A comparative study on quality, shelf life and sensory attributes of Atlantic salmon slaughtered onboard slaughter vessel against traditional land-based facilities

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Spring 2021

Acknowledgement

This master thesis was written as part of my education in Aquaculture at the University of Bergen. It was written in collaboration with Nofima, which allowed me to participate in an exciting research project funded by Regional Research Fund (RFF-Vest) (project ES612950). I would like to thank the employees in both Hav Line AS and Sekkingstad AS for their help with fish processing and transportation. I would also like to thank the team at Nofima AS, Stavanger namely Trond Karsten Løvdal, Bjørn Tore Rotabakk, Karin S Tranøy, Laila Budal, Sigurd Øines and Gro Haugvaldstad Kleiberg for their technical work, as well as Aase Vorre Skuland and Mette Risa Vaka in helping with the sensory analysis.

Writing this master thesis has been both challenging and exciting. It required a lot of effort and hard work in the writing process, and in the laboratory and fieldwork. It allowed me to work with something very relevant within the future of aquaculture, and it gave me a great amount of new knowledge. The thesis mainly consists of two published papers where I am the lead author in the first paper and a co-author in the second paper. The research project also gave me the opportunity to work alongside Sherry Stephanie Chan, a PhD student at NTNU, on one of her projects with Hav Line. I was therefore listed as one of her co-authors in her paper. In this regard, I have many people to thank for good cooperation and support along the way.

First, I would like to thank my supervisor from Nofima, Bjørn Roth, for his good guidance and high availability during the assignment. He has always been approachable, and I always received help whenever needed. I would also like to thank Sherry Stephanie Chan for her good cooperation, guidance, and conduction of the trial. I have learned so much and she is a big inspiration, so I am so thankful for getting the opportunity to work with her. Finally, I would like to thank my supervisor at UiB, Sigurd Olav Handeland, for his good guidance and high availability whenever I needed help.

Abstract

This thesis aims to compare two different slaughtering and storage methods of fish and study how these affect the quality and shelf-life of farmed Atlantic salmon. It is divided into three sections; The Hav Line method, Paper 1 and Paper 2. The Hav Line method introduces the slaughter vessel Norwegian Gannet and how its slaughter method can improve various challenges within the aquaculture industry. These include environment, infection and escape risk, fish health, better and longer quality and shelf life of the fish, and reduced waste and transport costs. Paper 1 investigates the quality and shelf life of whole Atlantic salmon slaughtered onboard Norwegian Gannet and stored in -0.8 ^{o}C refrigerated seawater (RSW) tanks compared to traditional land-based slaughtering facilities storing fish on ice. The shelf life and quality were measured on fresh and cold-smoked fillets including blood spot counts, fillet gaping, texture hardness, microbial counts, Quality Index Method (QIM) and sensory analysis. No significant differences were detected in blood spots counts nor texture hardness. Fresh fish slaughtered onboard the vessel had significantly lower QIM scores, fillet gaping scores, total mesophilic counts and H_2S producing bacteria at the end of storage (21d) than those from the facility. In Paper 2, the effect of different chilling technologies on quality and water holding parameters was investigated on Atlantic salmon throughout the entire value chain. In this study, all fish were slaughtered onboard Norwegian Gannet and divided into four different chilling methods; whole fish superchilling by RSW (S) or ice (I), followed by fillet chilling with liquid nitrogen (SS, IS) or ice (SI, II). The shelf life and quality were measured on fresh and cold-smoked fillets, including blood spot counts, fillet gaping, QIM, drip loss, water holding capacity and water content, colour and texture analysis, cathepsin B and L analysis and microbiological counts. Fish stored in RSW had lower H_2S producing bacteria for raw fillets, and lower gaping and blood spot counts after smoking. Firmness, breaking force and water holding capacity were higher for smoked than raw fillets, while colour parameters, muscle pH and water content were higher for raw than smoked fillets. Both papers concluded that fish slaughtered onboard vessels like Norwegian Gannet and transported in superchilled RSW presents good quality and improves shelf life over time.

Content

The Hav Line Method; Slaughtering at Sea

1. Background/Introduction

Over the last four decades, the salmon industry has gradually moved from a small side production in Norwegian agriculture to a big industry across the globe. The demand for salmon is high, and the list of guidelines and requirements to follow is long. Sustainability in the aquaculture industry is also receiving increased attention from farm to fork, which poses many challenges ranging from using small pelagic species as feed to utilizing the fresh cut and by-products from the farmed fish production (Naylor et al. 2021, Tacon and Metian. 2009).

Today the aquaculture industry faces two major problems: infestation of sea lice and high mortality (Naylor et al. 2021). Increasing the efficiency of the industry and focus on animal welfare and quality are significant steps towards solving the problems related to the increased health problems and mortality rate. Furthermore, the increase in Norwegian fish export imposes a greater demand for logistics transport to deliver good quality fish. Most of the seafood is currently being transported to Europe by road and to Asia by air transport (Rotabakk et al. 2020). Still, there are negative environmental, operational and financial impacts associated with these transportation methods. One way of contributing to more sustainable practice and significant socio-economic profitability in aquaculture is to move the slaughterhouses from land to sea. There are already existing vessels that stun and bleed the fish on site before it is transported to the land-based factory (Midling et al 2011), therefore utilizing the sea transport along the Norwegian coastline on a larger scale. This contributes to solving the following issues; environment, fish health, infection and escape risk, reduced transport costs, improved quality of the fish, achieve a longer shelf life of the fish and reduced waste during transport and in waiting cages (Midling et al. 2011, Skare et al. 2021, Chan et al. 2020, Philis et al. 2019, Rotabakk et al. 2020). To address the mentioned challenges within the aquaculture industry the Hav Line group AS has introduced a new concept of “The Hav Line method”.

2. Hav Line – Norwegian Gannet

The main idea of the Hav line method is to harvest fish directly from the cage before slaughtering the fish onboard the world´s largest slaughter vessel, Norwegian Gannet (Figure 1). The fish is then superchilled in refrigerated seawater (RSW) tanks onboard with ice slurry to below 0 ^{o}C, immediately after slaughter. This vessel (94 m long, 18 m wide) uses a diesel-electric hybrid engine (Wartsila, Norway) to reduce CO_2 emissions and contains 8 electric stunning machines, 14 gutting machines and 10 RSW tanks (Fishfarmingexpert.com, 2019). One load from the vessel can deliver as much as 1000 tons of fish to be transported to Hirtshals, Denmark, within 80 hours for further processing and delivery (Hav Line Metoden, 2018). The Hav Line method has been developed to create an innovation. Its goal is to contribute to the sustainability of the aquaculture industry by improving the traditional way of handling, slaughtering and transporting of farmed fish. The method can therefore deliver a product of high quality, as the welfare of the fish has been put into focus.

Figure 1; A picture of Norwegian Gannet (Retrieved from: own)

3. Fish welfare and Quality

Good fish welfare is an important prerequisite for good fish health, good quality, good profitability and low mortality and is gaining increased attention from both producers and consumers (Noble et al. 2018). Good welfare in aquaculture means that the fish showed normal behavior, have a high growth rate and remain healthy (Huntingford and Kadri, 2014). The Animal Welfare Act has requirements for good fish welfare, where several regulations are provided for both the establishment and operation of aquaculture facilities, as well as for the slaughter of farmed fish (Mattilsynet, 2021). Although the aquaculture industry is closely regulated, large numbers of fish can be injured, stressed or die during slaughter procedures and transport due to misfortunate actions. This can further lead to severe adverse effects such as lower quality of the product and large amounts of declassified fish (Santurtun et al. 2018).

Statistics show that an average of 24,000 salmonids gets declassified every year at the slaughterhouses, and the mortality rate has had an average of 43,000 deaths annually for the last decade (Fiskeridirektoratet, 2021). Based on the Fish Health report in 2020, the total mortality of farmed salmon was 60.3 million fish, where 52.1 million of these were reported as dead fish. Various factors such as bad conditions during transport and slaughter, stress, algal infections and infectious diseases are problems that contribute to these results every year (fiskehelserapporten, 2020).

The procedures involved in pre-slaughter are recognized as critical points in managing fish welfare and have important effects on meat quality (Lines and Spence, 2012). In the pre-slaughter phase, fish are stocked at high densities and procedures associated with crowding, pumping, transport, and harvest, result in stress from increased physical activity.

Pumping is a central part of fish retrieval but has been shown to cause stress. The problem with how fish is transferred today is not about the pumping itself but also the circumstances during the transfer. The environment around the process needs to be adapted to improve fish welfare during the pumping. The process with the most significant challenges considering applying stress to the fish is the crowding process. In the study of Roth et al. (2012), it was shown that increased pumping capacity would relieve the crowding density and thus also stress, giving a physiological status that is much better for the fish

in the cage. Based on Skare and Hernar (2019), lactate measurements were taken during the slaughter process onboard the slaughter vessel. The results showed fish in physiological balance post crowding, pumping and electrical stunning. This forms a belief that a large pumping capacity contributes to good welfare and less stressed fish since the need for crowding is reduced. Thus, fish welfare has major significant potential for improvements as the aquaculture industry will continue to expand, and several aspects of how fish are farmed are likely to change. Meeting biological needs, stock monitoring, and environmental control are all increasingly challenging technologically but needs to be prioritized to improve fish welfare (Huntingford and Kadri, 2014). These challenges are addressed during the recent development of the innovative Hav Line method.

Physiological factors such as increased glucose, chloride levels and plasma cortisol are used to determine the degree of stress in animals (Fantini et al. 2020). However, stress can make the metabolism more anaerobic, resulting in lower glycogen content, faster decrease in pH and an early onset of rigor mortis. Monitoring this process is crucial for the technical and sensory quality of the meat and shelf life. The sea temperature and how the fish are treated before, during and after slaughter will affect the time fish goes into rigor (Balevik and Slinde, 2004, Skare and Hernar, 2019). Filleting can only be carried out successfully when the fish is in pre- or post-rigor condition. Stress is, therefore, a harmful factor for the aquaculture industry. The onset and strength of rigor mortis affect the fillet quality due to faster autolysis and greater ruptures in connective and muscle tissues (Ageeva et al. 2018).

A proper stunning procedure is required to render fish unconscious before slaughtering to ensure good welfare and quality. There has been a rapid development of various anesthesia and killing methods over the last 10 years, either using electricity or percussive stunning (Grimsbø, 2016). Today, electricity is the most common method for stunning farmed fish, which is also practiced onboard Norwegian Gannet. Previous studies showed that electricity has an advantage as a fast and effective anesthesia within 0.5 seconds (Lamboojii et al 2010, Roth et al 2003). Still, there is a risk of spinal injuries and disruptors of large aorta and veins, which are the most severe consequences that can downgrade the fillet quality (Lamboojii et al 2010, Roth et al 2003). The risk depends on the types of current, strength and frequency. This also applies to percussion machines, when the risk to fish welfare and quality lies in the missing

punch percentage in automated systems (Lamboojii et al 2010). Although fish can be stunned unconscious within 0.5 seconds with electricity, commercial practices often involves exposing the animal for 5-15 seconds. This ensures a prolonged anesthesia where it is known that the duration of an unconscious condition and the likelihood of mortality increases with electrical current and duration (Robb and Roth, 2003). This is beneficial from a welfare point of view, but could pose negative consequences on the quality. Animals that are stimulated with electricity are known to empty their muscle of adenosine triphosphate (ATP), as well as stimulate anaerobic glycolysis and thus reduce muscle pH, which in turn can lead to an earlier outbreak of rigor mortis, softer texture, higher muscle tension, and higher color loss (Roth et al 2010). Through the Hav Line method, slaughtering the fish by the cage could give a longer pre-rigor time as it involves fewer operations that stress the fish compared to the traditional slaughter procedure (Midling et al 2011).

