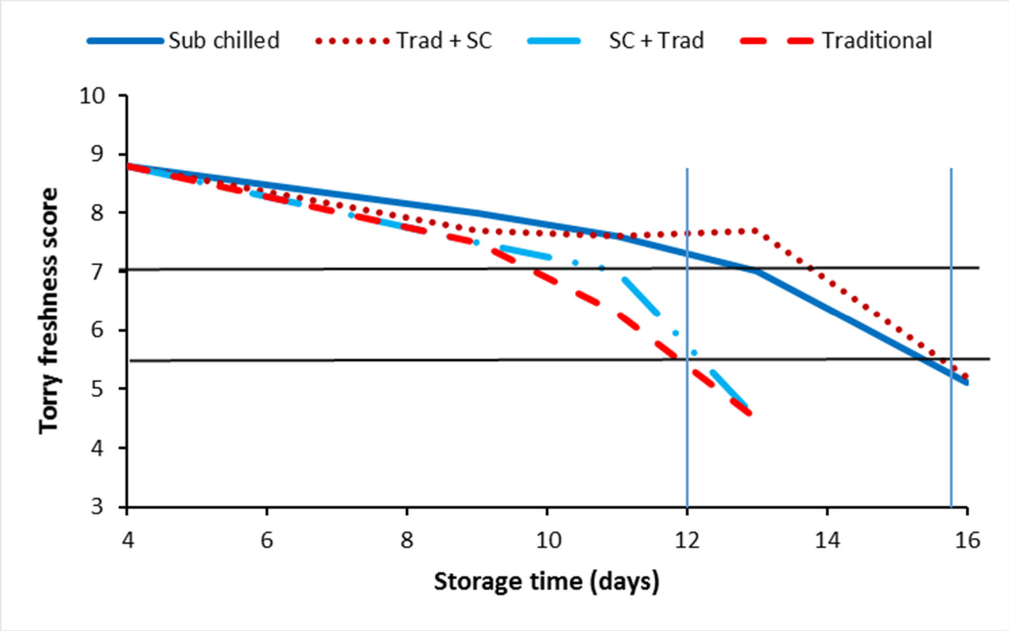


Figure 21. Torry freshness score during 16 days of storage of four different experimental groups within experiment #2: Sub chilled after catching and throughout processing and storing; sub chilled after catching and stored at 0 °C (SC + Trad); traditional chilled with ice after catching and sub-chilled during storage (Trad + SC); and traditional chilled after catching and throughout processing and storing. The two horizontal lines represent freshness limit (Torry score = 7) and acceptance limit for human consumption (Torry score = 5.5).

2.4.5 Production

In 2016, 300 kg of 5-6 kg cod was headed, filleted and skinned/trimmed. The product was weighed after each stage of the processing step to calculate the yield. The difference between the groups was minor but the sub chilled products had 1.5% higher processing yield. The sub chilled raw material is different from the traditional one, a little stiffer and that could require changes to the machinery settings during processing.

3 Logistics

Fresh salmon are packed in 30 litres EPS boxes with 27 placed on each pallet before stored in trucks for transportation. After the packing, extra ice is added to buffer heat load during distribution, around five kg for trucking and around three kg for airfreight. Approximately 23 pallets are loaded in each truck, each weighing around 20 tonnes. For airfreight, fresh salmon is trucked to Oslo or Helsinki for export to Asia. From Norway, about 660 thousand tonnes of salmon are being transported each year to Central Europe (Marin Harvest, 2015). Around 237 thousand tonnes of farmed salmon were shipped from Europe to Asia, all by airfreight. To keep the fish chilled during the transportation, 24 thousand tonnes of ice are required, the equivalent of 160 Jumbo jets each year (Marin Harvest, 2015). For trucking to European and Russian market, a product quantity of more than 900 thousand tonnes, 45 thousand tonnes of ice are needed to keep the product refrigerated during transportation.

With improved chilling and reliable operations throughout the cold chain, 20% of the export (melting ice) could be removed from the roads, equalling to 132 thousand tonnes (6,600 trucks). In addition, with the removal of inedible fish parts and transport of trimmed fillets only, the distribution volume could be reduced by half, from today's 6,600 trucks to 3,300 trucks.

Simulation test were conducted at Alta in Norway (experiment #14; Table 1) to monitor product temperature for six days (Figure 22). The idea behind this test was to estimate the need of ice though airfreight but iced at arrival in Asian destination. Salmon would be sub chilled and packed in EPS box and transferred by truck or/and air plane to customer. To simulate customary procedure, 3 kg of ice would be spread on top of salmon in each box at arrival on destination airport. Four 5 kg salmon was sub chilled and packed in 30 litres EPS box and stored in a cooling room with unstable temperature (ranging from 1 to 5 °C). Loggers were placed within the salmon, inside the box and outside the box (ambient temperature). This was performed to simulate a logistic chain from Alta to Asia where the fish is expected to be iced at reception.

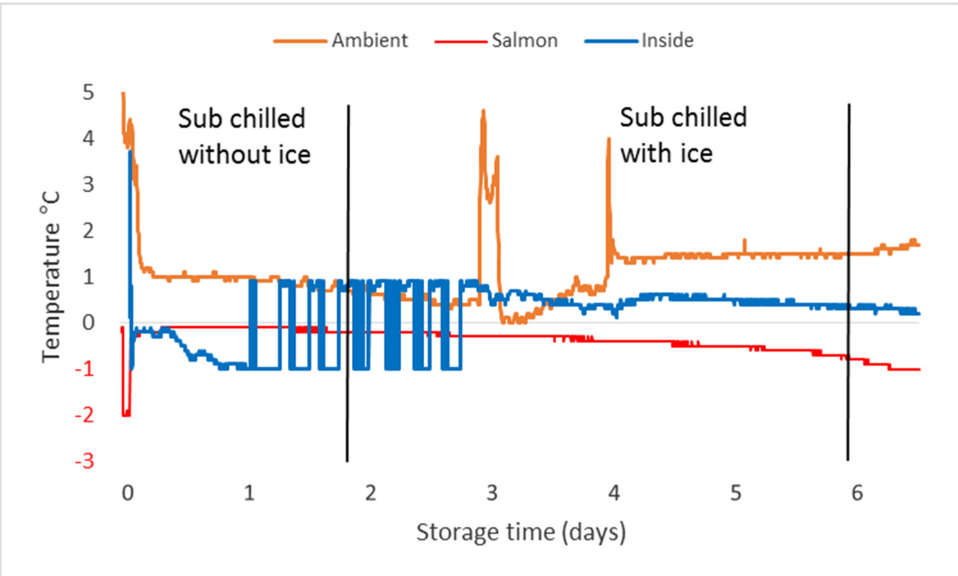


Figure 22. Temperature profile of sub chilled salmon as well as ambient temperature and temperature inside box during simulation test performed in December 2015 at Alta in Norway (n=1).

There were no negative effects from placing 3 kg of ice in each box. The ambient temperature was fluctuating between 0 °C to 5 °C, and the temperature inside the box was fluctuating between -1 °C to +1 °C. The salmon was at 1 °C which is a recommended temperature for this product.

Figure 23 shows temperature profiles from sub chilled and traditional chilled salmon, trucked from Alta in Norway to Oulu in Finland and stored there for three days prior to processing, total of four days. The temperature of sub chilled salmon was steady just below -1 °C while the traditional one was at 0 °C. The sub chilled salmons were transported and stored without ice.

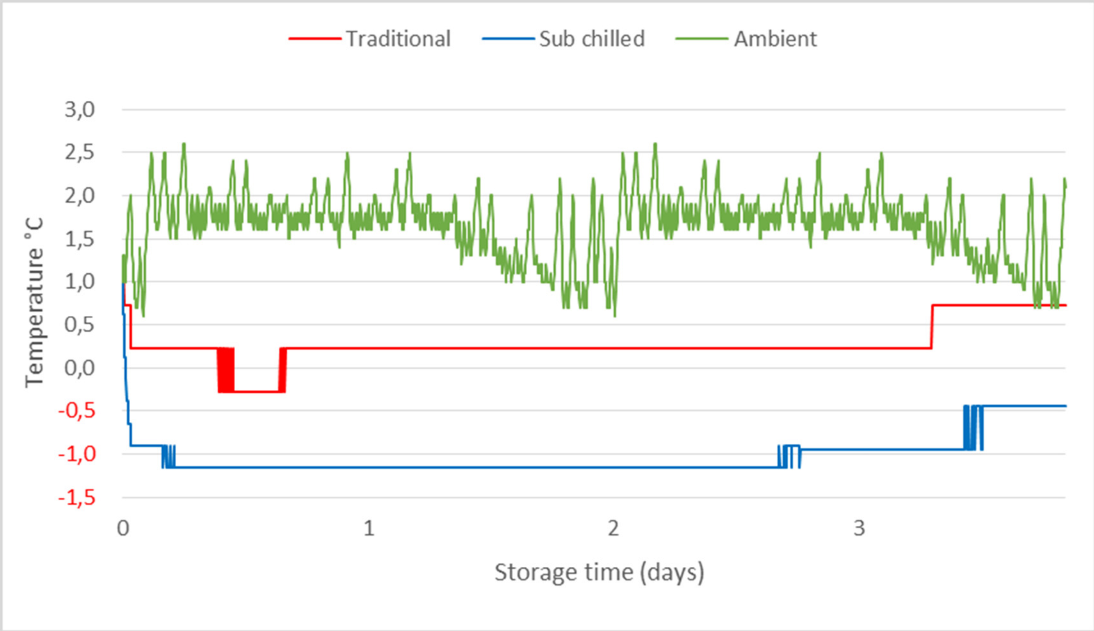


Figure 23. **Temperature profiles sub chilled (SC) and traditional chilled (Trad) salmon, as well as ambient temperature, during transportation from Alta in Norway to Oulu in Finland (12 hours) and 4 days storage at Oulu before processing (n=1).**

Six 30 litres EPS boxes, with 20 kg of sub chilled salmon in each, total 120 kg, was transported from Norway to Japan in November 2016 (experiment #15, Table 1). In Figure 24, the product and ambient temperatures are shown. The ambient temperature fluctuated from 3 °C to -4 °C while the product temperature starts at 1 °C and decrease to 0 °C in almost 3 days. Logging of temperature during the summer months was not available in this case.

It took almost 2 days to truck the salmon to Gardimor in Oslo where it was stored for couple of hours, and then trucked to Kastrup in Copenhagen where it was stored for almost one day. After 3,5 days the product were flown to Tokyo where it was delivered to customers at the Tokyo airport. Some of the product were re-iced at Tokyo airport, before delivered to customer.

The product was followed by quality expert and a sale person from the marketing company, Ocean Quality. Normally when fresh salmon arrives to Tokyo airport it is re-iced before trucked to smaller customers. Six of the sub chilled boxes had already been iced but the rest were send to customers without any ice. At arriving to the customer in Tokyo the temperature in the re-iced product was close to 0 °C but the rest, un-iced the whole time, were -0.9 °C. Ambient temperature at the airport was 15 °C. The experts used the FHF quality system to evaluate the fillets quality (appendix I – 9.3.3) The quality of the salmon had quality score of 0, same quality as expected from newly slaughtered salmon.