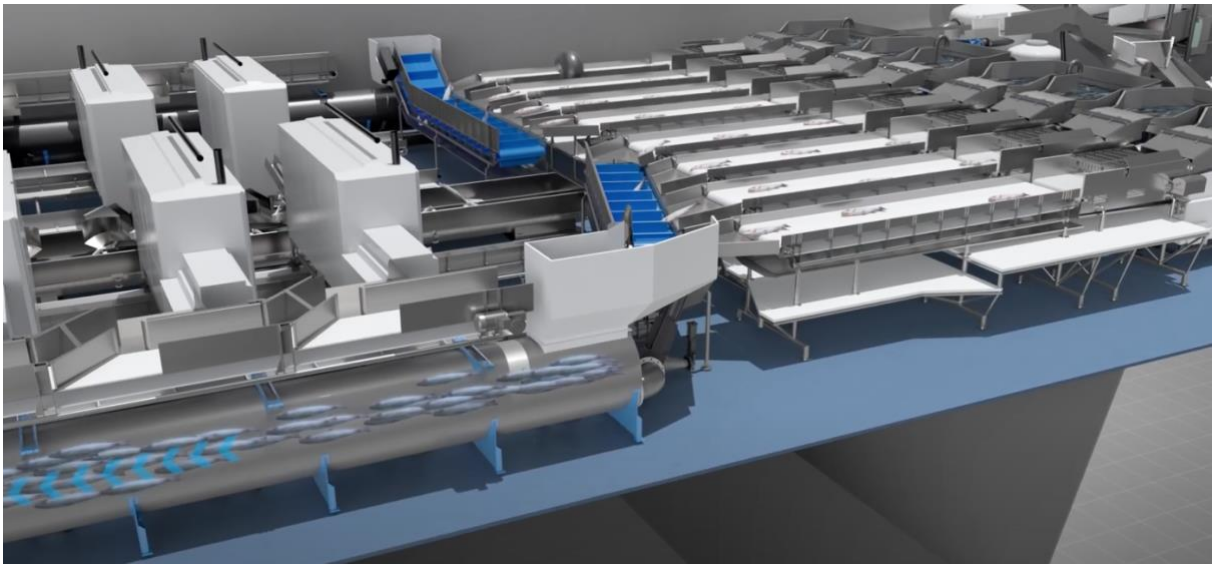

Figure 2; Drawing of the slaughter factory in Norwegian Gannet (Retrieved from: https://ilaks.no/animasjonsfilm-viser-hvordan-hav-lines-slaktebat-skal-fungere-i-praksis/)

The fish can have an overall better slaughter process (Figure 2) using the Hav line method. In a study where fish was directly pumped to Norwegian Gannet, the initial pH was found to be 7.22 (Chan et al 2020, Skare and Hernar 2019), which was within the same range as found in previous studies on unstressed fish (Lerfall et al 2015, Hultmann et al 2012, Erikson et al 2016). The lactate values in the study of Skare and Hernar 2019 were 1.70 mmol/L in contrast to Lerfall et al 2015, who measured 0.37 mmol/L from an uncrowded group in the seacage. Lerfall et al 2015 also measured 3.95 mmol/L from a crowded group in the seacage, which continued to increase when the fish were further chilled. These

results indicate that the fish pumped to Norwegian Gannet did not undergo hypoxia or expressed escape responses in the pumping process and therefore had an overall better slaughter process.

The traditional harvesting method involves several crowding and pumping steps through transport and slaughter. For the Hav Line method, the fish only undergoes one crowding and pumping step. The SeaQuest fish pump (SeaQuest Systems, Ireland) used by the vessel provides a large capacity and gently handles fish. The fish will also avoid a long starvation period during transport to land as they are directly slaughtered onboard. Slaughtering by cages provides less stress on the fish and thus better animal welfare and lower mortality.

4. Alternatively use and disease control

Transporting dead fish in closed systems could positively affect the environment in terms of preventing the spread of disease concerning the use of both waiting cages and transport in well boats with open systems.

Infectious diseases are a constant threat to industrialized farming, characterized by the high density of farm animals and farms (Aldrin et al. 2015). The rapid expansion of the aquaculture industry, where open-cage production has increased, has not occurred without challenges regarding the environment, fish health and fish welfare. Pancreas disease (PD) is a viral disease that has become a major problem in the last decade, with significant welfare and economic impacts due to poor growth, reduced harvest quality and high mortality rate (Jansen et al. 2015). These problems indicate that waiting cages cannot be used in the areas with a lot of spread, as the disease can be transmitted both within facilities, and during transport of live fish (Vetinst.no, 2019, Midling et al 2011). This presents an essential factor for using the alternative method of fish slaughter directly by the cage.

If an open system well boat sails through an area contaminated with fish pathogens, or if the boat is loaded with sick fish and sails through an area with fresh fish, contamination can happen (Iversen et al. 2005). A closed system is preferable concerning the water quality in the tanks if it cannot be shown that open transport is biosafe (Rosten, Kristensen, 2010). The size of the fish quantities in the transport tanks

is decisive for the result of the transport. An increase in biomass increases demands on oxygenation simultaneously as total ammonia excretion will increase. The weather affects the stress level of the fish in a well boat (Gunnes et al. 1998). These problems could be avoided with the Hav Line method as the fish is slaughtered before transportation in closed systems.

The vessel has proven to be of great support especially during the toxic algal blooms that hit the sea cages in Northern Norway in 2019 (Salmonbusiness.com, 2019). As the attacks of deadly algae can cause a significant volume in fish mortality, there was a tight race against time to retrieve the fish out from the pens quickly. The vessel could harvest the fish straight out from the cage within a few hours and reduce the damage and mortality rate, adding value to this slaughter method. Norwegian Gannet also sailed to Iceland to help slaughter a great volume of dead fish for Arnalax in Arnafjordur, February 2020 due to bad weather and low sea temperatures that resulted in challenging farming conditions and large amounts of dead fish (Berge, 2020). Therefore, the problems connected to the contamination that occurs if an open system well boat loaded with sick fish sails through an area with fresh fish, or if fresh fish is exposed to an area contaminated with fish pathogens can be avoided with Norwegian Gannet.

5. Transportation of slaughtered and chilled high-quality salmon to market

Superchilling is a food preservation method by partial ice-crystallization that lowers the temperature of the fish between conventional chilling and freezing, maintaining quality and extending the shelf life of food products (Banerjee and Maheswarappa 2019). Subzero storage temperature must be taken into account; if the shelf life of a particular fish product in ice is 14 days, the shelf life at -1, -2 and -3 ^{o}C is predicted to be 17, 22 and 29 days, respectively (Erikson et al. 2011). In relation to food processing, superchilling has presented many advantages. It minimizes labour, energy and transport costs and environmental impact (Kaale et al. 2011). The heat from the interior and the temperature equilibrates within the superchilled product are absorbed by the ice crystals formed at the surface layer. There is no need for external ice around the product during distribution or storage because the small amount of free water converted to ice will be used as an internal cold reservoir (Kaale and Eikevik 2014). In contrast, ice represents 20-30% of the total weight of each box of fish in traditional chilling. This directly incurs

extra costs to both consumers and producers (Magnussen et al. 2008). Therefore, the superchilling technology can reduce transport costs, the need for styrofoam boxes, and the use of a necessary amount of ice.

The superchilling concept has been under continuous development over the last 10-20 years, and today there are several methods of superchilling (Kaale and Eikevik 2014). One method is RSW slurry, a binary system consisting of water with microscopic ice crystals (Chan et al. 2020, Piñ Eiro, C., Barros-Velázquez, J., Aubourg, S.P., 2004). Seawater has a higher transfer coefficient than ice and therefore removes heat at a faster rate and maximizes the contact between seawater and fish, as used in the Hav Line method. As a result, the fish achieves a more even temperature distribution because of the rapid heat transfer. The seawater cleans the gutted fish in the RSW tanks, so that blood remnants do not remain. In addition, tanks are being thoroughly cleaned and process water filtered, ozone-treated and chlorinated after every slaughter process to ensure cleanliness (Chan et al. 2020, Hav Line Metoden, 2018). The RSW tanks (Figure 3) containing seawater onboard the vessel inhibits further growth of microorganisms and opens up the opportunity of transporting the fish to further processing and distribution without the use of ice as the fish is being chilled down to -1^{o}C (Erikson et al. 2011). By doing so, the temperature of the fish is already kept at superchilled conditions during the early stages of the value chain (Chan et al. 2020).

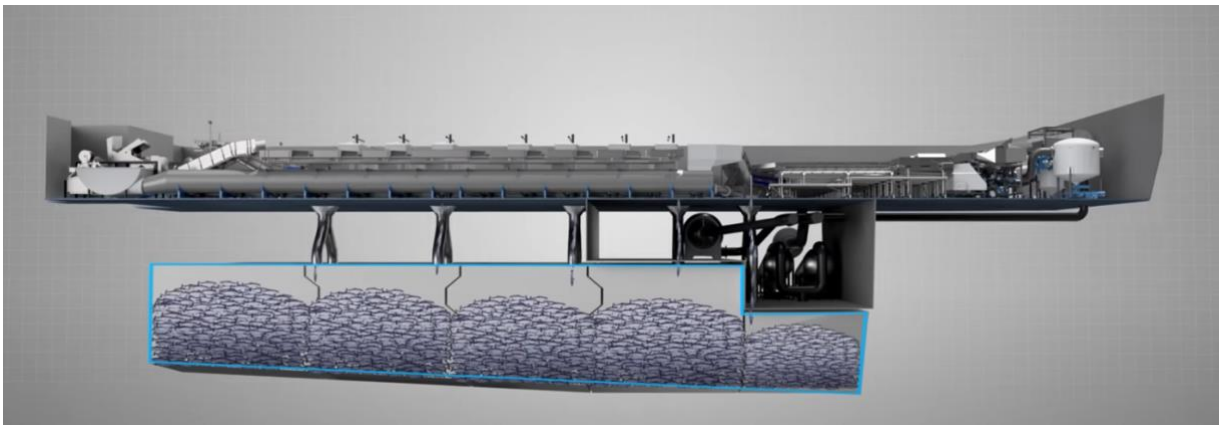

Figure 3; Illustration of the slaughter factory and RSW tanks in Norwegian Gannet (Retrieved from: https://ilaks.no/animasjonsfilm-viser-hvordan-hav-lines-slaktebat-skal-fungere-i-praksis/)

The method ensures pre-packed fish in Hirtshals, and the fish can be further transported by trains to large parts of Europe. The transport emissions will be minimized compared to the traditional method, where the fish is packed at facilities in Norway, transported to Europe then further out internationally. This method saves the roads in Norway from tons of heavy transport a year, which is better for the environment and safer for the population (Skare and Hernar 2019).

6. Concluding remarks

There are multiple benefits mentioned using the Hav Line method, which may potentially revolutionize the aquaculture industry without compromising fish quality. With a shorter harvesting and packing process, the fish can reach the market faster and be ready for delivery from Hirtshals. The transport options are many and efficient from Hirtshals, so it can take less time before the fish is on the market shelves than if it was shipped from Norway. The multiple benefits mentioned using the Hav line method are conductive to improve the welfare and quality of the fish as the escape and spread of disease and degree of stress applied to the fish is lowered. This results in fish being delivered fresher, providing a sustainable product with better quality and longer shelf life.

References

Aldrin, M., Huseby, R.B., Jansen, P.A., 2015. Space-time modelling of the spread of pancreas disease (PD) within and between Norwegian marine salmonid farms. Prev. Vet. Med. 121, 132–141. https://doi.org/10.1016/j.prevetmed.2015.06.005

Banerjee, R., Maheswarappa, N.B., 2019. Superchilling of muscle foods: Potential alternative for chilling and freezing. Crit. Rev. Food Sci. Nutr. https://doi.org/10.1080/10408398.2017.1401975

Beate Balevik, S., & Slinde, E. (2004). *Rigor mortis-dødsstivhet, avgjør kvalitet og økonomisk gevinst*. Havbruksrapport 2004. https://www.imr.no/filarkiv/2004/03/1.9_Rigor_mortis-dodsstivhet_avgjr_kvalitet_og_okonomisk_gevinst.pdf/nb-no

Berge, A., 2020. Høy dødelighet for Arnarlax - "Norwegian Gannet" på vei til Island for å hjelpe med utslakting. URL https://ilaks.no/hoy-dodelighet-for-arnarlax-norwegian-gannet-pa-vei-til-island-for-a-hjelpe-med-utslakting/

Chan, S.S., Roth, B., Skare, M., Hernar, M., Jessen, F., Løvdal, T., Jakobsen, A.N., Lerfall, J., 2020. Effect of chilling technologies on water holding properties and other quality parameters throughout the whole value chain: From whole fish to cold-smoked fillets of Atlantic salmon (Salmo salar). Aquaculture 526, 735381. https://doi.org/10.1016/j.aquaculture.2020.735381

Chan, S.S., Roth, B., Jessen, F., Løvdal, T., Jakobsen, A.N., Lerfall, J., 2020. A comparative study of Atlantic salmon chilled in refrigerated seawater versus on ice: from whole fish to cold-smoked fillets. Sci. Rep. 10, 17160. https://doi.org/10.1038/s41598-020-73302-x

Erikson, U., Gansel, L., Frank, K., Svendsen, E., Digre, H., 2016. Crowding of Atlantic salmon in net-pen before slaughter. Aquaculture 465, 395–400. https://doi.org/10.1016/j.aquaculture.2016.09.018

Erikson, U., Misimi, E., Gallart-Jornet, L., 2011. Superchilling of rested Atlantic salmon: Different chilling strategies and effects on fish and fillet quality. Food Chem. 127, 1427–1437. https://doi.org/10.1016/j.foodchem.2011.01.036

Fantini, L.E., Rodrigues, R.A., Honorato, C.A., Dos Reis Goes, E.S., Ferraz, A.L.J., Ferreira De Lara, J.A., Hanson, T., Meldau De Campos, C., 2020. Resting time before slaughter restores homeostasis, increases rigor mortis time and fillet quality of surubim Pseudoplatystoma spp. PLoS One 15. https://doi.org/10.1371/journal.pone.0233636

Fiskeridirektoratet, Akvakulturstatistikk: Matfiskproduksjon av laks, regnbueørret og ørret, 2021. URL https://www.fiskeridir.no/Akvakultur/Tall-og-analyse/Akvakulturstatistikk-tidsserier/Laks-regnbueoerret-og-oerret/Matfiskproduksjon

Fiskeridirektoratet, Akvakulturstatistikk: Svinn i produksjonen 2017-2019, 2021 URL https://www.fiskeridir.no/Akvakultur/Tall-og-analyse/Biomassestatistikk/Biomassestatistikk-etter-produksjonsomraade/svinn-i-produksjonen-2017-2019

Fiskehelserapporten 2020. URL https://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2021/fiskehelserapporten-2020

Grimsbø, E. (2016). *Title: Measuring methods for fish welfare during slaughter based on electrical impedance, EEG, ECG and blood parameters Scientific environment*. Retrieved from http://bora.uib.no/bitstream/handle/1956/12226/dr-thesis-2016-Endre-Grimsbø.pdf?sequence=1&isAllowed=y

Gunnes, K., Hansen, T., Lie, Ø., Rønningen, K., Staurnes, M., Åsgård, T., 1998. Oppdrett av laksesmolt – Nasjonalbiblioteket, URL https://www.nb.no/nbsok/nb/aafd1de840484f1fac038ee402dd947e?lang=no#167

Hultmann, L., Phu, T.M., Tobiassen, T., Aas-Hansen, O., Rustad, T., 2012. Effects of pre-slaughter stress on proteolytic enzyme activities and muscle quality of farmed Atlantic cod (Gadus morhua). Food Chem. 134, 1399–1408. https://doi.org/10.1016/j.foodchem.2012.03.038

Huntingford, F.A., Kadri, S., 2014. Defining, assessing and promoting the welfare of farmed fish. OIE Rev. Sci. Tech. 33, 233–244. https://doi.org/10.20506/rst.33.1.2286

Iversen, H (2013). Stress and its impact on animal welfare during commercial production of Atlantic salmon (Salmo salarL.)2013) Faculty of Bioscience and Aquaculture. Retrieved from https://brage.bibsys.no/xmlui/bitstream/handle/11250/299967/Haugmo Iversen.pdf?sequence=1&isAllowed=y

Iversen, M., Finstad, B., Nilssen, K.J., 1998. Recovery from loading and transport stress in Atlantic salmon ž Salmo salar L . / smolts. Aquaculture 168, 387–394.
Jansen, M.D., Jensen, B.B., Brun, E., 2015. Clinical manifestations of pancreas disease outbreaks in Norwegian marine salmon farming - variations due to salmonid alphavirus subtype. J. Fish Dis. 38, 343–353. https://doi.org/10.1111/jfd.12238
Kaale, L. D., & Eikevik, T. M. (2014). The development of ice crystals in food products during the superchilling process and following storage, a review. *Trends in Food Science and Technology*, *39*(2), 91–103. Retrieved from https://doi.org/10.1016/j.tifs.2014.07.004
Daniel Kaale, L., Eikevik, T.M., Rustad, T., Kolsaker, K., 2011. Superchilling of food: A review. https://doi.org/10.1016/j.jfoodeng.2011.06.004
Lambooij, E., Grimsbø, E., de Vis, J.W. van, Reimert, H.G.M., Nortvedt, R., Roth, B., 2010. Percussion and electrical stunning of Atlantic salmon (Salmo salar) after dewatering and subsequent effect on brain and heart activities. Aquaculture 300, 107–112. https://doi.org/10.1016/j.aquaculture.2009.12.022
Lerfall, J., Roth, B., Skare, E.F., Henriksen, A., Betten, T., Dziatkowiak-Stefaniak, M.A., Rotabakk, B.T., 2015. Pre-mortem stress and the subsequent effect on flesh quality of pre-rigor filleted Atlantic salmon (Salmo salar L.) during ice storage. Food Chem. 175, 157–165. https://doi.org/10.1016/j.foodchem.2014.11.111
Magnussen, O.M., Haugland, A., Torstveit Hemmingsen, A.K., Johansen, S., Nordtvedt, T.S., 2008. Advances in superchilling of food - Process characteristics and product quality. Trends Food Sci. Technol. 19, 418–424. https://doi.org/10.1016/j.tifs.2008.04.005
Mattilsynet | Fiskevelferd, 2021.
URL https://www.mattilsynet.no/fisk_og_akvakultur/fiskevelferd/
Midling, K.Ø., Mejdell, C., Olsen, S.H., Tobiassen, T., Aas-Hansen, Ø., Aas, K., Harris, S., Oppedal, K., Femsteinevik, Å., 2008. Slakting av oppdrettslaks på båt, direkte fra oppdrettsmerd.
Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao, L., Klinger, D.H., Little, D.C., Lubchenco, J., Shumway, S.E., Troell, M., 2021. A 20-year retrospective review of global aquaculture. Nature 591, 551–563. https://doi.org/10.1038/s41586-021-03308-6
Noble, C., Nilsson, J., Stien, L.H., Iversen, M.H., Kolarevic, J., Gismervik, K., 2018. Velferdsindikatorer for oppdrettslaks: Hvordan vurdere og dokumentere fiskevelferd 312.
Philis, G., Ziegler, F., Gansel, L.C., Jansen, M.D., Gracey, E.O., Stene, A., 2019. Comparing life cycle assessment (LCA) of salmonid aquaculture production systems: Status and perspectives. Sustain. 11. https://doi.org/10.3390/su11092517
Piñ Eiro, C., Barros-Velázquez, J., Aubourg, S.P., 2004. Effects of newer slurry ice systems on the quality of aquatic food products: a comparative review versus flake-ice chilling methods. https://doi.org/10.1016/j.tifs.2004.09.005
Robb, D. H. ., & Roth, B. (2003). Brain activity of Atlantic salmon (Salmo salar) following electrical stunning using various field strengths and pulse durations. *Aquaculture*, *216*(1–4), 363–369. https://doi.org/10.1016/S0044-8486(02)00494-5
Rosten, T.., Kristensen, T., 2010. Best practice in live fish transport. Aquaculture 22.
Rotabakk, B.T., Bergman, K., Ziegler, F., Skåra, T., Iversen, A., 2020. Climate impact, economy and technology of farmed Atlantic salmon Documentation of the current state for fresh and frozen products exported to Asia and fresh products to Europe 14.
Roth, B., 2016. Avlusing av laksefisk med Optilice: effekt på avlusing og fiskevelferd, Nofima rapport 59/2016.
Roth, B., Grimsbø, E., Slinde, E., Foss, A., Stien, L. H., & Nortvedt, R. (2012). Crowding, pumping and stunning of Atlantic salmon, the subsequent effect on pH and rigor mortis. *Aquaculture*, *326–329*, 178–180. Retrieved from https://doi.org/10.1016/j.aquaculture.2011.11.005
Roth, B., Nortvedt, R., Slinde, E., Foss, A., Grimsbø, E., Stien, L.H., 2010. Electrical stimulation of Atlantic salmon muscle and the effect on flesh quality. Aquaculture 301, 85–90. https://doi.org/10.1016/j.aquaculture.2010.01.008
Santurtun, E., Broom, D.M., Phillips, C.J.C., 2018. A review of factors affecting the welfare of Atlantic salmon (Salmo salar). Anim. Welf. 27, 193–204. https://doi.org/10.7120/09627286.27.3.193

Skare, M., Chan, S.S., Handeland, S.O., Løvdal, T., Lerfall, J., Roth, B., 2021. A comparative study on quality, shelf life and sensory attributes of Atlantic salmon slaughtered on board slaughter vessels against traditional land-based facilities. Aquaculture 540. https://doi.org/10.1016/j.aquaculture.2021.736681

Skare, M., Hernar, M., 2019. Bacheloroppgave; The effect of superchilling on quality and shelf life of farmed salmon slaughtered at production site.

Tacon, A.G.J., Metian, M., 2009. Fishing for feed or fishing for food: Increasing global competition for small pelagic forage fish. Ambio 38, 294–302. https://doi.org/10.1579/08-A-574.1

HAV LINE METODEN Et nytt konsept innen havbruk – PDF, 2018. URL http://docplayer.me/110108886-Hav-line-metoden-et-nytt-konsept-innen-havbruk.html

Veterinærinstituttet; Pankreassykdom (PD) 2021. URL https://www.vetinst.no/sykdom-og-agens/pankreassykdom-pd

"Norwegian Gannet" comes to rescue after toxic algae attack, 2019. URL https://salmonbusiness.com/__trashed/

Norway welcomes world's largest slaughter boat - FishFarmingExpert.com, 2019. URL https://www.fishfarmingexpert.com/article/norway-welcomes-worlds-largest-slaughter-boat/

Aquaculture 540 (2021) 736681

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

A comparative study on quality, shelf life and sensory attributes of Atlantic salmon slaughtered on board slaughter vessels against traditional land-based facilities

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ARTICLE INFO

Keywords:
Atlantic salmon
Superchilling
Slaughter vessel
RSW

ABSTRACT

The purpose of this study was to investigate the shelf life and quality of Atlantic salmon (*Salmo salar*) slaughtered onboard vessels and shipped to Denmark in −0.8 °C refrigerated seawater (RSW) as compared to traditional land-based slaughtering facilities having fish on ice. The quality and shelf life were measured on fresh and smoked fillets including blood spot counting, fillet gaping, texture hardness, microbiological counts, Quality Index Method (QIM) and sensory analysis. Blood spot counting and fillet gaping were measured on smoked fillets. Fresh fish slaughtered onboard the vessel had significantly lower fillet gaping scores as compared to those slaughtered at the facility, while no difference was found on smoked fillets. There were no significant differences in blood spots counts nor texture hardness between any of the groups. Salmon slaughtered on the vessel had a significant lower QIM score. The total mesophilic count and H_2S producing bacteria for fish slaughtered onboard vessels were significant lower at the end of storage (21d). Sensory analysis after 18 days of storage revealed minimal differences between the groups, whereas fish from the vessel had lower protein precipitation. We conclude that fish slaughtered onboard vessels and transported in superchilled RSW onboard a slaughter vessel presents good quality and improves shelf life over time.