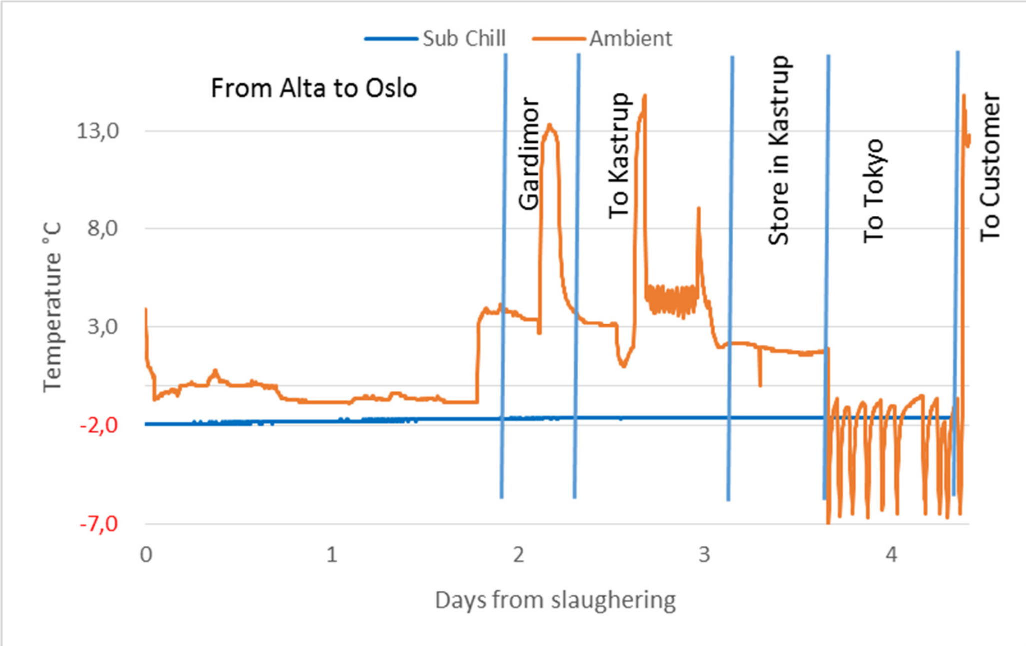


Figure 24. **Temperature profiles of sub chilled (S.C.) salmon and the environment during transportation from Norway to Japan (Alta-Oslo-Copenhagen-Tokyo). The logistic from Alta to Copenhagen airport was by trucks and cooing rooms but by airfreight from there to Tokyo (n=1).**

In November 2015, four tonnes of sub chilled and ten tonnes of traditional chilled salmon were trucked from Norway to Denmark for secondary production (experiment #11; Table 1). The trucking time was around 24 hours but the product was kept for 11 days at refrigerated temperature before production in order to estimate the time sub chilled product could keep freshness without the need of external ice for chilling (Figure 25).

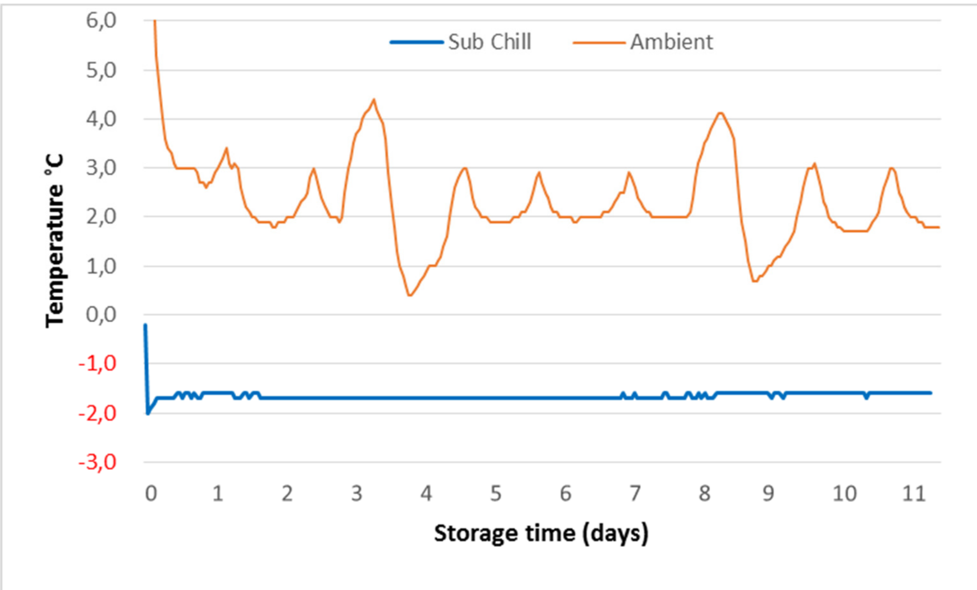


Figure 25. **Temperature profiles of sub chilled (SC) salmon and the environment during transported from Norway to Denmark and storage for 11 days before production in a refrigerator (n=1).**

Two shipment with traditional- and sub chilled rainbow trout were shipped from Arctic Fish in Isafjordur to Słupsk in Poland; in April and June 2017 (experiment #18 & 19; Table 1). The fish were exported by temperature controlled containers from Iceland to Gdansk via Rotterdam. The first shipment took 12 days but the latter one took 9 days from slaughtering to quality inspection in Poland. The aim was mainly to compare sub chilled trout exported without ice and traditional product chilled with ice (Figure 26). The former shipment included 700 kg of trout, which were manually sub-chilled in 660 litres tub while the rest of the containers were filled up with traditionally iced trout in 460 and 250 litres tubs, manufactured by Sæplast Iceland. There was no ice on the sub chilled product but it was sub chilled below -1 °C in core temperature while the traditional one were packed at 0 °C. The container was set at -1 °C during the transportation. The transportation took 12 days from packing to inspection at Milarex zp in Słupsk, Poland. The second shipment included five 460 litres tubs including 300-326 kg each, four 220 litres tubs with 175-183 kg each and two 40 litres EPS boxes with around 21 kg of fish in each; all with super chilled rainbow trout without ice. The sea container was filled up with traditionally chilled trout iced in 40 litres EPS boxes.

Figure 26 Sub chilled rainbow trout in container packed Sæplast tubs and EPS boxes, without ice.

The former experiment came out quite decisive but the temperature of the sub chilled trout was around -1.5 °C when it arrived in Poland while the traditional one was at around 0 °C °C. The temperature in the sub chilled product was steady close to -1.5 °C the whole time, slightly going up from day three, as can be seen in Figure 27. The quality of the traditional chilled trout was starting to degrade at arrival in Poland, the surface was markedly wrinkled after the ice cubes and the soft tissue in fillets and the belly muscle could easily be ripped. Visual quality inspection turned out much better for the sub chilled trout but numerical results are not available. The only problem was the skinning process of the sub chilled fillets where the texture of the fillets was rather firm and some of the skin was not removed. Temperature of fillets after skinning was around -1.5 °C.

The second shipment took only nine days from slaughtering to production/inspection and this time a quality inspection by FHF system were used for all groups of trout; traditional, and sub chilled. The sub chilled were grouped in ten group; nine tubs and two EPS boxes with no drain holes. This was to test different depth of tubs (220 litres – 440 litres), also test if there were different of quality of fish from bottom of tub and on top of it and if lid on the tubs make difference in quality. Quality of the traditional was basically good but excellent of the sub chilled product.

Temperature of sub chilled were stable and was around -1.5 °C, same as after the chilling process. Best score for quality is zero but worst is 2 for elasticity and 4 for gaping. At least 20 fillets were examined from each group, 10 groups of sub chilled and one of traditional. The outcome of the quality test done at Milarex zp in Słupsk in June 2017 is presented in Figure 27. The sub chilled product turned out with better quality, even though it came from tubs, but the traditional from 30 litres EPS boxes.

Table 3 Quality outcome for one traditional group (25 fillets) and sub chilled groups (10x25 fillets). Best score is zero and worst is 2/4

Sub chilled	**Elasticity %**	**Softness %**	**Gaping %**
0	94	99,5	100
1	6	0,5	0
2	0	0	0
3			0
4			**0**
Total	**100**	**100**	**100**
Traditional	**Elasticity %**	**Softness %**	**Gaping %**
0	56	84	96
1	40	16	4
2	4	0	0
3			0
4			0
Total	**100**	**100**	**100**

Temperature in product were staple (figure 27) in both sub chilled and traditional and ambient temperature was stable at around -1 °C.

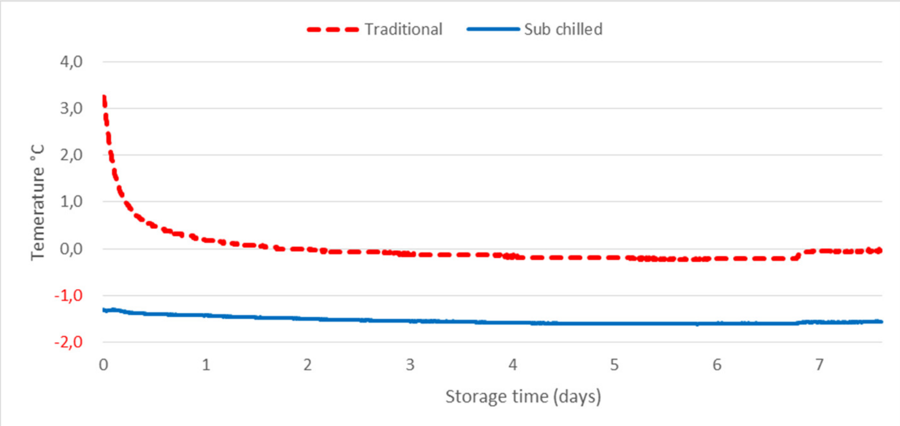


Figure 27 Temperature profiles of sub chilled (SC) and traditional chilled trout during chilling and transportation from Iceland to Poland. The sub chilled trout were transported in 460 and 250 litres tubs but the traditional chilled trout were transported in 30 litres EPS boxes.

3.1 Reducing the carbon footprint

The sub chilling method can have an enormous positive environmental effect within the fisheries**.** The technique is based on the usage of the frozen internal water as the cooling media, instead of external ice. The method can lower the energy used substantially. Building small ice crystals within the fish instead of using external ice saves a lot of weight and energy. Ice production is power intensive and the norm is to use only fraction of it for chilling. The rest is often melted by expensive external energy and requires intensive cleaning before drained to the environment. Sub-chilling saves large energy effort in logistics, with no ice which normally weighs up to 15-20% of the cargo. Skipping the ice can also save substantial amount of water, which is an important environmental matter in many countries. This ice can therefore be substituted for fish, allowing more volume of products to be transported each time.

There is a lot of energy and carbon footprint to be saved by using the sub chilling within the logistics chain. On top of that the sub-chilling method will give the consumer more value with better quality and more food safety and possibly reduce food waste. Temperature is the most important factor in all handling and storage of fresh fish, and without precise control, its original quality is easily lost. For example, shelf life of fish is halved by raising the storage temperature from 0 °C to 4 °C. Several biochemical processes taking place post mortem are highly temperature dependent such as blood coagulation, rigor and other autolytic changes. With rising temperature, these processes are greatly accelerated resulting in less blood removal, gaping, drip loss and overall poorer quality and shelf life.

Maintaining the cold-chain from harvesting to market is therefore critical for the end-product quality and value. In relation to this fact, the temperature sensitivity often forces fresh fish exporters to choose airfreight as the only viable option for transportation of fresh products to market. However, airfreight has several disadvantages compared to, for example, sea freight such as high cost, large amount of ice needed to maintain adequate temperature and environmental drawbacks (Margeirsson *et al*, 2012).

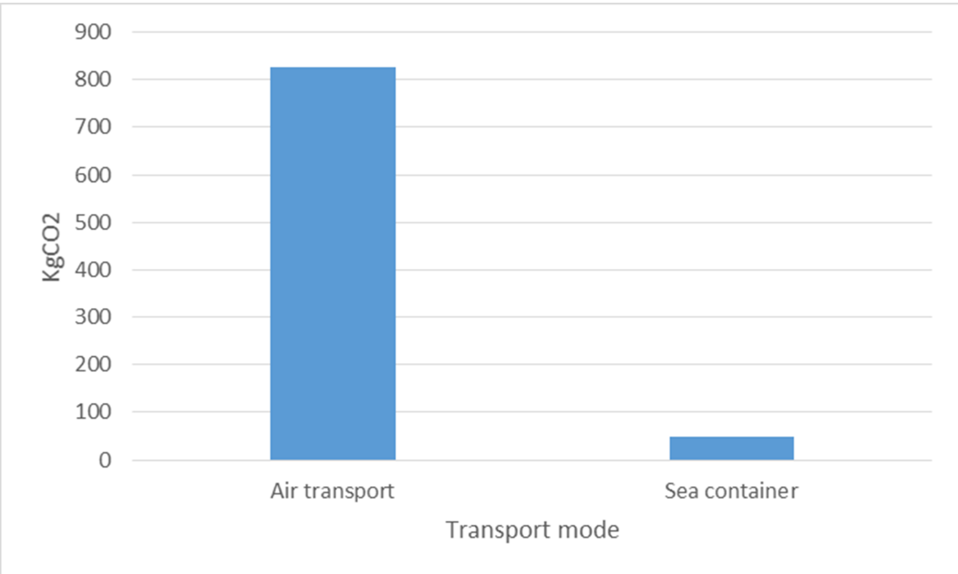
With ice no longer being a part of the cooling, storage and transport, it's clear that a new area is being entered. To begin with, handling becomes less demanding and there are new opportunities for packaging. Until now it has not been advisable to transport whole salmon iced in tubs, as the ice can damage the fish, but by using sub chilling that problem is no longer present (Figure 28).