1. Introduction

The history of the Norwegian aquaculture industry has had an explicit development over the past five decades, evolving from a small experimental scale to becoming a global research-based industry (Haaland et al., 2014). Farming of Atlantic salmon (*Salmo salar*) is still a relatively young industry, characterized by rapidly increasing production from 230 thousand metric tons (mt) in 1990 to 2.2 million mt in 2018 on a world basis (Iversen et al., 2020).

The traditional method of using wellboats to transport today's volume of fish to average size slaughter facilities with a capacity of approximately 150 tons/day can be time consuming. This means that the fish has to spend a longer time in the waiting cages, in addition to being crowded several times due to the insufficient capacity of the wellboats to transport the whole biomass from the cage. The traditional slaughter and processing routine involves several comprehensive steps to transport the fish from the cage and onto the market shelves. This process starts with a fasting period to empty the gut before the major operations that follows. After starvation, the salmon is more robust against stress and thus provides better harvest quality (Hvas et al., 2020). Further, the fish is crowded to 200–300 kg/m^3 at the farm site, before it is pumped alive into large tanks onboard the well-boat and transported to new waiting cages located near the slaughterhouse or the processing line (Merkin et al., 2010; Nortvedt et al., 2006). This gives the fish time to rest between the operations. Concerning animal welfare and quality, a proper stunning procedure is required to render fish unconscious before slaughtering (Roth et al., 2002). After the salmon is pumped into the slaughterhouse, it is either stunned with a percussive blow to the head or with electricity prior to slaughter (Lambooij et al., 2010), and the operation ends with packed head on gutted (HOG) fish and is transported to market by vehicle.

The farmed Atlantic salmon produced in Norway is usually traded as

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https://doi.org/10.1016/j.aquaculture.2021.736681
Received 16 October 2020; Received in revised form 22 March 2021; Accepted 24 March 2021
Available online 26 March 2021

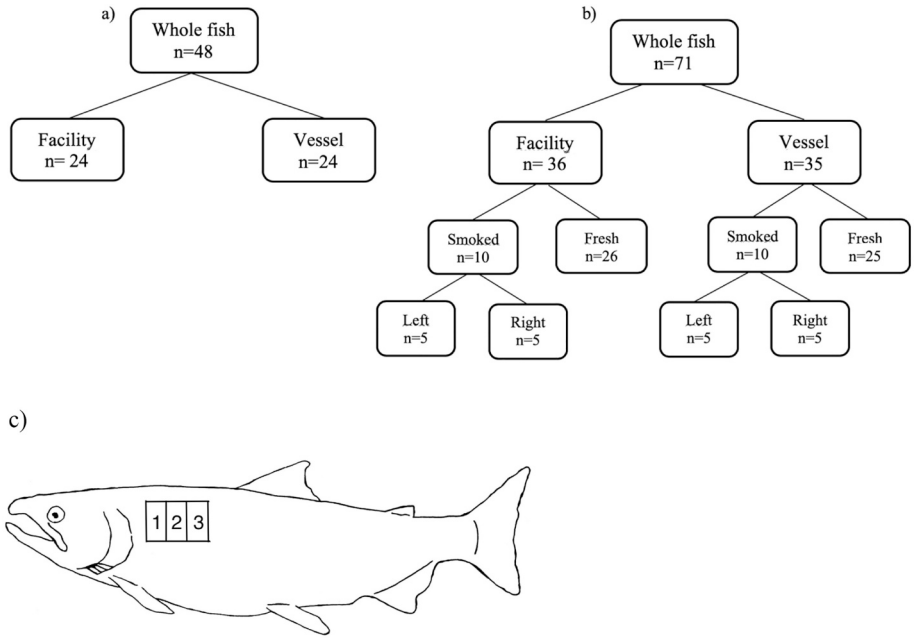

Fig. 1. (a) Experimental overview of the experiment conducted in December 2018, where texture, colour, gaping and blood spot counts were done (b) Experimental overview of the experiment conducted in October 2019. 10 fish from each group were filleted, dry salted and cold-smoked. The remaining 26 fish for each group was used for microbiological, QIM and sensory analysis. All smoked fillets were analyzed for gaping and blood spot counts. (c) Schematic illustration showing the areas where analysis was done on raw whole fish. 1, 2, 3. Microbiological analysis.
Facility = Well-boat live transport and processing at plant in NO.
Vessel = Slaughtered on-site and transported in RSW to sorting and packing factory in DK.

bled and gutted whole fish packed in ice. The seawater temperature varies from 4 to 20 °C, and at packaging the core temperature of fish must be less than 2 °C (Chan et al., 2020a). One way to achieve this temperature is by the use of superchilling technology (Erikson et al., 2011; Chan et al., 2020a), which can be done using several methods (Kaale and Eikevik, 2014), such as supercooling, deep-chilling, partial ice formation and immersion in refrigerated sea water (RSW) slurry. The RSW system is commonly used in fishing vessels to cool the catch to −1 °C in large seawater tanks until processing (Piñeiro et al., 2004). As an alternative to the aforementioned slaughter method, a large slaughter vessel with a slaughter and transport capacity of over 1000 tons per loading is being introduced in the salmon industry. The main idea of this method is to harvest fish directly from the cage before slaughtering the fish onboard the slaughter vessel and immediately superchilling the fish below 0 °C in refrigerated seawater (RSW) tanks onboard with ice slurry. The fish is then transported from Norway to Hirtshals, Denmark where the commercial processing plant is located. With the shorter harvesting and packing process, in addition to many efficient transport options from Hirtshals, less time is needed before the fish is on the market shelves compared to if it was shipped from a land-based facility in Norway. The fish is therefore delivered fresher, providing a product with better quality and longer shelf life. This allows the industry to increase production efficiency in logistics and economic benefits, reducing the need for styrofoam boxes before processing and meeting futures demands on slaughter capacity. This method also reduces stress on the fish, and thus better animal welfare as the method only requires one pumping and crowding stage (Chan et al., 2020a).

As the fish is being chilled down to −1 °C, RSW tanks can inhibit further growth of microorganisms (Fogarty et al., 2019). Food quality and shelf life are important properties to both producers and consumers. Still, there are variabilities that can significantly influence the shelf life of the product (Rasmussen et al., 2002). It is therefore important to keep the quality of the fish at a high level throughout the whole complex fishery chain to get a healthy, fresh and high-quality product (Nielsen and Hyldig, 2004). The method using RSW tanks after slaughter is beneficial where the temperature of salmon can be kept at superchilled conditions during the early stages of the value chain (Chan et al., 2020a).

Previous studies on salmonids showed that both quality and welfare can be affected by severe stress (Iversen et al., 1998; Skjervold et al., 2001; Merkin et al., 2010). Conditions during the slaughter process have a major impact on the quality of the salmon meat, and it has previously been shown that particularly crowding and pumping are stressful operations (Roth et al., 2012; Lerfall et al., 2015). Fasting fish prior to transport or slaughter is a common routine in the aquaculture sector which reduces metabolic activity, reduces oxygen demand, and empties the gut to avoid waste contamination (López-Luna et al., 2013; Lines and Spence, 2012). Mørkøre et al. (2008) concluded that prolonged fasting improves the ability of salmon to withstand stress during harvesting. Stress can increase the risk of various factors such as faster bacterial growth, softer texture as well as the degree of gaping and freshness. It is therefore important to reduce ante- and post mortem handling that can accelerate the loss of quality (Hansen et al., 2012). As freshness is the most fundamental and important factor to assess fish quality (Itoh et al., 2012), fish should be properly processed and stored at low temperatures before to packing because biochemical degradation and bacterial growth are easily inhibited (Hansen et al., 2009).

Since the idea of having the slaughter line onboard a vessel is new, few studies have been conducted to compare the sensory quality and shelf life of fish slaughtered onboard vessels against the traditional method of slaughtering on land. Therefore, the aim of this study was to investigate the sensory attributes and shelf life from the effect of slaughter on vessel using RSW compared to traditional slaughter on land using ice as cooling methods. Blood spots, gaping, texture, Quality Index Measurements, microbiology and sensory profiling were the quality attributes assessed in this study.

2. Materials and methods

2.1. Raw material and experimental design

The study was done on 5th December 2018 and 25th October 2019 with a total of 48 and 71 Atlantic salmon, respectively. Fish were starved for 7 days and transported Skaganeset, Sund, Hordaland, Norway. Temperature at sea was October/December: ~11.7 °C/~8.0 °C and weight October/December: ~4.14 kg/~4.23 kg. At Skaganeset the population was split into 2 half, where one part of the cage was pumped into the slaughter facility (Facility) and the other half was pumped onboard the slaughter vessel Norwegian Gannet (Vessel). At slaughter all fish underwent same procedures with electrical stunning (Stansas,

Optimar, Norway), bleeding in tanks/tubes prior to gutting (Baader 144, Baader Food Processing Machinery, Germany).

On both occasions (October and December) a full factorial design (Fig. 1a and b) was carried out; whole fish (slaughtered at vessel versus slaughtered at facility), resulting in two different groups. A group of HOG salmon ($n = 24$, $n = 36$) was slaughtered on land and stored in wet ice in expanded polystyrene (EPS) boxes and sent to Nofima AS, Stavanger for further quality analyses. Another group ($n = 24$, $n = 35$) was slaughtered by the cage onboard the vessel and immediately superchilled in RSW with ice slurry to −0.8 °C in storage tanks onboard for around 48 h. The superchilled fish were then taken out from the tanks and placed in EPS boxes with wet ice before transporting all the fish from Hirtshals to Stavanger in 8 h. Upon arrival, fish stored in ice and superchilled fish (RSW) were stored equally in a 0 °C cooling room with ice until day 21 post mortem to maintain both chilled and superchilled conditions.

In the December study, twentyfour fish from the facility and vessel group were kept as fresh fish, and analysis was carried out for texture and surface appearance, blood spots and gaping score 5 days post mortem. Twentysix and twentyfive fish from the facility and vessel group in the October study respectively were also kept as fresh fish for 3 weeks, where analysis was carried out on day 4, 10, 14, 18 and 21 post mortem for microbiology, QIM and sensory assessments. Analysis for sensory profiling was carried out on day 17 post mortem, while blood spots and gaping was carried out on day 4. To also assess gaping and blood spot counts of cold-smoked salmon, the remaining 10 fish from each group were filleted and dry salted with refined salt (GC Rieber, Norway) on day 4 for 18 h at 0 °C. They were then rinsed briefly and gently dried before cold-smoking on day 5 using the protocol of Birkeland and Skåra (2008) before vacuum packaging with 99% vacuum and stored at 4 °C.

2.2. Sensory analysis

2.2.1. QIM – Quality index method

QIM was carried out on days, 4, 10, 14, 18 and 21 post mortem with 4 trained panelists all in accordance to Hyldig and Green-Petersen (2005). The scheme is based upon well-defined characteristic changes of 4 quality attributes of raw fish; skin, eyes, gills, abdomen using a 4 -scale demerit scoring system (0: best, 3: worst). Every parameter is described in the schematic illustration of QIM. The scores for all the attributes are summed up to give a total sensory score, with a total possible demerit point of 24. The quality index is increasing linearly with the storage time on ice and the total QIM score is used to predict the remaining shelf life (QIM Eurofish, 2001).