Figure 28. **Salmon transported in tubs with ice and in sub chilled condition without ice. On the figure to right the sub chilled product is on the left side but traditional with ice is on the right.**

The extended shelf life ushers in notable changes in transporting fish to markets, opening the way for rationalisation. A longer shelf life means that fish can be transported by sea rather than by air. This represents a saving in transport costs, as well as a substantial reduction in the carbon footprint. Until now, salmon has mainly been transported in single-use packaging, but sub-chilling means that tubs can be used instead as a multifunctional packaging.

Carbon footprint is defined as the total amount of carbon dioxide and other greenhouse gas emissions emitted over the full life cycle of a product or product system. It is measured in equivalent kilograms or tonnes of CO_2. Figure 29 shows a calculated CO_2 emission pr/kg of product of fresh fish exported from Iceland to Europe, by two different packaging and by sea container and airfreight.

Figure 29. **Carbon footprint of two fresh fish groups transported from Iceland to Europe in kg CO_2: by air freight and by container ship (Margeirsson *et al*, 2012).**



The carbon footprint was calculated for each group, considering the recycling and reuse-phases of the containers (Margeirsson *et al*, 2012). The packaging used are 30 litre ESP boxes using 5 kg of ice for ship and 3 kg for air. No ice is used for sub chilled product. The calculation of carbon footprint shows that Sea-SC scores the lowest number, only 53 kg CO_2 for the whole process but traditional product 75 kg CO_2. The air transport traditional group are using almost 1000 kg CO_2, but sub chilled 60 kg CO_2.

4 Process and marketing

4.1 Promotion and marketing of the Sub-Chilling methodology

Promotion and marketing to introduce sub-chilling method to the fishery industry:

1. Introduced at Brussel Expo Global 2015 and 2016.
2. Introduced at Boston Seafood Exhibition 2016 and 2017.
3. Introduced in Bergen, North Atlantic Seafood Forum Conference 2016.
4. Introduced in The Seafood Conference Iceland 2015 and 2016.
5. Introduce to Fishery Minister of Norway and her delectation in 2015.
6. Introduce to specialists and scientists from COFASP and Rannis in September 2016.
7. Introduced to Aquaculture Cluster of Westfjords 2015.
8. Numbers of visits to fishery companies and salmon aquaculture companies for the last two years in Iceland, Norway, Poland, Finland, Faroe Islands, and Denmark.
9. The products have been introduced to retail shops in Finland.
10. The Sub-Chill process has been prominent in the media in Iceland.
11. Appling for “Svifaldan” the progressive idea of Fisheries Conference 2016, and winning the first price.
12. Number of media coverage in Iceland and Norway about the project “Sub chilling of fish”.
13. Two master degree students were involved in this project, Sigurdur Orn Ragnarson and Hildur Inga Sveinsdottir.

5 Main outcome of the project

5.1 Economic potential of Sub-Chilling

For over two years, Fisk Seafood, a partner in this project, have been using the sub chilling method on board the trawler Malmey SK1 as well as through production for the same raw material. On board the fishing vessels are three Rotex screw conveyors for the chilling process. No ice is used on-board with the fish-hold kept at -1 °C. This storage temperature is maintained as well at the receiving end on land, their fish plant in Saudarkrokur. The trawler is catching around 7.500 tonnes a year, roughly 30 a day in 6 or7 hauls. Given these numbers, the trawler would normally be using around 30 tonnes of ice a day if not for the Rotex equipment. Instead the trawler uses around tree tonnes of salt for each trip for processing the brine for the chilling process. To sail with extra 50 tonnes of ice for each trip will cost some extra energy, but so will the chilling process on-board. In Table 3 is estimation on the energy cost for sub chilling on-board a trawler. The energy needed to chill one tonne of whole fish from 6 °C down to -1 °C is around 7 kWh. The normal catch for one trip is 150 tonnes, a total over the year of 7,500 tonnes in 50 trips. The price of ice is around NOK 0.21 pr/kg. The cost of producing power using diesel fuel is around NOK 0.12 pr/Kw.

Table 4. Comparison for cost of chilling on board using the sub chilling method and the traditional method by using ice.

Item	Tonnes pr. trip	Tonnes pr. year	Price NOK	Cost NOK
Salt	3	150	1,429	214,286
Ice	50	2,500	214	-535,714
kWh	350	17,500	2	30,000
Subtotal a year				**-291,429**

Table 3 shows a rough comparison of a trawler using ice or the Rotex chilling method. It should be noted that borrowing cost or maintenance is not included. However, it should be considered that the system saves quite some workforce as well as giving better flow during processing. Malmey doesn't need ice, saving NOK 214 thousand a year. With the extra cost of NOK 536 thousand for salt and another NOK 30 thousand for energy cost, the total savings per year given this estimation are NOK 291 thousand a year.

A Rotex sub-chill equipment is now in use at Arnarlax salmon production in Bildudalur, the largest aquaculture processor in Iceland and it has been showing excellent results. However, the company is still using ice as a refrigerant but it does so in cooperation with the customer, reducing the overall ice usage. The salmon is packed in 30 litres EPS boxes with around 5 kg of ice in each.

Table 5. Comparison for cost of sub chilling and traditional chilling in salmon production.

Item	Tonnes pr. trip	Tonnes pr. year	Price NOK	Cost NOK
Salt	1.2	50	20,000	71,429
Ice	4.5	1,125	3,000	-241,071
kWh	210	6,300	24	10,800
Subtotal a year				**-158,843**

Arnarlax is slaughtering around 30 tonnes a day for 250 days a year, totalling around 7,500 tonnes a year. If the company was using sub chilling method there would be no need for ice, reducing the cost by NOK 71 thousand like seen in table 3. The company need less ice for chilling, around 1,125 tonnes a year an estimated cost of NOK 241 thousand, but extra energy cost would be around NOK 11 thousand a year. This would be the subtotal per year around NOK 159 thousand.

5.2 Economic advantage

The quality comparison between sub chilled and traditional salmon products in this project is unsubstantiated with substantial difference on gaping, elasticity and softness, sub chilled product in favour. The result for the cod is more critical with one result of sub chilling in favour but the second one there were no substantial different between

Research has also included comparisons of the cold chains in handling both sub chill and conventionally produced salmon. The former was transported without ice and conventionally produced salmon in ice, for further processing in Finland and Norway. Similar comparative studies were carried out on sub chilled and conventional salmon shipments to Iceland via Oslo and to Tokyo via Oslo.

The trawler Malmey SK 1 has been using the sub chilling method along this project for two years, with excellent success. New trawlers build for Icelandic fisheries are designed with sub chilling capability supplied by Skaginn3X, a participant in this project.

These studies have confirmed that sub chilled salmon holds its water content better throughout the production and storage processes, and it has a better culinary yield, such as when poached. The qualities and the firmness of the fish remain for longer, maintaining quality more effectively through production.

Microbiological analysis has also confirmed that the fish stays fresher for longer than conventionally chilled fish, also confirming that sub chilling can extend the shelf life of the finished product by three days for cod and up to five days for salmon product. Research indicates that sub chilling after catching/slaughtering but storing by traditional temperature (0-2 °C) does not extend self-life substantially. The storing temperature is the most important factor of longer self-life and reduced bacterial growth. Storage of sub chilled products is challenging, however, with urgent need of steady temperature to avoid damaging crystal build up within the muscle.

5.2.1 Improved handling

With ice no longer a part of cooling, storage and transport, it’s clear that we are entering a new era. To begin with, handling becomes less arduous and there are new opportunities for packaging. Until now it has not been though advisable to transport whole salmon iced in tubs, as the ice can damage the fish. But the best quality can be achieved using sub chilling.

The extended shelf life ushers in notable changes in transporting fish to markets, opening the way for rationalisation. A longer shelf life means that fish can be transported by sea rather than by air. This represents a saving in transport costs, as well as a substantial reduction in the carbon footprint. Until now, salmon has mainly been transported in single-use packaging, but sub chilling means that tubs can be used instead.

5.2.2 Reducing the carbon footprint

Sub chilling does not just have economic benefits. This approach demonstrates a notable contribution in environmental terms with a reduced carbon footprint in production and transport.

Approximately 20% of the overall weight in salmon transport is ice. Sub chilling makes ice redundant and reduces the strain on much of the transport chain, by air, road or by sea. The extended product shelf life brings in possibilities to ship larger volumes in containers to replace the amount of fresh whitefish exported from Iceland by air.

Production of farmed salmon in Norway, the Faroe Islands and Iceland has in recent years topped the million tonne mark, while the domestic market for these products is estimated to be only around 36,000 tonnes. A million tonnes of salmon are shipped to other markets every year.

In rough terms, we can estimate that in the region of 200,000 tonnes of ice are shipped with this salmon. Approximately 240,000 tonnes of salmon every year are freighted by air to Asia, which means that an estimated 48,000 tonnes of this weight are ice – so a saving equivalent to 1000 Jumbo jet flights could be made.

5.2.3 A green future – ahead of the market

Arnarlax, a salmon farming and processing enterprise in Arnarfjordur, Iceland have made it plain the media that their fish farming is a long way from their markets, but sub chilling approach brings them a lot closer to their customers. At the same time, this gives them a strong lead, especially in more distant markets. This has also opened people's eyes to the positive environmental aspects of this method just as around the world people are wondering how to reduce carbon footprints. Sub chilling ensures better usage of the resources that nature provides us with, and puts it on the consumer's plate at a lower cost and in a greener way.

5.3 Market potential

Fresh food is gaining advantage on the marked with consumer ready to pay higher prices for fresh instead of frozen product. The disadvantage of fresh food is short self-life and the need for notable temperature control in the cooling chain; through fishing, primary processing, secondary processing, retailing and consumer.

The most important market opportunities for sub chilled product is better quality of fresh fish and longer self-life. It will give fresh fish producers' stronger marketing position against the rivals of other fresh food on the market. Better quality will always give stronger marketing position but the slang of fish "smell and taste" is a well-known Achilles heel for introducing fish as coveted food. But fresh quality fish doesn't smell or taste bad, but that is caused by too high bacterial growth caused be wrong treatment. Better chilling and better control of logistic temperature is highly important marketing tool for the fresh fish market in the future, and sub chilling could be excellent mechanism for success.

But there are additional factors on quality and in this project the relationship between slower gentler rigor mortis process is introduced. The quality difference in sub chilled and traditional salmon fillets are well-defined from our tests in this project. The fillets have less gaping, better elasticity, and are firmer. The results in cod were not as decisive, both due to fewer samples taken and with highly variable raw material from wild fish stocks.

The ice free logistic could give market potential in the future, with lower carbon foot-print on fresh fish marketing. The environmental impact from food production will more coverage in the future with more concern consumers. Ice-less logistic will also lower transportation cost with less weight and also make ship containers more viable against air transport, takes longer time but is a lot cheaper and also have better temperature control. Sub chilling processing is also making use of tubs instead of EPS disposable packaging, saving both money and carbon foot-print.