2.2.2. Microbiological analysis

Microbiological analysis was carried out for the October experiment, with procedures done in accordance to the NMKL method No. 184 (% National Veterinary Institute, 2006) to determine total psychotropic count (TPC), total mesophilic bacterial count (TMC) and H_2S producing bacteria (HSPB). The analysis was done on the first day of sampling, day 4, and further on days 10, 14 and 18 until the last sampling day (day 21) for raw fish ($n = 12$). Three muscle pieces, (~10 g, without skin) were excised from the anterior part of the epaxial muscle (Fig. 1). Pieces 1 and 2 were used directly in the analyzes, while the third piece was frozen as a backup sample. The samples were placed in Stomacher bags with filter and weighed. Sterile buffered peptone water (Merck, Germany) was added to make a 1:10 dilution, and samples were homogenized in a Smasher® stomacher (AES Laboratorie, bioMérieux Industry, USA) for 120 s. Dilution series of the homogenates were made and 49.2 µl of each dilution was transferred to the Long and Hammer (L&H) plates using the Eddy Jet 2 W Spiral Plater (IUL micro, Spain) while 1 ml of each dilution was transferred to the iron agar, supplemented with 0,04% L-cysteine (Sigma-Aldrich, Norway). The iron agar plates were incubated at 25 °C for 72 ± 6 h before TMC and HSPB were determined by counting the total and black colonies, respectively, while L&H plates were incubated at 15 °C for 5 days to quantify for TPC. Microbial concentrations were expressed as log cfu g^{-1}.

2.2.3. Sensory assessment

For the October experiment, sensory evaluation was carried out on cooked salmon samples ($n = 5$ from each group) on day 18 by a panel of 4 assessors trained according to ISO 8586-1 (2012). All sensory evaluations were carried out in randomized order of coded samples. The left fillets were used and cut into pieces of 2 cm before skin and bones were removed. The cooked salmon samples were packed in cook-plastic pouches (PA/PE 70my 160 × 200 mm, LietPak, Lithuania) under slight vacuum (90%) and cooked without any salt or spice addition in steam (80 °C in 10 min). Evaluation of sensory attributes within appearance, odour, flavour and texture were assessed using a descriptive sensory test modified from Quantitative Descriptive Analysis (QDA®) (ISO 13299, 2016). A total of 12 different attributes were selected according to ISO 5492 (2008) giving a score for each key attribute (protein precipitation, colour intensity, discoloration, fresh odour, rancid odour, off odour, fresh flavour, rancid flavour, off flavour, hardness, juiciness and adhesiveness). The criteria for all the key attributes was graded using a 1 to 9 nonstructured scale (1 = low intensity and 9 = high intensity). The 4 assessors were given 11 samples consecutively to give individual scores at their own pace on a computerized system for direct recording of data from the modified QDA, collected by the software program EyeQuestion version 4.11.67 (Logic8 BV, the Netherlands).

2.2.4. Flesh quality analysis

For both the December and October experiment, the extent of fillet gaping was visually inspected according to Andersen et al. (1994), in addition to the number of blood spots on both raw and smoked fillets on days 5 and 21, respectively. The gaping score was determined according to the severity of gaping on a scale from 0 to 5; where 0 means no gaping and 5 means severe gaping.

Colorimetric analysis was performed on day 5 on the top loin of raw fillets using a digital colour imaging system (DigiEye full system, VeriVide Ltd., Leicester, UK). The fillets were placed in a standardized lightbox (daylight, 6400 K) and photographed with a calibrated digital camera (Nikon D80, 35 mm lens, Nikon Corp., Japan). The software Digipix (version 2.8, VeriVide Ltd., Leicester, UK) was used to calculate L*a*b* values from RGB values obtained from the fillet image, where L* represents lightness of the sample (L* = 0 = black, L* = 100 = white). The a* value changes from -a (greenness) to +a (redness) while b* value changes from -b (blueness) to +b (yellowness). Chrome and hue values were calculated using the formulas; $C^* = (a^2 + b^2)^{1/2}$ and $h^* = \arctan(b^*/a^*)$.

For the December experiment, texture analysis was measured with a Texture Analyzer TA-XT® plus (Stable Micro Systems Ltd., UK), equipped with a 5 kg load cell on day 4 post mortem. To make triplicate punctures above the mid-line of the Norwegian quality cut (NQC, NS1975), a 12.7 mm P/0.5 flat-ended cylinder probe was used. This was done directly on raw fillets transverse to the muscle fiber orientation. The force-time graph was recorded by a computer equipped with the Texture Exponent light software (Stable Micro Systems) to analyze the data. The resistant force (N) was recorded with a constant speed of 2 mm s^{-1}, where the surface breaking strength (fracturability, i.e. force at first breaking point), maximum force, 80% and 60% compression force from the original sample height were recorded.

2.3. Statistical analysis

Statistical analysis was done using the software Statistica (Dell inc, USA). To test continuous dependent variables against independent and fixed variables a *t*-test was used for comparing two independent different groups, while analysis of variance (ANOVA) was used above 2 variables. In case where dependent and continuous variables were tested

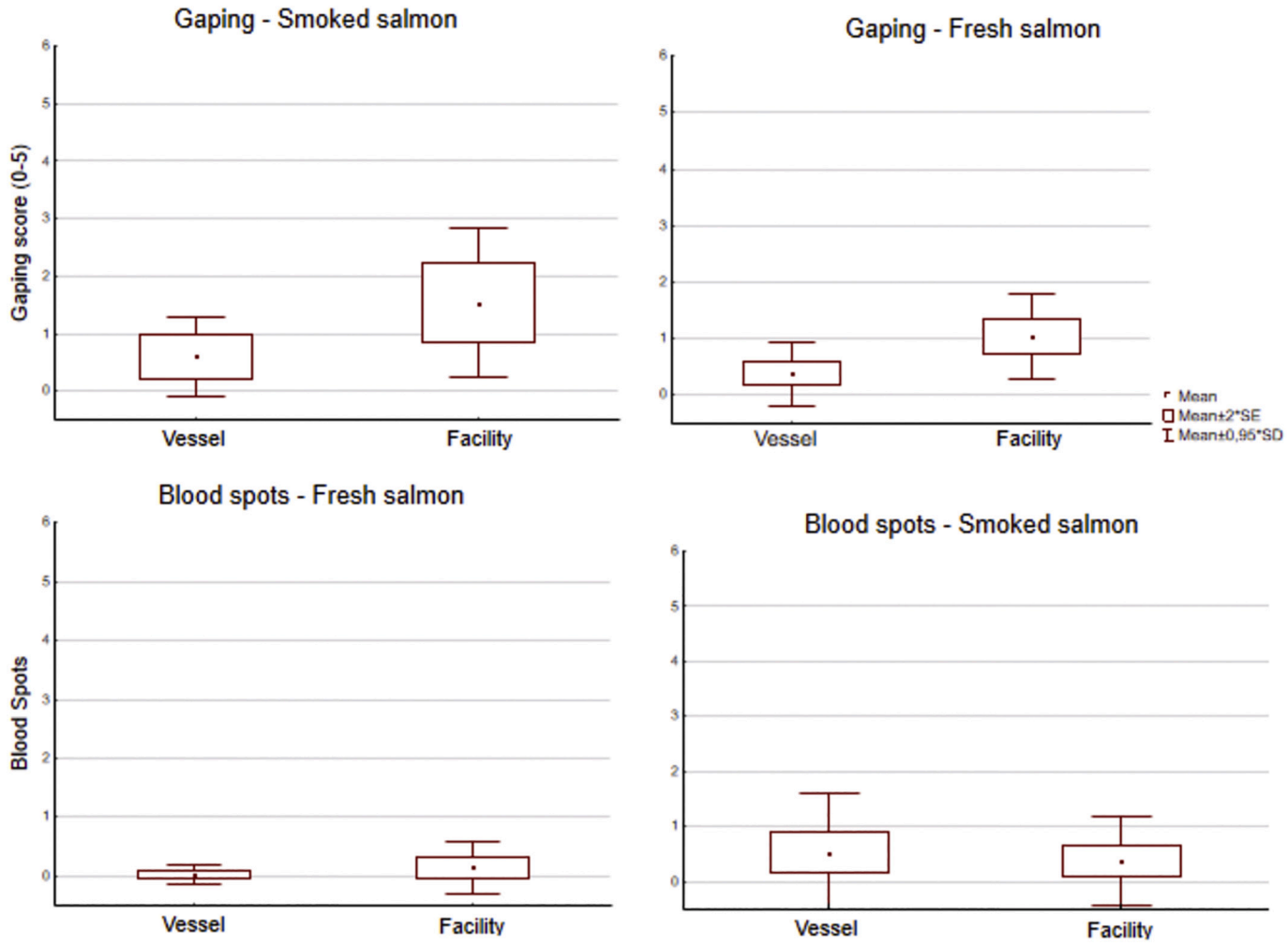

Fig. 2. Weighted result of gaping and blood spot counts from both fresh ($n = 24$) and smoked salmon ($n = 10$).

against fixed and continuous independent variables such as time (microbiology) or fillet height (texture), a general linear model (GLM) and ANCOVA was used respectively. A log transformation of the dependent variable was conducted to obtain a linear relationship and normal distribution of the residuals on bacterial growth. To obtain a normal distribution of the residuals a nested ANOVA was used to test average QIM scores against and fixed variable treatment nesting time as an independent variable. Prior to all variance analysis the homogeneity of the variance was tested (Levene's test of homogeneity of variances) along with testing correlation between covariates and dependent variables. For post hoc test, Bonforroni was used for testing pairs. A non-parametric (2 Sample Kolmogorov-Smirnov) test was used to analyze fillet gaping and blood spot count. The alpha level for statistical difference was set to ($p = 0.05$). All results are presented as mean ± standard deviation.

3. Results

3.1. Surface appearance

There was a significant difference in fillet gaping score ($p < 0.01$; 2 Sample Kolmogorov-Smirnov test) among the fresh fish between the groups, where fish slaughtered onboard the vessel had significantly lower gaping score at the end of storage (on average 0.4 ± 0.60) as compared to fish slaughtered at facility (on average 1.0 ± 0.79). There was no significant difference between the groups in blood spots ($p >$ 0.10; 2 Sample Kolmogorov-Smirnov test), where fresh fish slaughtered at facility (on average: 0.1 ± 0.45) had a slightly higher blood spot count compared to fish slaughtered onboard vessel (on average: 0.0 ± 0.14). Among the cold-smoked fish, there was no significant difference in fillet gaping score ($p > 0.10$; 2 Sample Kolmogorov-Smirnov test) nor the number of blood spots ($p > 0.10$; 2 Sample Kolmogorov-Smirnov test) between the two groups. Cold-smoked fish slaughtered at facility had a slightly lower blood spot counts (on average: 0.4 ± 0.84) than slaughtered at vessel (on average 0.5 ± 1.14) (See Fig. 2).

Table 1
Texture analyses of raw fillets from both groups on day 5 post mortem.

Hardness of texture (N)					
Group	Breaking force	Max force	80% compression	60% compression	n
Vessel	21.5 ± 9.4	60.6 ± 3.2	58.5 ± 9.6	24.3 ± 4.9	24
Facility	20.4 ± 17.9	60.1 ± 5.1	58.4 ± 17.5	20.4 ± 6.6	24
ANCOVA[a]	$p > 0.35$	$p > 0.89$	$p > 0.98$	$p > 0.40$	

[a] ANCOVA, analysis of covariance with fillet groups as factors and fillet height as covariant.

3.2. Texture

The results from the compression test (Table 1) on raw fillets showed no significant differences between the groups. There was no effect of

Table 2
Colour measured as L*, a*, b* of raw fillets from both groups on day 5 post mortem from the December experiment.

	Colour measurements					
Group	L*	a*	b*	C*	h*	n
Vessel	46.4 ± 2.0	46.6 ± 0.8	25.4 ± 1.4	53.07 ± 1.2	0.50 ± 0.01	24
Facility	43.8 ± 1.6	44.4 ± 1.0	24.8 ± 1.3	50.86 ± 1.4	0.51 ± 0.01	24
t-test[a]	$p < 0.00$	$p < 0.00$	$p > 0.12$	$p < 0.00$	$p > 0.08$	

[a] Two-way *t*-test comparing fresh fillets from both groups as factors.

slaughtering method on breaking force ($p > 0.35$), maximum force ($p > 0.89$), nor at 80% compression ($p > 0.98$) and 60% compression ($p > 0.40$).

3.3. Colour analysis

As shown in Table 2, the lightness (L*, $p < 0.00$) and redness (a*, $p < 0.00$) of the fillets were significantly higher for the fish slaughtered onboard vessel compared to those slaughtered at facility. There was no significant difference between the groups in yellowness (b*, $p > 0.12$). Fish from the vessel had significant higher colour saturation (C*, $p < 0.00$) than fish from the facility. No difference in hue was measured (h*, $p > 0.08$).

3.4. QIM

The QIM score (Table 3) for fish slaughtered onboard the vessel and facility increased through storage duration on all measured attributes ($p < 0.00$). Analysis of the total score show that fish slaughtered at facility had a significantly higher QIM score than fish slaughtered at vessel ($p < 0.03$). Of all the measured attributes, the mucus on the gills and skin along with smell, were in particular different.

3.5. Microbiology

The initial TMC measured on day 4 was below detection level except for one fish from the facility, providing an estimated average of 2.1 ± 0.10 log cfu g^{-1} (Fig. 3a). The TMC increased in both groups along with storage time ($p < 0.0005$, F = 313, GLM). Fish slaughtered at the vessel had generally lower TMC as compared to fish slaughtered at the facility ($p < 0.05$, F = 10, GLM), By the end of storage, at 21 days post mortem, fish from the facility had an significant higher TMC with 8.0 ± 0.36 log cfu g^{-1} as compared to the vessel with 6.4 ± 0.36 log log cfu g^{-1} ($p < 0.00$, post hoc test).

There was a significant difference increase in the amount of H_2S producing bacteria (Fig. 3b) as a function of storage time ($p < 0.00$, F = 69, GLM) and slaughter method ($p < 0.05$, F = 5, GLM). There were no detectible levels of HSPB until 10 days post mortem. After 21 days, fish slaughtered at facility had the highest counts (7.0 ± 0.47 log cfu g^{-1}) than those slaughtered on vessel (6.0 ± 0.37 log cfu g^{-1}, $p < 0.00$; post hoc test).

On TPC (Fig. 3c), 2 samples from the vessels were below detection level at 4 days post mortem, providing an average of 2.4 ± 0.29 (vessel) and 2.9 ± 0.33 (facility). The TPC levels is significantly dependent on storage time ($p < 0.00$, F = 578, GLM), but not on the slaughter method ($p > 0.26$, F = 1, GLM). At 21 days post mortem, the final TPC was 7.6 ± 0.37 and 8.1 ± 0.17 log cfu g^{-1} for fish slaughtered at the vessel or facility respectively.

3.6. Sensory assessment

The sensory profile (Fig. 4) showed no differences between the two groups on all attributes ($p > 0.64$; ANOVA) except for protein precipitation. The fish slaughtered on facility had a significantly higher score on protein precipitation (6.8 ± 1.24), than those slaughtered at vessel (5.8 ± 1.02), ($p < 0.01$; ANOVA). The juiciness for the fish slaughtered at vessel had a slightly higher score (6.1 ± 0.94), compared to fish slaughtered on facility (5.8 ± 0.72), although a significant difference was not detected ($p > 0.30$; ANOVA).

Table 3
QIM score provided as mean ± SD for both groups over time for each quality attribute and total score.

Attributes	Group	Vessel					Facility					Nested ANOVA[a]	
	Day	4	10	14	18	21	4	10	14	18	21	Group	Day
Skin	Colour	0.7 ± 0.17	0.7 ± 0.21	0.7 ± 0.14	1.0 ± 0.13	1.0 ± 0.41	0.3 ± 0.34	0.7 ± 0.14	0.7 ± 0.14	0.8 ± 0.16	1.0 ± 0.21	$p > 0.27$ F (1.25)	$p < 0.01$ F (3.20)
	Mucus	0.0 ± 0.00	0.7 ± 0.17	0.3 ± 0.21	0.5 ± 0.19	0.7 ± 0.14	0.0 ± 0.00	0.7 ± 0.25	0.7 ± 0.21	0.8 ± 0.21	0.7 ± 0.27	$p < 0.02$ F (6.16)	$p < 0.00$ F (9.62)
	Smell	0.2 ± 0.18	0.7 ± 0.25	0.7 ± 0.14	0.5 ± 0.19	1.0 ± 0.50	0.2 ± 0.18	0.7 ± 0.14	0.7 ± 0.39	0.8 ± 0.29	1.2 ± 0.30	$p > 0.56$ F (0.35)	$p < 0.00$ F (7.38)
	Texture	1.0 ± 0.14	1.0 ± 0.25	1.0 ± 0.14	1.0 ± 0.00	1.2 ± 0.57	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	$p > 0.68$ F (0.18)	$p > 0.76$ F (0.62)
Eyes	Pupils	0.7 ± 0.14	1.2 ± 0.33	1.2 ± 0.27	1.1 ± 0.38	1.3 ± 0.65	0.5 ± 0.33	1.0 ± 0.34	1.2 ± 0.39	1.3 ± 0.29	1.3 ± 0.37	$p > 0.94$ F (0.01)	$p < 0.00$ F (4.91)
	Shape	0.8 ± 0.18	1.2 ± 0.18	1.2 ± 0.27	1.5 ± 0.43	1.5 ± 0.68	1.0 ± 0.28	1.3 ± 0.25	1.7 ± 0.46	1.4 ± 0.34	1.7 ± 0.40	$p > 0.19$ F (1.80)	$p < 0.00$ F (4.2)
Gills	Colour	0.2 ± 0.42	1.0 ± 0.30	1.0 ± 0.44	1.8 ± 0.53	2.0 ± 0.81	0.3 ± 0.34	1.3 ± 0.58	1.2 ± 0.61	1.3 ± 0.79	1.7 ± 0.50	$p > 0.58$ F (0.31)	$p < 0.00$ F (3.90)
	Mucus	0.2 ± 0.39	0.7 ± 0.00	0.7 ± 0.33	0.6 ± 0.20	0.0 ± 0.56	0.7 ± 0.34	1.5 ± 0.56	1.3 ± 0.34	0.9 ± 0.41	0.5 ± 0.78	$p < 0.00$ F (17.9)	$p < 0.00$ F (5.11)
	Smell	0.0 ± 0.28	0.7 ± 0.27	1.5 ± 0.40	1.8 ± 0.19	2.2 ± 0.91	0.3 ± 0.25	1.3 ± 0.53	1.8 ± 0.27	1.8 ± 0.33	2.2 ± 0.46	$p < 0.00$ F (10.78)	$p < 0.00$ F (21.13)
Abdomen	Blood	0.0 ± 0.00	0.3 ± 0.39	0.3 ± 0.25	0.3 ± 0.26	0.7 ± 0.27	0.0 ± 0.00	0.3 ± 0.25	0.5 ± 0.40	0.5 ± 0.21	0.3 ± 0.34	$p > 0.98$ F (0)	$p < 0.01$ F (2.78)
	Smell	0.0 ± 0.00	1.3 ± 0.54	1.7 ± 0.30	2.0 ± 0.46	1.8 ± 0.75	0.2 ± 0.18	0.8 ± 0.42	2.0 ± 0.52	1.8 ± 0.29	2.5 ± 0.27	$p > 0.35$) F (0.9)	$p < 0.00$ F (22.42)
Total score	All	3.5 ± 1.11	9.7 ± 1.70	10.8 ± 1.61	10.8 ± 1.83	13.5 ± 1.80	4.5 ± 1.27	11.1 ± 1.99	12.5 ± 2.28	11.4 ± 2.04	14.3 ± 2.15	$p < 0.03$ F (5.19)	$p < 0.00$ F (19.3)

[a] Nested ANOVA analyses of dependent variables on slaughtering method with time nested into the design. Provided are the p and F values.

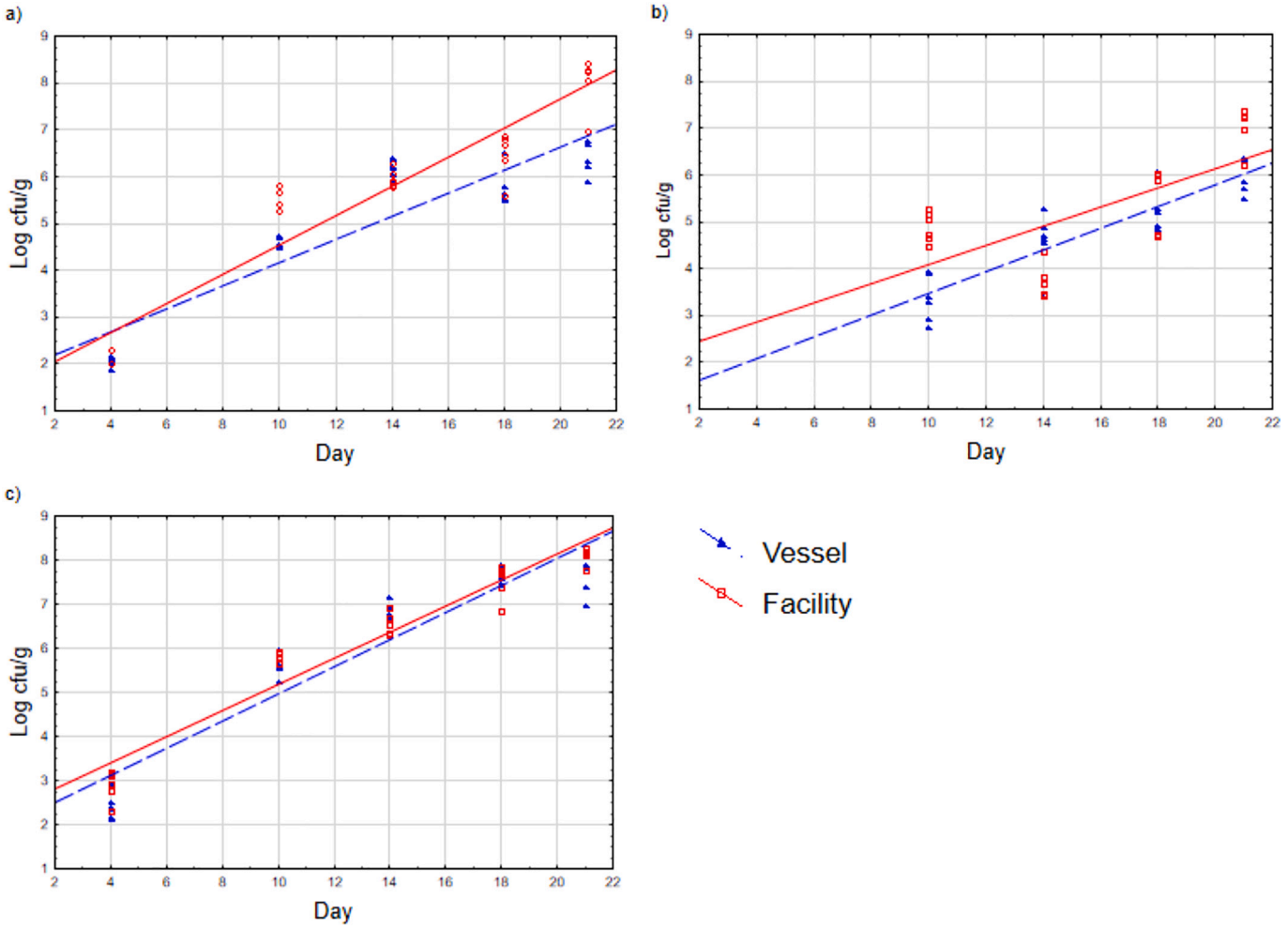

Fig. 3. (a) Total mesophilic counts (log cfu/g) (b) Hydrogen sulphide producing bacterial counts (log cfu/g) and (c) Total psychotropic count (log cfu/g) from day 4 to day 21 post mortem of fresh fish.