6 Discussion

The sub chilling process on an industrial scale has been successful and sup chilling without causing quality problems because of large ice crystal build up in the fish muscle has not been a problem. On the contrary the conclusion of this project is a better fillet quality and longer self-live of fresh product from salmon and cod. It is technically possible to sub chill salmon and cod, farmed and caught fish, with acceptable result. It is also clear that technically it is possible to produce sub chilled products and transfer them through the logistic chain without using ice as a preservation medium. This project shows that slower and easier contraction through rigor mortis is decisive factor for fillet quality including gaping. But more researches are however needed on this matter, especially on the effect of filleting before rigor mortis on the fillet quality. It could be interesting to investigate the possibility of pre-rigor filleting, but the contraction effect on the muscle quality is not known.

The relationship between temperature and shelf life of fresh cod has been explored thoroughly in recent years (Haugland, 2002), however, no comparable scientific findings are available for salmon. Such information would be very informative for the global salmon industry. Present project demonstrates the important of low and stable temperature in order to slow down the spoiling process of fresh fish product and hence gaining of longer shelf.

To maximize the benefits of using sub chilling through the value chain, the procedure must be introduced to the industry to adapt the method. The environmental impact on ice free logistic must be introduced as well, but the marked advance in the future is enormous. There is also the possibility of using tubs instead of EPS boxes, especially in the transportation from primary producer to the secondary processor. It will both reduce cost and carbon foot-print for the product. Two delivery of sub chilled rainbow trout from Iceland to Poland delivered excellent quality of fish in 440 litre saeplast tubs, without any Ice. The former shipment took 11 days from harvesting to production and the later one seven days. There were no measurable difference in quality of fillets from fish at the bottom of the tub or from the top. Using tubs instead of EPS boxes can save enormous shipping cost and also carbon footprint in the fresh fish industry.

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9 Appendix I – Research methodology

9.1 Chilling

The fish was sub chilled by using 1000 litres tub (Figure 30) with circulation pump-system using 5-6% salinity brine and pouring ice in to it for controlling temperature. Cod was chilled down by keeping temperature at -1 °C for 50 minutes and then lowered down to -3 °C until core temp reached -0.5 °C. During a few hours' storage time the ice build-up absorbed interior heat and reached equilibrium around -0.8 °C. Salmon was sub-chilled in -1 °C for 90 minutes and then by pouring more ice in the tub the temp was lowered to -3 °C until core temp of -1 °C was reached. The sub chilled salmon and cod was then stored at around 1 to 1.5 °C below zero, providing an internal ice reservoir so no external ice for storage was needed.

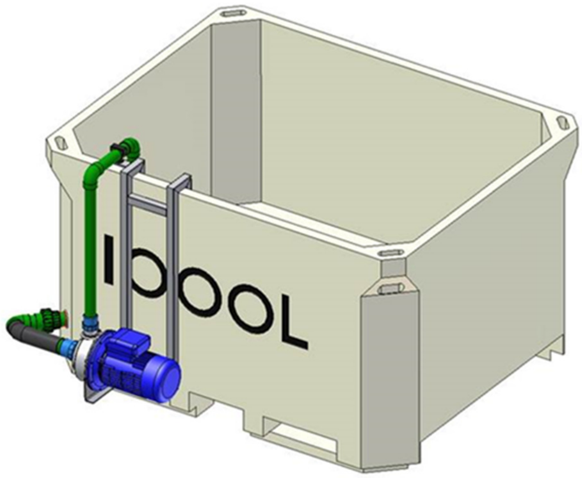

Figure 30. **Schematic figure of the 1000 litres sub-chilling tub equipped with a centrifugal pump to recycle the chilled brine.**

For larger test at Grieg Seafood in Simanes, between eight and ten tonnes were chilled in a large 30,000 litres screw conveyer by using 5-6% brine strength and ice slush poured in until the right temperature was reached. The temperature of the brine was around -2 °C and the salmon was kept in it until reaching -1 °C core temperature.

The chilling process was started right after slaughtering and bleeding, as fast as possible to minimize crystallization within the muscle cells. The difference in thickness of fish (loins, middle and tail of fish) has to be kept in mind, as well as differences in water- and fat content in different part of the fish. The size of fish is also a decisive factor in proper chilling processes. After the chilling, the surface of the fish will be colder than the core of the thickest part but the temperature will even out in a few hours storage. Traditional salmon and cod were chilled by ice, using around 5 kg of ice for 20 kg fish.

9.2 Temperature monitoring

Testo 176 data loggers thermometer from Testo, were used to record the chilling process of the salmon. The thermometer has four sensors to measure temperature and time during the process. A bracket was built to hold the sensors at the right positions under the fish skin, 15 mm deep at the core of the fish.

IButton data loggers (DS1922L) from Maxim Integrated Products (Figure 31A) were used for monitoring temperature in the trials. These loggers have an accuracy of ±0.5 °C, a resolution of 0.0625 °C and an operating range of -40 to 85 °C. The diameter is 17 mm and the thickness is 5 mm. All temperature loggers were factory calibrated and re-calibrated in thick mixture of fresh crushed ice and water. These temperature loggers were placed within the product, and on the outside of the boxes to monitor the ambient temperature.

Tempsen ITAG –PDF temperature data loggers (Figure 31B) were used to monitor the temperature of product and the ambient temperature. These loggers can collect data temperature for periods of 15 and 25 days. The ITAG-PDF is ideal for temperature monitoring of long distance transportation. The measurement range is -30°C~70°C with the accuracy in the range of ±0.3°C. The temperature resolution is 0.1 °C/°F and the data storage capacity is 3,800 readings.

Figure 31. A) IButton DS1922L temperature loggers used to monitor the temperature within the product and the ambient temperature. B) Tempsen ITAG-PDF single use temperature logger used to monitor the temperature of product and the ambient temperature.

9.3 Analytical parameters

9.3.1 Microbiological analysis

Total viable psychotropic counts (TVC) were performed on iron agar (IA) as described by Gram *et al* (1987) with the exception that 1% NaCl was used instead of 0.5% with no overlay. Counts of H_2S producing bacteria, forming black colonies on IA, were also evaluated. Plates were spread-plated and incubated at 17 °C for 4 to 5 days. Two replicates were analysed on each day of sampling.

9.3.2 Physicochemical analysis

9.3.2.1 Water content

Water content was determined by difference in weight of homogenized muscle samples before and after drying for 4 h at 102 °C to 104 °C (ISO 1993). Results were calculated as g water/100 g muscle. Total lipids (TL) were extracted from 25 g samples (80±1% water) with methanol/chloroform/0.88% $KCl_{(aq)}$ (at 1/1/0.5; v/v/v) according to the Bligh & Dyer (1959) method. The lipid content was determined gravimetrically and the results were expressed as grams lipid per 100 g wet muscle.

9.3.2.2 Lipid content

Lipid content (%) was evaluated after extraction of lipids by two different solvent systems, Soxhlet (AOCS, 1998) and Bligh & Dyer (1959) with some modifications (Hanson and Olley, 1963). In Bligh & Dyer, extraction of lipids was carried out by chloroform/methanol extraction system and with butylated hydroxytoluene (BHT) admixed into all solvents (50 - 100 mg/L).

9.3.2.3 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_3 solution. The solution was then titrated with 0.1 N $AgNO_3$ in a 716 DMS Titrino device.

9.3.2.4 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).

9.3.2.5 Total volatile basic nitrogen (TVB-N)

The method of Malle and Tao (1987) was used for measurements of Total Volatile Base-Nitrogen (TVB-N) and Trimethylamine (TMA). TVB-N was measured by steam distillation (Struer TVN distillatory, STRUERS, and Copenhagen) and titration, after extracting the fish muscle with 7.5% aqueous trichloroacetic acid solution. The distilled TVB-N was collected in boric acid solution and then titrated with sulphuric acid solution. TMA was measured in trichloroacetic acid (TCA) extract by adding 20 ml of 35% formaldehyde, an alkaline binding mono- and diamine, with TMA being the only volatile and measurable amine.

9.3.2.6 Free fatty acids (FFA)

Free fatty acid content (FFA) was determined on the TL extract according to Lowry & Tinsley (1976), with modification from Bernardez *et al* (2005). The FFA concentration was calculated as µmolar quantities of oleic acid based on standard curve spanning 2-22 µmol range. Results were expressed as grams FFA / 100 g of total lipids

9.3.2.7 Water Holding Capacity (WHC)

The WHC was determined by a centrifugation method (Eide and others 1982). The saithe samples (n=3) were coarsely minced in a mixer (BraunElectronic, Type 4262, Kronberg, Germany) for approximately 20 s at speed. Approximately 2 g of the minced saithe muscle was weighed accurately into Sample glass and centrifuged at 210 * g for 15 min. The weight of liquid expelled from the muscle during centrifugation was subtracted from the weight of water in the sample before centrifugation. The WHC was calculated as the ratio of the water remaining after centrifugation compared to the initial water content of the sample before centrifugation and expressed as % WHC.

9.3.2.8 Cooking yield

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

9.3.3 Sensory/ fillet evaluation

To evaluate quality of salmon and to compare sub-chilled fillets to traditional fillets, a guidance from Fishery and Aquaculture Industry Research Fund (FHF) was used (Figure 32). An expert in quality checking executed the test and the whole process was filmed by a GoPro camera. In the test the inelasticity, softness and quality tests of sub-chilled products and traditional products were carried out. Salmon from same production at Simanes was used as a sample in the project. During processing, a part of it was packed traditionally in normal EPS boxes (Expanded Polystyrene) or tubs, and iced in a traditional way. From the same sequence of production, a part of it was sub-chilled and packed in EPS boxes (airfreight type with no drip-holes) or tubs. These two groups were compared in several quality tests.

The quality test measuring softness, elasticity and gaping were built on instruction from FHF (Appendix II), (Erikson, Bye, & Oppedal, 2009) using the following grade with zero being the best quality:

1. Elasticity (0-2)
2. Softness during finger test (0-2)
3. Gaping in loin, belly and tail (0-4)

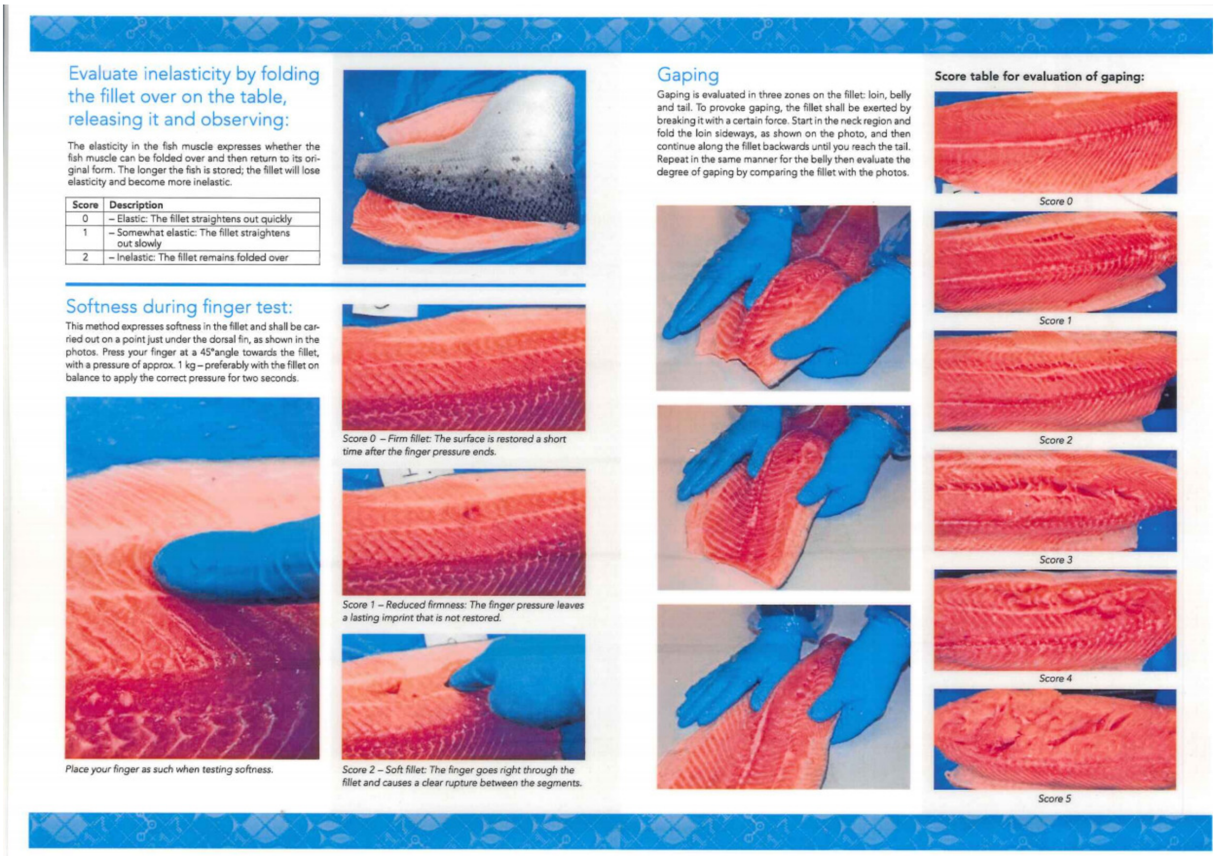

Figure 32. Quality valuation for salmon fillets

Fillet evaluation of cod fillets was carried out in relation to the project “Ofurkæling á afla smábáta” (Thordarson, Hognason, & Flosason, Ofurkæling á afla smábáta, 2015) supported by Atvest. The goal of this evaluation was to look at the texture and gaping in sub-chilled cod fillets compared to traditionally chilled fillets. Ten fillets from each group were evaluated according to conventional grading scale (Table 5). All fillets were skinned and coded with a three-digit number. Before the sensory evaluation took place, all fillets were placed at random on a white table. They were then evaluated by the judges who all carried out their evaluations at the same time. Nine judges took part, all specially trained in sensory evaluation (ISO 8586:2008). To check for differences between groups and see if it was notable, the process of general linear models was used, where the judges’ usage of scale was corrected. The confidence interval was set at 95% and therefore the difference was notable for $p<0.05$.

Table 6 Quality valuation for cod fillets.

Quality attribute		Description	Grade	Sample number									
				1	2	3	4	5	6	7	8	9	10
Flesh	***Texture***	Firm, springy	3										
		Firmness gained slowly after pressure	2										
		Soft texture, no springiness	1										
	Colour	Shining, bright colour according to specie	5										
		Matte colour, characteristic for specie	4										
		Small yellow dots, colour very matte/dull	3										
		Large yellow dots, characteristic colour vanishing	2										
		Yellow and mucous	1										
	Smell	Fresh, seaweedy, metallic	5										
		Neutral	4										
		Fishy, trace of thawing odour	3										
		Obvious thawing odour, sour, trace of ammonia	2										
		Strong ammonia, off-odour	1										
	Gaping	No visible gaps	5										
		Gaping less than 20% (1-3) longitudinal cracks	4										
		Minor gaping on one area (20%) or >3 longitudinal cracks	3										
		Some gaping, 25-75% of the fillet	2										
		Deep cracks or gaping in more than 75% of the filler	1										
	Grade (18-0) TOTAL SCORE												

The sensory evaluation of cod fillets were performed by the sensory panel at Matís. Torry freshness score sheet (Shewan *et al*, 1953) were used to assess cooked samples. Twelve panellists participated in the sensory evaluation. They had all been trained according to international standards (ISO 8586, 1993); including detection and recognition of tastes and odours, use of scales and in development, and use of descriptors. The members of the panel were experienced in using the Torry freshness score.

Portions weighing about 40 g were cut from the loins and placed in aluminium boxes coded with three-digit random numbers. The samples were cooked for 6 minutes in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany) at 95–100 °C with air circulation and steam, and then served warm to the panel. Each panellist evaluated duplicates of each test group in a random order in six sessions (maximum four samples per session). A computerised system (FIZZ, Version 2.0, 1994-2000, Biosystèmes) was used for data recording.

The sensory program Panelcheck V1.3.2 (Nofima, Tromsø, Norway) was used to evaluate panel performance. The statistical program NCSS 2000 (NCSS, Utah, USA) was used to analyse difference between groups with ANOVA (glm) and Duncan´s test. Differences between groups were considered notable when $p < 0.05$. Results from each sampling day were treated as a separate data set.

9.3.4 Production

By sub chilling the salmon and cod the muscle tissue strength is increased, giving advantage over traditional warmer raw material for rough treatment caused pressure, bending and tension in the processing machinery, often causing reduced yield and poorer quality. This will cause gaping in fillets, one of the common quality abnormality in the fish industry. Warmer fish is softer leading to faulty slicing and cutting in filleting process and with pressure causing increased fluid losses.

9.3.4.1 Production Yield

Around 250 – 300 kg of fish, sub chilled and traditional, were weighed after gutting and storage for 3-4 days; cod for three days and salmon for four to six days. Weighing accounting were kept through production, after heading, filleting, skinning (cod) and trimming. For record the heading yield, filleting yield, and product yield were kept

9.4 Bleeding

The temperature effects on bleeding was tested on cod, to find out if the sub chilling process could be started during the bleeding process. Samples were taken (experiment #18) on-board Malmey SK 1 using three different temperatures in the Rotex bleeding tank; 6 °C, 2 °C and –1°C. The hauling time during catching was 180 min. and the hauling size was 9.7 tonnes.

Bleeding test were also made on salmon at a salmon farm in Bildudalur, Iceland. Bleeding were tested at four different temperature; 0 °C, 5 °C, 9 °C. The sea temperature was 7 °C.

9.5 Rigor mortis

To groups of salmon and cod were compered, sub chilled and traditional chilled. Same definitions were used for each group as in other tests in this research. The fish was filleted right after slaughter and chilling and kept at defined temperature for each group. For salmon, the sub chilled fillets were stored at -1.5 °C and the traditional chilled one at 0 °C. The sub chilled cod were stored at -0.8 °C and the traditional chilled one at 0 °C. Go-Pro camera was used to film the contractions of the fillets, throughout the rigor process. The time of rigor constrictions and the amount were recorded.

The right fillet of both groups of salmon and cod were detached from the vertebrae just after slaughter and chilling but the left one kept on. After the rigor process, the left fillet was cut off and the difference of contraction from the right fillet were compered and photographed (Figure 33).

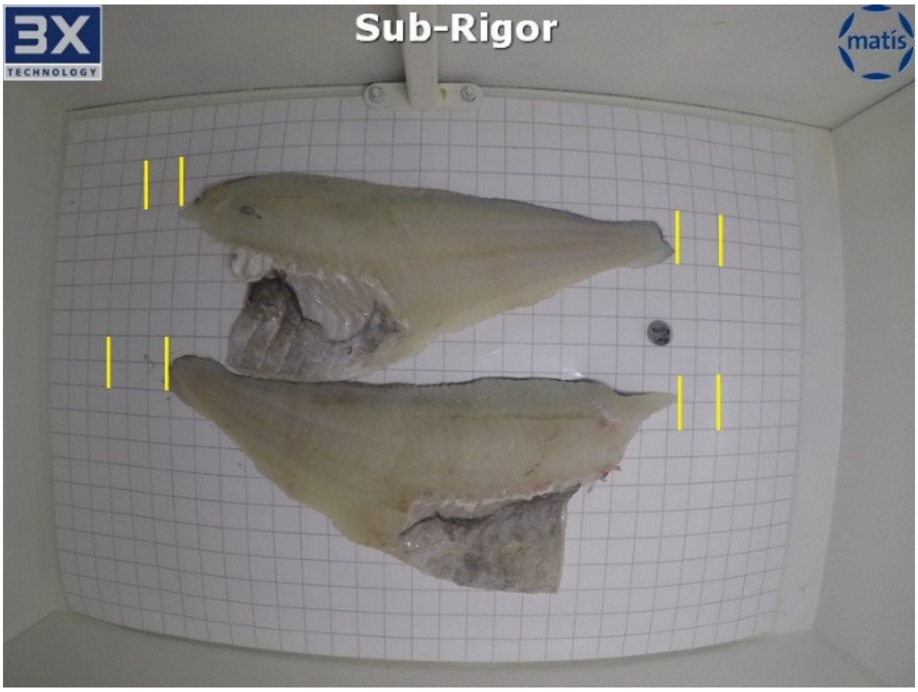

Figure 33. **Fillets filmed in simulation unit through the rigor mortis process.**

10 Appendix II – Theoretical discussion

The sub-chilling process is the method of preserving food by partial ice-crystallization. With proper implementation, the procedure could become one of the most important parameters that define the quality of fresh fish products during production and storage (Bahuaud *et al*, 2008) (Fernandez *et al*, 2008) (Kiani & Sun, 2011). The partial freezing, or sub-chilling, of products as a preservation process is dependent upon the amount of water that is frozen in the fresh material. These boundaries can range from 5% up to 30% depending on the product type (Kiani & Sun, 2011) but usually the target temperature is around 1-3 °C under the initial freezing point (Duun & Rustad, 2008) (Hemmingsen, 2002).

The crystallization build-up phase is caused by molecular aggregation in the solution, forming nuclei and then subsequent crystal growth (Delgado & Sun, 2012). The overall process can be divided into two stages. Firstly, the temperature of the respective products has to be brought down to the initial freezing point. Secondly, additional energy has to be removed from the product to reach the pre-determined crystallization temperature with respect to 5-30% of the water being frozen (Kaale L. *et al*, 2013).

Reaching the limit of 30% water being frozen in the products is the absolute boundary with regards to sub-chilling. This is due to the fact that with higher ratio of water being frozen, factors contributing to lower quality in sub-chilled food become more prominent. This becomes most relevant when reviewing the effects of ice crystal formation within the cells of the products but limiting the formation of these crystals is the most important factor in reducing muscle damage and maintaining quality (Kaale L. D., 2014) (Petzold & Aguilera, 2009). Faster chilling produces better products, as the freezing rate of the water affects the size of the ice crystals forming in the muscles and, therefore, the overall quality (Kaale L. D. *et al*, 2013).

By optimal sub-chilling of the product, the quality is promising with almost the same sensory attributes and nutritional value as the original product. This factor becomes important considering that fish has for a long time been renowned for its peculiar taste which often is simply due to incorrect product handling. With insufficient cooling prior to processing as well as no active procedures to improve quality, bacteria and enzyme growth will accelerate and contribute to these undesirable traits. By improving the logistics of the processing and transportation of fish, these problems can be reduced notably (Magnussen O. M. *et al*,2008).

Keeping temperature fluctuations during storage as low as possible is essential to maintain the product quality after proper cooling, as ice crystals have a natural tendency to grow with increased storage time (Alvarez, 2009);(Hagiwara *et al*, 2002). While most laboratories can maintain ambient temperatures at +/- 0.5 °C from a given average, the deviation must be brought down to at least +/- 0.3 °C from average in order not to affect the ice crystal formation that took place during cooling (Kaale L. D., Eikevik, Bardal, Kjorsvik, & Nordtvedt, The effect of cooling rates on the ice crystal growth in air-packed salmon fillets during superchilling and superchillde storage, 2013). High fluctuations in temperature cause fusing of smaller crystals to larger ones due to repeated melting and freezing (Kaale L. D. *et al*, 2013b) ; Pham & Mawson, 1997; Roos, 1995; Zaritzky, 2012; (Hagiwara *et al*, 2011) (Hagiwara, Suzuki, & Takai, 2002). This will in turn have negative effects on the quality as the location and size of the crystals is a key factor (Martino & Zaritzky, 1986); (Alizadeh *et al*, 2007) Alizadeh, Chapleau, Lamballerie, & Bail, 2007).

The Atlantic salmon is a highly delicate product, as well as most fish species, when it comes to chilling and storing. When adequately cooled and stored on ice, the shelf life of fresh salmon can range up to 21 days (Thordarson, Karlsdottir, Pedersen, Johannsson, & Hognason, 2015). This storage time can be extended should the products be sub-chilled and stored at respective temperatures (Huss, 1995) (Ronsivalli & Baker, 2014) (Duun & Rustad, 2008). The most notable losses of quality during chilling are associated with the loss of functionality of proteins as well as the aforementioned ice crystal formation (Kaale L. D. *et al*, 2013b). General findings of (Thrordarson *et al*, 2015) were that according to microbial-analyses, the storage life of salmon fillets can be extended up to 5 days by effective precooling and sub chilled storage.