4. Discussion

In line with Erikson et al. (2011), the results from this present study demonstrated that farmed salmon immersed into RSW, followed by storage in ice, results in better gaping scores, QIM scores, lower microbial counts as compared to the traditional slaughtering method with the use of ice only. Storing and chilling fish in RSW is a common chilling method for fish, whether it is live chilling (Skjervold et al., 2001; Erikson, 2008), carcass cooling or superchilling (Erikson et al., 2011). This fish however was kept in subzero temperatures also during transport, very much similar to the pelagic industry (Anders et al., 2019). Like the pelagic industry, temperature during transport may affect on the quality. This was shown in Espe et al. (2004), were both harvest time and storage conditions affected the gaping score and the softness in the fillets of Atlantic salmon.

Gaping negatively affects texture, causing flesh softening from the collapse of muscle tissue and the increasing amount of soluble collagen in the extracellular matrix (Espe et al., 2004). Jacobsen et al., 2017 stated that immediately chilling after slaughter leads to a better quality, and the difference in storage temperature after slaughter does not seem to affect gaping. In this present study, the gaping score was significantly lower for the fresh fish slaughtered at vessel than those slaughtered at facility. This was also observed in Chan et al. (2020a) and Chan et al. (2020b), which was explained by the consistency in regular cleaning of the RSW tanks. A higher gaping score is highly correlated to improper cleaning of fish where remnants like fluid and blood are left in the abdomen of the fish (Jacobsen et al., 2017).

The presence of blood spots in fish has become more frequent, leading to unacceptable appearance and eventually rejection and financial loss (Balaban et al., 2011; Olsen et al., 2006). Pre-slaughter stress due to crowding, stunning, exsanguination techniques and chilling are important factors contributing to blood spot formation (Roth et al., 2005; Robb et al., 2003). In the present study, the standard deviation of bloodspot counts was low for both smoked and fresh salmon in both groups. Smoked salmon slaughtered on vessel had a slightly higher count compared to both fresh and smoked salmon slaughtered at facility. Blood spots are caused not only on the surface but also within the fillets, as residual blood is often left in the blood vessels after bleeding. Efficient removal is dependent on gravity and vasodilation in peripheral tissues and muscle contraction (Lambooij et al., 2004; Robb et al., 2003).

Texture of fish is an important quality parameter known to decrease throughout storage time (Huff-Lonergan and Lonergan, 2005). The texture measured in this study was measured on day 5 post mortem. The results gave a good indication of the meat quality after two different slaughtering methods were conducted. In this present study, there was a minimal difference between the groups, where the fish slaughtered at vessel showed a slightly better texture than fish slaughtered at facility. As expected, due to the minimal differences, the texture values of fresh fillets were not affected by the slaughtering method after 5 days of storage. Bahuaud et al. (2008) also found no significant differences between the superchilled group and the iced group in texture measurements after 1 week of storage. The thickness of the fillet can be

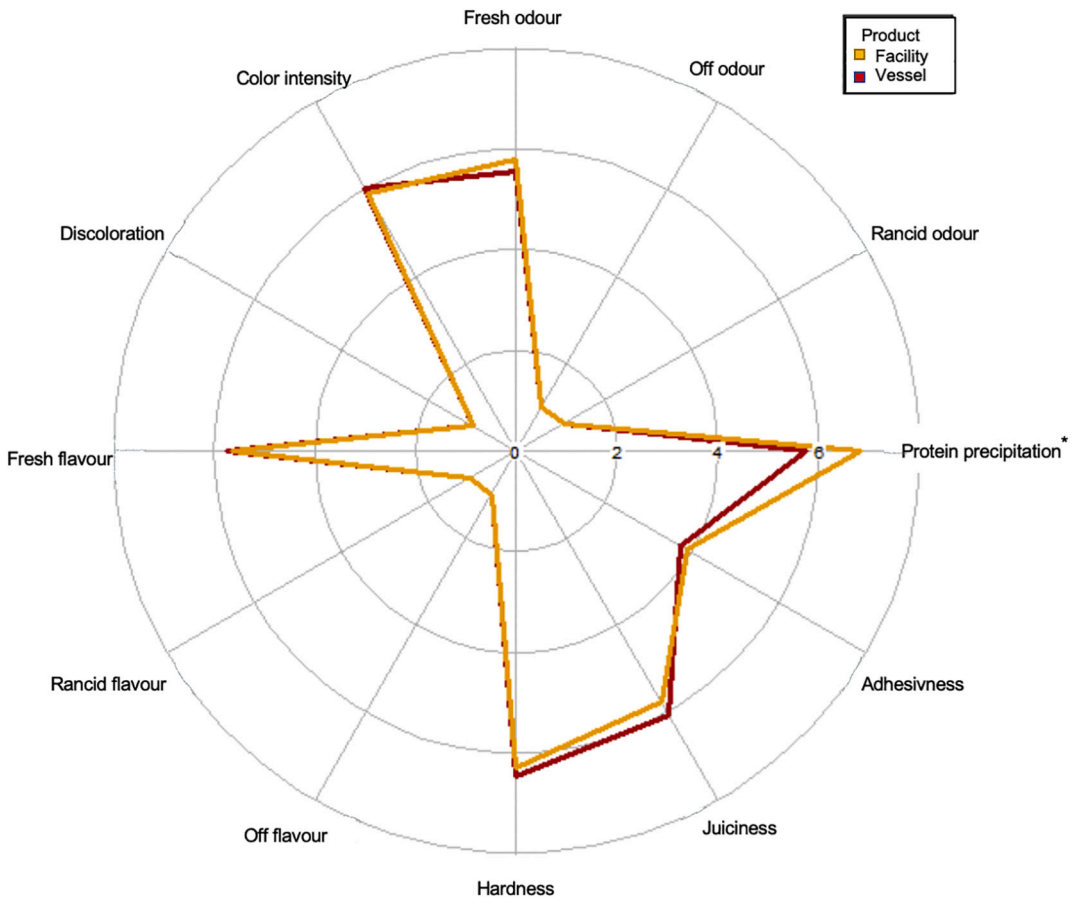

Fig. 4. Spider Plot showing the score for each attribute for both groups ($n = 5$ from each group), Day 18 post mortem (October experiment). *significant levels with $p < 0.05$.

considered as a source of variation as the probe was directly applied on the fillet. Therefore, its textural properties varied, and the comparison became uncertain.

Previous studies on how fillet colour is affected by superchilling showed inconsistent results. In this present study, the lightness was generally high for both groups, but a lighter, more intense red colour was observed in the fish slaughtered onboard vessel. This was similar to Chan et al. (2020b), where RSW stored fish was significantly lighter in colour than ice stored fish after 4 days, although this effect was statistically insignificant. Espe et al. (2004) found that raw fillets stored on ice had a more reddish colour. In contrast, Erikson et al. (2011) and Chan et al. (2020b) indicated decreased redness in ice storage, and in addition, Chan et al. (2020b) indicated a decrease in yellowness through storage.

QIM is a quality control system for the freshness of seafood. The method creates a way to measure the quality rapidly and reliably and provides the users with more accurate information about the freshness of the product (QIM Eurofish, 2001). The QIM test performed in this present study was conducted as a blind test, and various reasons relating to the judges on the sampling days can explain why the total score on day 18 dropped to the same score as day 11. The results observed in the present study were similar to the study of Erikson et al. (2011), which reported that after 11 days post mortem, the QIM scores of the fish stored continuously in slurry at −2 °C were lower than those stored in ice. External quality was better maintained with superchilling in RSW than with ice storage in the present study. Eye form, gill mucus, gill odour and skin all scored lowest for the superchilled fish. A QI score between 16 and 20 indicates that the salmon is becoming rancid and sour, while above 20 indicates spoilage (Sveinsdottir et al., 2002). The QI scores in this present study were lower than 16 up to day 21 in both groups. A greater variation may occur among panelists as storage time increases, as observed by Sveinsdottir et al., 2002 where some panelists tended to score lower or higher than average. As individual fish have different spoiling rates, using a minimum of 3 fish is recommended for QIM assessment with a ± 2 days of buffer time.

Spoilage is a complex process involving chemical, enzymatic and microbiological changes, where microbiology is proven to be a primary determinant of shelf life (Anacleto et al., 2011). TMC is usually used, but the levels to detect the end of shelf life varies greatly between TMC and HSPB (Dalgaard et al., 1997). Based on the results in the present study, fish slaughtered at the vessel and stored in RSW was more effective in limiting the growth of HSPB. This is in contrast to Erikson et al. (2011), who reported that superchilling was successful inhibited TMC, but not HSPB. Although a high bacterial count can be found in spoiling fish, only some are considered active spoilers (Erikson et al., 2011). Fogarty et al. (2019) found that HSPB may be a better indicator of shelf life rather than general bacterial counts such as TMC growth, which indicated spoilage of the fish with a count of 5–6 log cfu g^{-1}. The HSPB observed may indicate a longer shelf life for fish slaughtered onboard vessel compared to fish slaughtered at the facility, although there is no consensus as to which bacterial species should be used to monitor the shelf life of the fish.

If HSPB counts were used as spoilage indicator, both groups have exceeded the limit after day 18 from this study. However, as the growth of HSPB developed later, this gave a slight discrepancy from the observed QIM values, as the QI scores suggest that fish have a longer shelf life than 21 days. The results obtained from the sensory evaluation was in line with the QIM scores, suggesting acceptable quality on day 18 where the two groups showed minimal differences concerning the different attributes. This was in contrast to the observation of Sivertsvik

et al. (2003), where the salmon chilled traditionally on ice was not evaluated due to spoilage after day 17. However, they found that both air and modified atmosphere (MA) exposed superchilled salmon had a considerably longer shelf life than traditional chilling and MA chilled salmon and was acceptable after 21 days of storage. The juiciness for the air superchilled salmon observed by Sivertsvik et al. (2003) also had a score of 6.1, similar to the results in this study where fish chilled in RSW had a score of 6.05.

Due to the rapid expansion of the aquaculture industry, the welfare of farmed fish has been set in focus. Fish welfare is an important issue within the industry, not just for marketing and profitability, but also for fish health, quality, production efficiency and low mortality (Ashley, 2007). From a welfare point of view, the methods used to handle fish during transfer to the slaughtering facility and up to the point of stunning and immediate loss of consciousness are the most important factors in slaughter technique (Southgate and Wall, 2001). Slaughter vessels might be conducive to improve the fish welfare, because spread of diseases and lower escape risk, in addition to better quality and longer shelf life are some of the parameters this method can help improve. Based on the results in this present study and in accordance to Chan et al. (2020a), slaughtering onboard a vessel with the use of RSW tanks to store whole gutted fish can potentially become the future method of fish slaughter and storage.

5. Conclusion

This study shows that slaughtering at land-based facilities and post mortem storage at 0 °C gives shorter shelf life based on a higher QIM score and microbiological count compared to those slaughtered onboard the vessel and stored at −0.8 °C in RSW for 48 h. Moreover, the results also showed that fish slaughtered onboard the vessel had better gaping scores and gave lighter and more reddish fillets. Otherwise, there were minimal differences in quality including blood spots, texture and sensory analysis. Fish slaughtered and chilled in RSW onboard a slaughter vessel, therefore gives good quality and shelf life over time and can potentially be a more sustainable slaughtering method. An interesting aspect that could be explored in further studies is to focus more on biosecurity measures and fish welfare during the harvest operation for fish slaughtered onboard the vessel, and how this affects the shelf life and other quality parameters when they are processed and packed for direct selling to consumers in the markets.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This project was funded by the Regional Research Fund (RFF-Vest) (project ES612950). The authors would like to thank employees in both Hav Line AS and Sekkingstad for their help with fish processing and transportation. We would also like to thank the team at Nofima AS, Stavanger, namely Karin S Tranøy, Laila Budal, Sigurd Øines and Gro Haugvaldstad Kleiberg for their technical work, as well as Aase Vorre Skuland and Mette Risa Vaka in helping with the sensory analysis. The authors declare that there is no conflict in interest.