By measuring the water holding capacity (WHC) of the fish, these degrading quality effects can be quantified to some extent. WHC is the muscles' 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). Much of the water is entrapped in the structures of each cell, including intra- and extra myofibrillar spaces and therefore, all changes that the intracellular architecture of the cells experience will affect the muscle's ability to retain water (Huff-Lonergan & Lonergan, 2005). WHC 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). The WHC generally decreases with more water being frozen in the muscle during the chilling process but the formation of the ice within the muscle is largely dependent upon how rapid the chilling process is. It has been observed that large intra- and extra-cellular ice crystals form at the borders of myofibrils in sub-chilled fillets, reducing the overall quality compared to controlled fillets (Bahuaud *et al*, 2008). However, it should be noted that those fillets were being chilled by blast-air current at -25 °C, lasting 45 minutes. With higher sub-chilling rates, an even distribution of fine ice crystals inside and outside cells can be observed, leading to higher product quality (Clevalier *et al*., 2001; Dincer, 1997; Fernandez *et al*., 2008; Kiani & Sun, 2011; Martino & Zaritzky, 1986; Martino *et al*., 1998; Petzold & Aguilera, 2009). Furthermore, sub-chilling should not affect the WHC notably, as the drip loss experienced with sub-chilled salmon fillets stored at -1.4 °C and -3.6°C, was observed to be 1-2% and less than 0.3% of total sample weight, respectively, according to (Duun & Rustad, 2008).

Minimizing drip loss is therefore an essential part of maintaining the product as close to its original state as possible. With higher drip loss the product becomes visually unattractive, it loses soluble nutrients and flavour compounds as well as there is an economy loss during transport. Changed texture of the muscle can also contribute to higher drip and therefore the state of the fillets prior to chilling is determinant on the final product. Chilling the fish as soon as possible after catching or slaughtering is essential as well as the handling during processing is important to be able to deliver high quality end products to the market. That will, in turn, deliver better products with higher WHC to the consumer, resulting in less water loss during cooking and better textures.

When transporting the sub-chilled fillets to the market, the partial formation of ice crystals inside the muscles acts as a buffer for the thermal loads the fillets might experience along the way. By letting these ice crystals handle the miniature temperature fluctuations, the need for ice during transport is eliminated. This has a positive effect on the logistics of fresh fish transportation, as higher quantities can be shipped with higher quality products. The miniscule drip observed in sub-chilled fillets by (Duun & Rustad, 2008), also implies that the weight loss during transport of sub-chilled fillets is of a small degree.

The increased firmness of the fillets after sub-chilling is also an advantage. Because of the fact that fish live in almost zero-gravity environment contrary to land animals, the meat is much more tender and delicate. This raises some complications during processing and transportation as it is easy to spoil the meat by incorrect handling. By sub-chilling the fish and, at the same time, increasing the ratio of frozen water in the muscles, the meat becomes firmer, giving it advantage through the production. Project research shows substantial difference in quality and yield in processing cod and salmon by using the method (Thrordarson, Karlsdottir, Pedersen, Johannsson, & Hognason, 2015). More of the cod ends up in the exclusive mix of products on the market giving it a better value.

By sub-chilling, both microorganism growth and enzymatic build up will slow down spoilage processes, prolonging shelf life and quality. Lower temperatures have also been shown to prevent toxin producing microorganisms' growth and thereby increase food safety (Magnussen O. M. *et al*, 2008). Therefore, optimal cooling followed by correct product handling and packaging will reap excellent quality food products. Sub-chilled salmon stored at -2 °C while being packaged by atmosphere packaging managed to show good qualities for 24 days based on both sensory and microbial analyses (Siversvik, Rosnes, & G., 2003).

Moreover, studies show that notably larger ice crystals seem to form in fillets post rigor mortis than prior. This indicates the importance of chilling as soon as possible after the fish is slaughtered (Kaale L. D., 2014). Immediately after death, specific chemical reactions start occurring within the fish muscle, gradually, the muscle tightens and the fish becomes stiff. Low levels of ATP (adenosine triphosphate), the energy of the muscle cells, is the reason for why the rigor happens. ATP is produced in the presence of oxygen so when the organism dies and the flow of oxygen stops, the ATP will diminish (Thordarson *et al*, 2016). The chilling is important with respect to the fact that it slows down the rigor process and while normal chilling at 0 °C shows good results, the sub-chilling produces even better quality products in this regard with less contractions of muscle fibres being observed (Thordarson *et al*, 2016). This minimizes the gaping often formed in fish muscles during rigor mortis due to contraction forces between the muscle layers and the vertebrae. The rigor process affects the WHC as well, as large contractions in the fish muscle can reduce the space for water being held in the myofibrils and fluid is forced into the extra myofibrillar spaces, and is therefore more easily lost as drip. Lateral shrinkage of myofibrils during the rigor process can impact the whole cell if proteins linking myofibrils together and to the cell membrane are not degraded. Therefore, limited degradation of cytoskeletal proteins may result in increased shrinking of the overall muscle cell, which is ultimately translated into drip loss (Huff-Lonergan & Lonergan, 2005). Postponing the end of the rigor is also desirable due to the fact that the muscle does not start to spoil due to bacteria until after the rigor process. By elongating the progression of the rigor and bleeding the fish adequately, the products can therefore stay fresher for longer periods of time (Sveinsdóttir, 2016). By sub-chilling and storing fillets of salmon at -1.5 °C, a shelf life of at least 15 days has been achieved but this fact is an important parameter regarding transportation over long distances (Olafsdottir *et al*, 2006).

For sub-chilled products, it is assumed that less than 30% of the water within the muscles is in a frozen state but for salmon, this is at the range of 5-15% for 70% water content of muscle (Rha, 1975). Having a higher ratio of frozen water would result in damages on a microscopic level, as mentioned earlier. For salmon, this temperature is usually around -1.5 °C, even though the distribution of water within the salmon is not equal (Thrordarson *et al*, 2015). Dealing with Cod in this regard is simpler, since its water content is relatively even throughout the whole volume, but sub-chilling temperatures for cod are in the range of -0.8 °C to -0.9 °C. The Sub-Chill project (Sub-Chill of Salmon, 2015) shows that unique combination of temperature control during bleeding and sub-chilling of salmon in pre-rigor state will give excellent quality products. It is important to note that when dealing with these sub-chilling temperatures both for cod and salmon, the target temperatures are not absolute freezing temperatures but rather the point at which the first ice crystals start to form. This key aspect has sometimes been ignored and the fish considered fully frozen at these temperatures. It is therefore key to realize that the cod has the initial freezing temperature of roughly -0.9°C (Rahman, 2009) and therefore temperatures lower than -1°C should never be achieved if the fish is to be sub-chilled in modern industrialized applications (Valtýsdóttir *et al*, 2010) (Margeirsson, Bjarnason & Arason, 2013). The same goes for salmon, with the initial freezing temperature of -1.5 °C, approximately.

The successful result of the sub chilling methodology could be important for the Nordic marine industry, fishing and aquaculture, by gaining strong market position on the fresh food market. The future is in the fresh fish industry by distinguishing from the bulk of frozen products on the market but a well-controlled cold chain is essential. The sub-chilling method offers fresher products with longer shelf life, less hint of spoilage, fish smell and taste. Sub-chilling will also increase the yield by protecting weight losses during transport through excessive drip and by having part of the water inside the muscle frozen, a buffer is created to deal with amber temperature fluctuations during transport and storage. With fish being an important source of proteins, lipids and other nutrients and with consistent rise in high-paying customers expecting high quality products, the need for a way to get these sub-chilled products on the market is high. A successful implementation of these processes on an industrial scale with a secure cool-chain and stable temperatures during storage and transportation will therefore strengthen the market position of fresh fish in the future.

11 Appendix III – Research results

11.1 Salmon

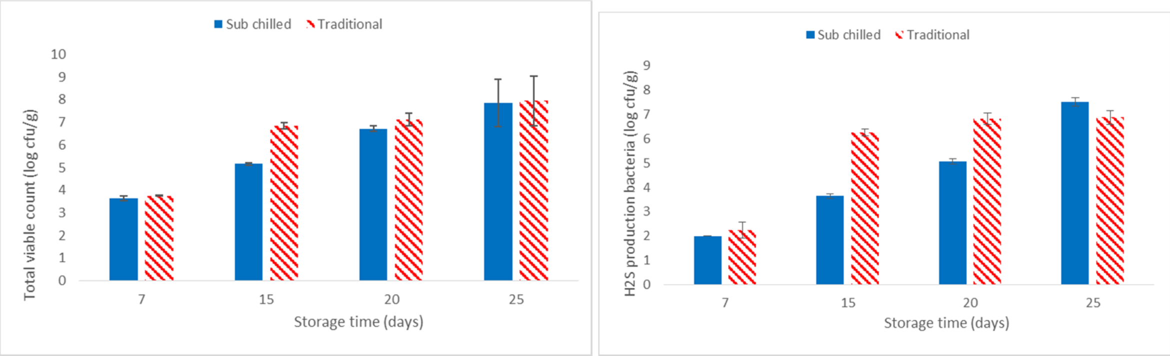

Figure 34. Total viable count and H_2S producing bacteria (log cfu/g) of sub-chilled and traditional salmon fillets from experiment #12, stored for up to 25 days from slaughtering (n = 2; Mean±SD). Sub-chilled fillets were kept at around -1 °C the whole time, while traditional fillets were chilled with ice and kept at 1 °C the whole time.

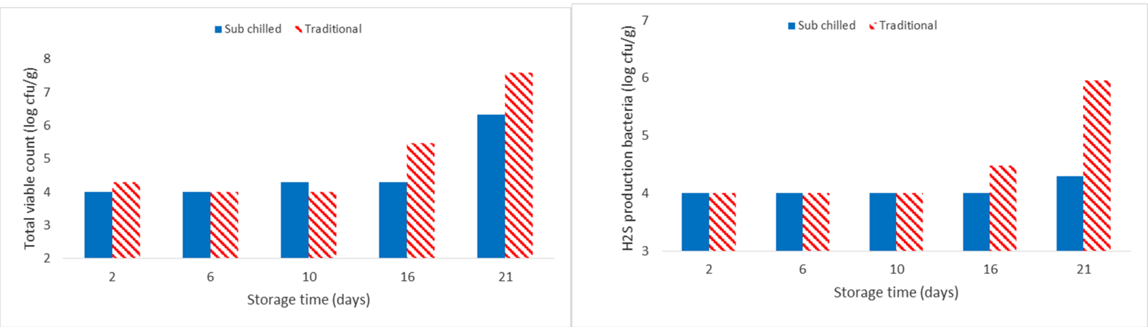

Figure 35. Total viable count and H_2S producing bacteria (log cfu/g) of sub-chilled and traditional salmon fillets from experiment #2, stored for up to 21 day from slaughtering (n = 1). Sub-chilled fillets were kept at around -1 °C the whole time, while traditional fillets were chilled with ice and kept at 1 °C the whole time.

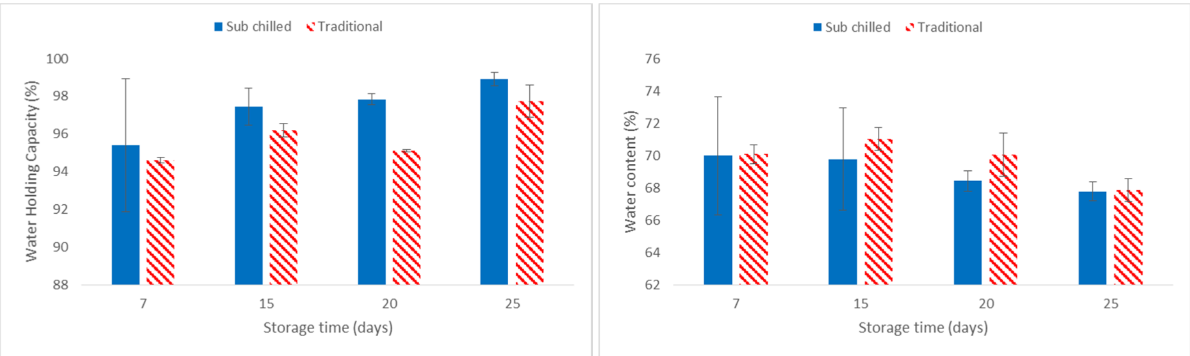

Figure 36. Water holding capacity (WHC; %) and water content (%) of sub chilled and traditional chilled salmon fillets from experiment #12. Sub-chilled fillets were kept at around -1 °C the whole time, while traditional fillets were chilled with ice and kept at 1 °C the whole time (n=2; Mean ±SD).

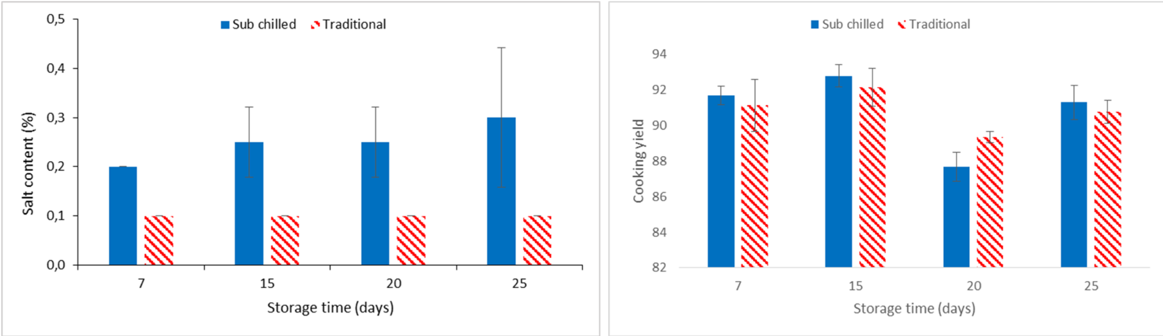

Figure 37. Salt content (%) and cooking yield (%) of sub chilled and traditional chilled salmon fillets from experiment #12. Sub-chilled fillets were kept at around -1 °C the whole time, while traditional fillets were chilled with ice and kept at 1 °C the whole time (n=2; Mean ±SD).

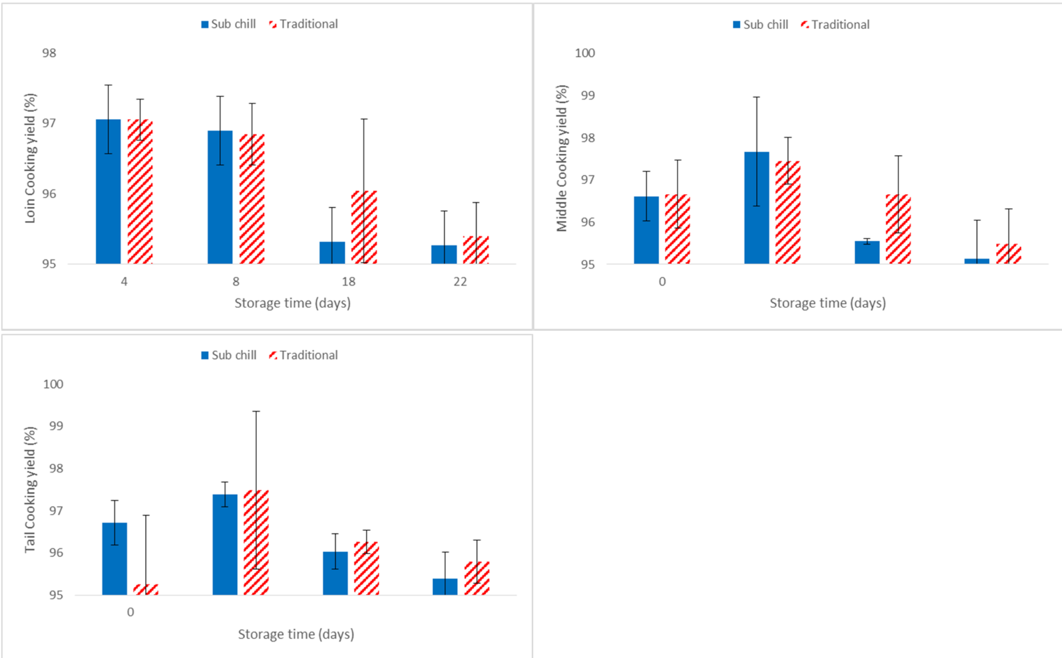

Figure 38. Cooking yield (%) of loin, middle and tail part of sub chilled and traditional chilled salmon fillets from experiment #2 (n = 3 Mean ±SD). There is no significant difference in cooking yield between sub chilled and traditional salmon. This indicate that the tail, thinnest part of the fillet is not freezing in the chilling process.

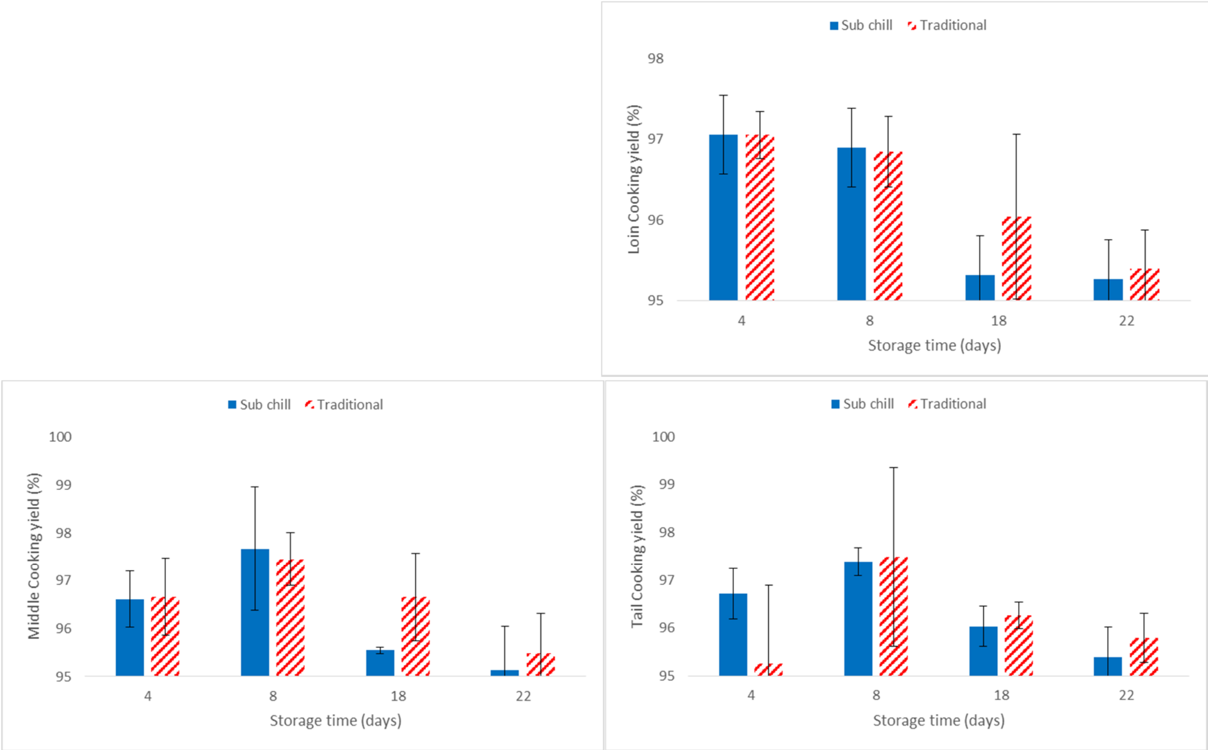

Figure 39. Cooking yield (%) of loin, middle and tail part of sub chilled (SC) and traditional chilled (trad) salmon fillets from experiment #3 (n = 3 Mean ±SD). There is no significant difference in cooking yield between sub chilled and traditional salmon. This indicate that the tail, thinnest part of the fillet is not freezing in the chilling process.

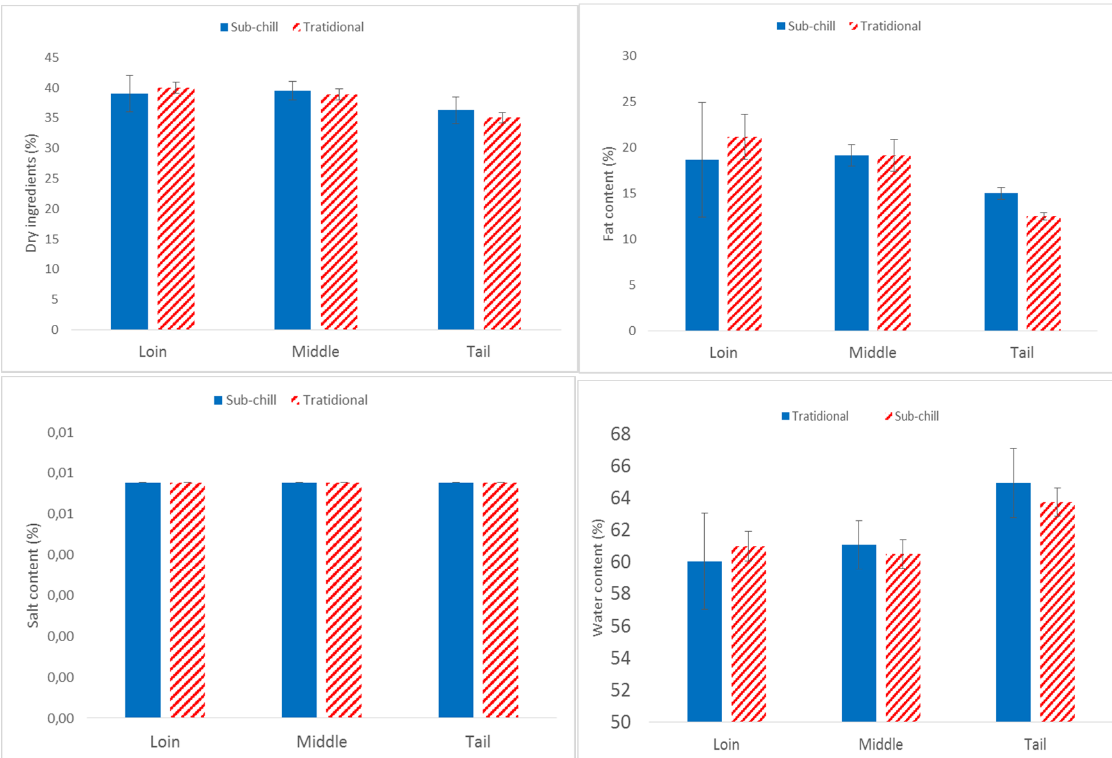

Figure 40. Dry ingredients (%), fat content (%) salt content (%) and water content (%) of sub chilled and traditional chilled salmon fillet from experiment #2. The analysis were performed on the loin, middle and tail part of the salmon fillet (n = 3 Mean ±SD).

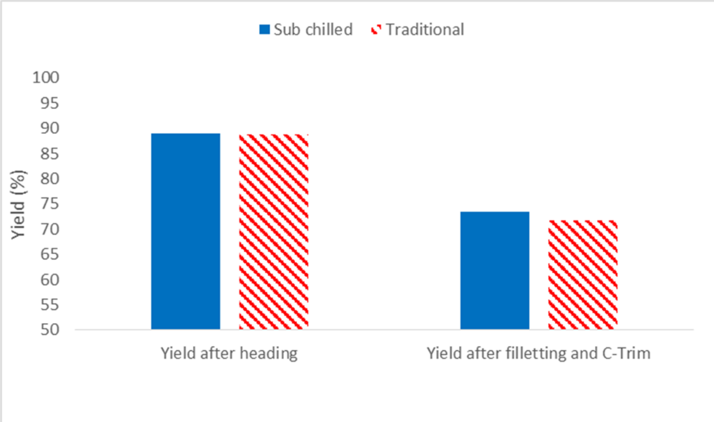

Figure 41. Yield test was made from 2x300 kg of salmon of each group, sub chilled and traditional (n=1) from experiment (3). Yield after heading and also after filleting C trim. The yield of sub chilled were slightly better.

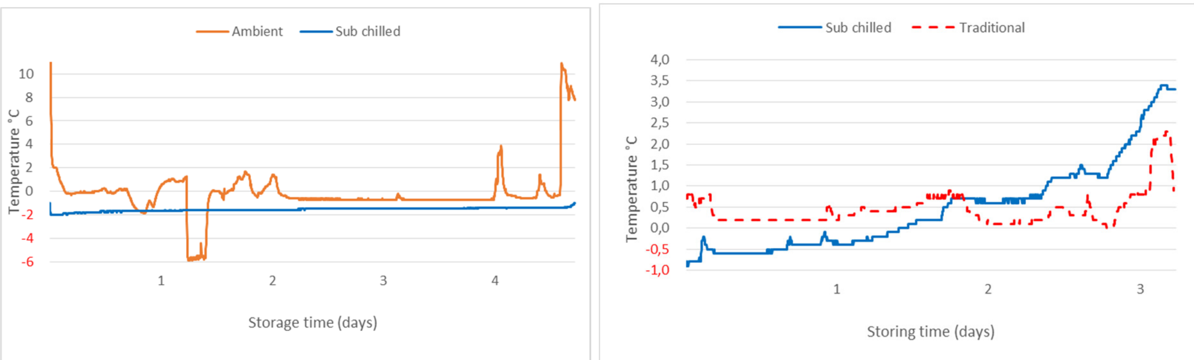

Figure 42. **Temperature profiles during transportation of sub chilled and traditional chilled salmon, including ambient temperature, from Norway to Dubai (to the left; experiment #16) and from Iceland to San Francisco (to right; experiment #17).**

11.2 Cod

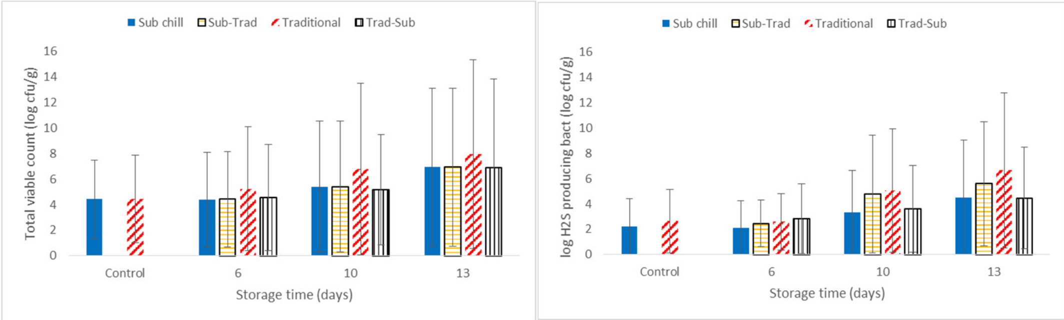

Figure 43. **Total viable count and H_2S producing bacteria (log cfu/g) during 13 days of storage of four different experimental groups within experiment #1: Sub chilled after catching and throughout processing and storing; sub chilled after catching and stored at 0 °C (SC + Trad); traditional chilled with ice after catching and sub-chilled during storage (Trad + SC); and traditional chilled after catching and throughout processing and storing. (n=2; Mean±SD).**

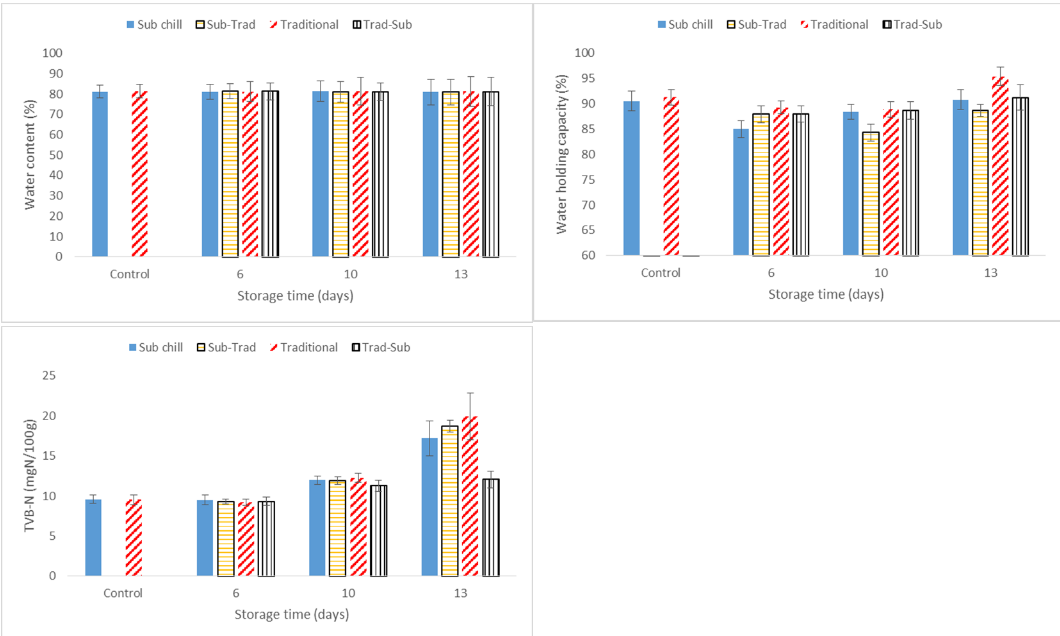

Figure 44. **Water content (%), water holding capacity (%) and total volatile basic nitrogen (TVB-N; mg N/100 g) during 13 days of storage of four different experimental groups within experiment #1: Sub chilled after catching and throughout processing and storing; sub chilled after catching and stored at 0 °C (SC + Trad); traditional chilled with ice after catching and sub-chilled during storage (Trad + SC); and traditional chilled after catching and throughout processing and storing. (n=3; Mean±SD).**

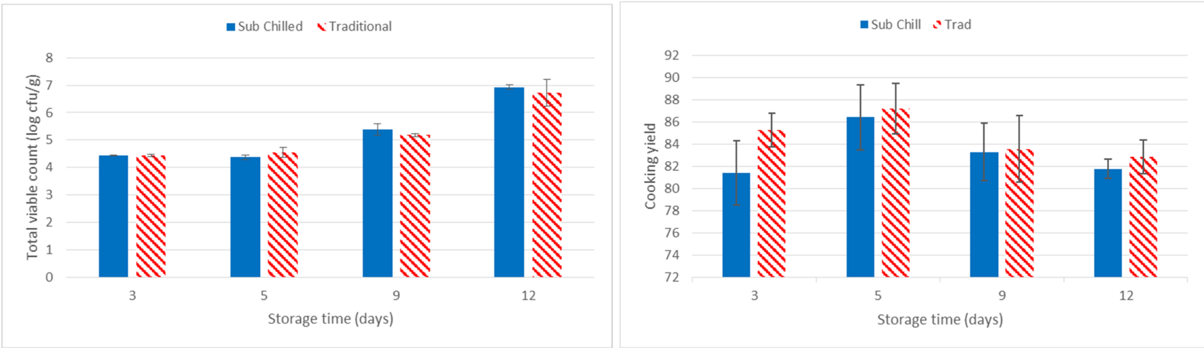

Figure 45. **Total viable count (log cfu/g) and cooking yield during 12 days of storage of two different experimental groups within experiment #1: Sub chilled after catching and throughout processing and storing; and traditional chilled after catching and throughout processing and storing. (n=2; Mean±SD).**

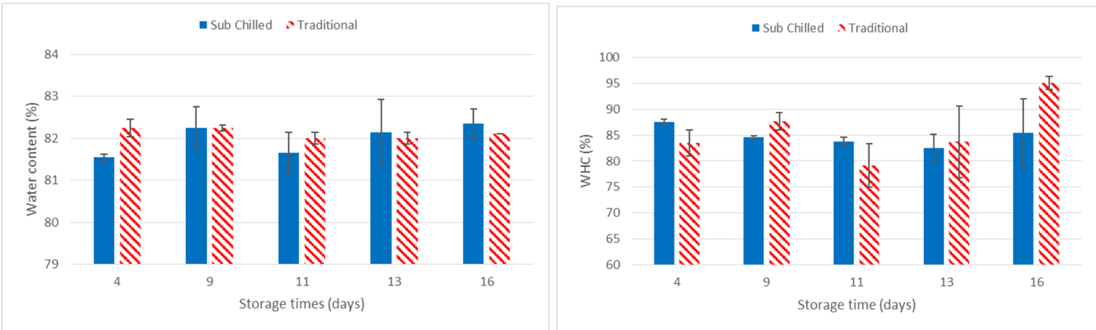

Figure 46. Water content (%) and water holding capacity (WHC; %) during 16 days of storage of two different experimental groups within experiment #2: Sub chilled after catching and throughout processing and storing; and traditional chilled after catching and throughout processing and storing. (n=2; Mean±SD).

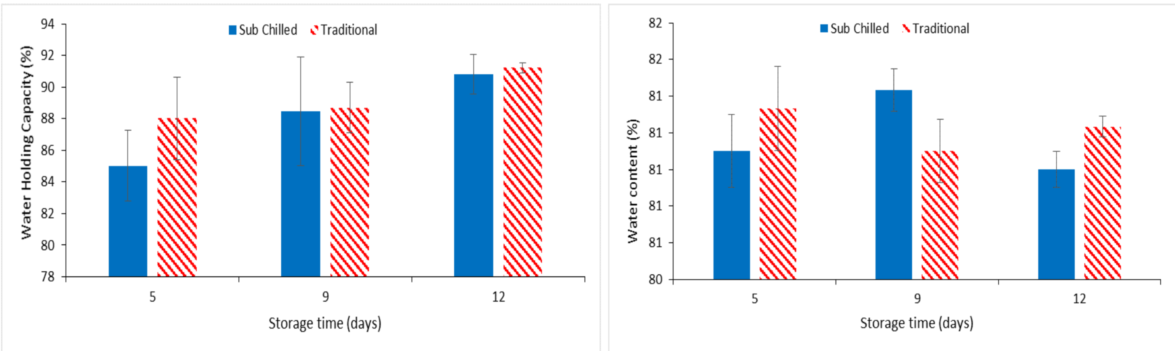

Figure 47. **Water holding capacity (WHC; %) and water content (%) during 12 of storage of sub chilled and traditional chilled cod from experiment #1. Sub chilled after catching and throughout processing and storing; and traditional chilled after catching and throughout processing and storing. (n=2; Mean±SD).**

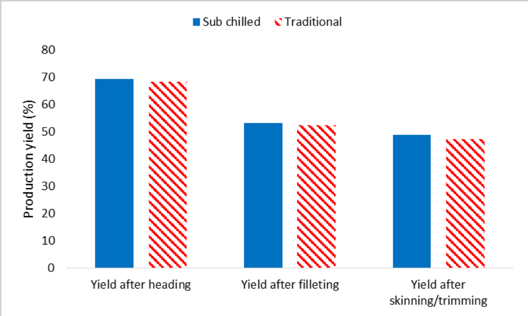

Figure 48 Cod experiment #3 product yield taken from 300 kg lot of each group; yield after heading, filleting and skinning/trimming.

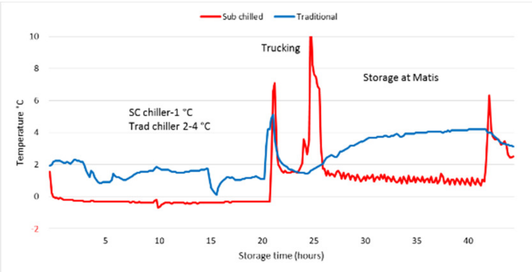

Figure 49 Cod experiment #3 Temp logging of ambient temp for sub chilled and traditional groups