References

Anacleto, P., Teixeira, B., Marques, P., Pedro, S., Nunes, M.L., Marques, A., 2011. Shelf-life of cooked edible crab (Cancer pagurus) stored under refrigerated conditions. LWT Food Sci. Technol. 44 (6), 1376–1382. Retrieved from. https://doi.org/10.1016/j.lwt.2011.01.010.

Anders, N., Roth, B., Grimsbø, E., Breen, M., 2019. Assessing the effectiveness of an electrical stunning and chilling protocol for the slaughter of Atlantic mackerel (Scomber scombrus). PLoS One 14, 1–11. https://doi.org/10.1371/journal.pone.0222122.

Andersen, U.B., Steinsholt, K., Stroemsnes, A.N., Thomassen, M.S., 1994. Norwegian Journal of Agricultural Sciences 8, 165–179.

Ashley, P.J., 2007. Fish welfare: current issues in aquaculture. Appl. Anim. Behav. Sci. 104 (3–4), 199–235. Retrieved from. https://doi.org/10.1016/j.applanim.2006.09.001.

Bahuaud, D., Mørkøre, T., Langsrud, Ø., Sinnes, K., Veiseth, E., Ofstad, R., Thomassen, M. S., 2008. Effects of −1.5 °C super-chilling on quality of Atlantic salmon (Salmo salar) pre-rigor fillets: Cathepsin activity, muscle histology, texture and liquid leakage. Food Chem. 111 (2), 329–339. Retrieved from. https://doi.org/10.1016/J.FOODCHEM.2008.03.075.

Balaban, M.O., Ünal Şengör, G.F., Soriano, M.G., Ruiz, E.G., 2011. Quantification of gaping, bruising, and blood spots in salmon fillets using image analysis. J. Food Sci. 76 https://doi.org/10.1111/j.1750-3841.2011.02060.x. Retrieved from.

Birkeland, S., Skåra, T., 2008. Cold smoking of Atlantic salmon (Salmo salar) fillets with smoke condensate - an alternative processing technology for the production of smoked salmon. J. Food Sci. 73 (6) https://doi.org/10.1111/j.1750-3841.2008.00850.x. Retrieved from.

Chan, S.S., Roth, B., Skare, M., Hernar, M., Jessen, F., Løvdal, T., Lerfall, J., 2020a. Effect of chilling technologies on water holding properties and other quality parameters throughout the whole value chain: from whole fish to cold-smoked fillets of Atlantic salmon (Salmo salar). Aquaculture *526* (April), 735381. Retrieved from. https://doi.org/10.1016/j.aquaculture.2020.735381.

Chan, S.S., Roth, B., Jessen, F., Løvdal, T., Jakobsen, A.N., Lerfall, J., 2020b. A comparative study of Atlantic salmon chilled in refrigerated seawater versus on ice: from whole fish to cold-smoked fillets. Sci. Rep. 10, 17160. Retrieved from. https://doi.org/10.1038/s41598-020-73302-x.

Dalgaard, P., Mejlholm, O., Christiansen, T.J., Huss, H.H., 1997. Importance of ***Photobacterium phosphoreum*** in relation to spoilage of modified atmosphere-packed fish products. Lett. Appl. Microbiol. 24 (5), 373–378. Retrieved from. https://doi.org/10.1046/j.1472-765X.1997.00152.x.

Erikson, U., 2008. Live chilling and carbon dioxide sedation at slaughter of farmed Atlantic salmon: a description of a number of commercial case studies. J. Appl. Aquac. 20, 38–61. https://doi.org/10.1080/10454430802022078.

Erikson, U., Misimi, E., Gallart-Jornet, L., 2011. Superchilling of rested Atlantic salmon: different chilling strategies and effects on fish and fillet quality. Food Chem. 127 (4), 1427–1437. Retrieved from. https://doi.org/10.1016/j.foodchem.2011.01.036.

Espe, M., Ruohonen, K., Bjørnevik, M., Frøyland, L., Nortvedt, R., Kiessling, A., 2004. Interactions between ice storage time, collagen composition, gaping and textural properties in farmed salmon muscle harvested at different times of the year. Aquaculture *240* (1–4), 489–504. Retrieved from. https://doi.org/10.1016/j.aquaculture.2004.04.023.

Fogarty, C., Whyte, P., Brunton, N., Lyng, J., Smyth, C., Fagan, J., Bolton, D., 2019. Spoilage indicator bacteria in farmed Atlantic salmon (Salmo salar) stored on ice for 10 days. Food Microbiol. *77*, 38–42. Retrieved from. https://doi.org/10.1016/j.fm.2018.08.001.

Haaland, A., Hersoug, B., N. K. og, D.M., 2014. Norges fiskeri- og kysthistorie » Band 5: Havbruk. Retrieved from. https://norges-fiskeri-og-kysthistorie.w.uib.no/bokverket/bind-5-havbrukshistorie/.

Hansen, A.Å., Mørkøre, T., Rudi, K., Langsrud, Ø., Eie, T., 2009. The combined effect of superchilling and modified atmosphere packaging using CO_2 emitter on quality during chilled storage of pre-rigor salmon fillets (*Salmo salar*). J. Sci. Food Agric. 89 (10), 1625–1633. Retrieved from. https://doi.org/10.1002/jsfa.3599.

Hansen, A.Å., Rødbotten, M., Eie, T., Lea, P., Rudi, K., Mørkøre, T., 2012. The effect of crowding stress on bacterial growth and sensory properties of chilled Atlantic Salmon fillets. J. Food Sci. 77 (1), S84–S90. Retrieved from. https://doi.org/10.1111/j.1750-3841.2011.02513.x.

Huff-Lonergan, E., Lonergan, S.M., 2005. Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. Meat Sci. 71 (1), 194–204. Retrieved from. https://doi.org/10.1016/j.meatsci.2005.04.022.

Hvas, M., Stien, L.H., Oppedal, F., 2020. The metabolic rate response to feed withdrawal in Atlantic salmon post-smolts. Aquaculture 529. https://doi.org/10.1016/j.aquaculture.2020.735690. Retrieved from.

Hyldig, G., Green-Petersen, D.M.B., 2005. Quality index method—an objective tool for determination of sensory quality. J. Aqua. Food Product Technol. 13 (4), 71–80. Retrieved from. https://doi.org/10.1300/J030v13n04_06.

ISO 13299, 2016. Sensory Analysis - Methodology - General Guidance for Establishing a Sensory Profile, 2. ed. International Organization for standardization.

ISO 5492, 2008. Sensory Analysis - Vocabulary, 2.ed. International Organization for standardization.

ISO 8586-1, 2012. Sensory Analysis - Genreal Guidance for the Selection, Training and Monitoring of Assessors - Selected Assessors, 2. Ed. International Organization for Standardization.

Itoh, D., Sassa, F., Nishi, T., Kani, Y., Murata, M., Suzuki, H., 2012. Droplet-based microfluidic sensing system for rapid fish freshness determination. Sensors Actuators B Chem. *171–172*, 619–626. Retrieved from. https://doi.org/10.1016/j.snb.2012.05.043.

Iversen, M., Finstad, B., Nilssen, K.J., 1998. Recovery from loading and transport stress in Atlantic salmon ž *Salmo salar* L / smolts. Aquaculture 168 (1–4), 387–394. Retrieved from. https://www-sciencedirect-com.pva.uib.no/science/article/pii/S0044848698003640.

Iversen, A., Asche, F., Hermansen, Ø., Nystøyl, R., 2020. Production cost and competitiveness in major salmon farming countries 2003–2018. Aquaculture 522. https://doi.org/10.1016/j.aquaculture.2020.735089. Retrieved from.

Jacobsen, Á., Joensen, H., Eysturskarð, J., 2017. Gaping and loss of fillet firmness in farmed salmon (Salmo salar L.) closely correlated with post-slaughter cleaning of the abdominal cavity. Aquac. Res. *48* (1), 321–331. Retrieved from. https://doi.org/10.1111/are.12884.

Kaale, L.D., Eikevik, T.M., 2014. The development of ice crystals in food products during the superchilling process and following storage, a review. Trends Food Sci. Technol. 39 (2), 91–103. Retrieved from. https://doi.org/10.1016/j.tifs.2014.07.004.

Lambooij, E., Kloosterboer, R., Gerritzen, M., van de Vis, J., 2004. Head-only electrical stunning and bleeding of African catfish (Clarias gariepinus): assessment of loss of consciousness. Anim. Welf. 13 (1), 71–76. https://www.ingentaconnect.com/content/ufaw/aw/2004/00000013/00000001/art00011.

Lambooij, E., Grimsbø, E., de Vis, J.W., van Reimert, H.G.M., Nortvedt, R., Roth, B., 2010. Percussion and electrical stunning of Atlantic salmon (Salmo salar) after dewatering and subsequent effect on brain and heart activities. Aquaculture 300, 107–112. https://doi.org/10.1016/j.aquaculture.2009.12.022.

Lerfall, J., Roth, B., Skare, E.F., Henriksen, A., Betten, T., Dziatkowiak-Stefaniak, M.A., Rotabakk, B.T., 2015. Pre-mortem stress and the subsequent effect on flesh quality of pre-rigor filleted Atlantic salmon (Salmo salar L.) during ice storage. Food Chem. 175, 157–165. https://doi.org/10.1016/j.foodchem.2014.11.111.

Lines, J.A., Spence, J., 2012. Safeguarding the welfare of farmed fish at harvest. Fish Physiol. Biochem. 38 (1), 153–162. Retrieved from. https://doi.org/10.1007/s10695-011-9561-5.

López-Luna, J., Vásquez, L., Torrent, F., Villarroel, M., 2013. Short-term fasting and welfare prior to slaughter in rainbow trout, Oncorhynchus mykiss. Aquaculture *400–401*, 142–147. Retrieved from. https://doi.org/10.1016/j.aquaculture.2013.03.009.

Merkin, G.V., Roth, B., Gjerstad, C., Dahl-Paulsen, E., Nortvedt, R., 2010. Effect of pre-slaughter procedures on stress responses and some quality parameters in sea-farmed rainbow trout (Oncorhynchus mykiss). Aquaculture 309, 231–235. https://doi.org/10.1016/j.aquaculture.2010.08.025.

Mørkøre, T., Mazo, T.P.I., Tahirovic, V., Einen, O., 2008. Impact of starvation and handling stress on rigor development and quality of Atlantic salmon (salmon Salar L). Aquaculture 277 (3–4), 231–238. Retrieved from. https://doi.org/10.1016/j.aquaculture.2008.02.036.

Nielsen, D., Hyldig, G., 2004. Influence of handling procedures and biological factors on the QIM evaluation of whole herring (Clupea harengus L.). Food Res. Int. *37* (10), 975–983. Retrieved from. https://doi.org/10.1016/j.foodres.2004.06.006.

NMKL - Metoder - Mikro, 2006. Retrieved from. https://www.nmkl.org/index.php/en/publications.

Olsen, S.H., Sorensen, N.K., Stormo, S.K., Elvevoll, E.O., 2006. Effect of slaughter methods on blood spotting and residual blood in fillets of Atlantic salmon (Salmo salar). Aquaculture 258 (1–4), 462–469. Retrieved from. https://doi.org/10.1016/j.aquaculture.2006.04.047.

Piñeiro, C., Barros-Velázquez, J., Aubourg, S.P., 2004. Effects of newer slurry ice systems on the quality of aquatic food products: a comparative review versus flake-ice chilling methods. Trends Food Sci. Technol. 15, 575–582. https://doi.org/10.1016/j.tifs.2004.09.005.

QIM-ditt ideelle verktøy for å måle fiskens ferskhet, 2001. Retrieved from. www.qim-eurofish.com.

Rasmussen, S.K.J., Ross, T., Olley, J., McMeekin, T., 2002. A process risk model for the shelf life of Atlantic salmon fillets. Int. J. Food Microbiol. 73 (1), 47–60. Retrieved from. https://doi.org/10.1016/S0168-1605(01)00687-0.

Robb, D.H.F., Phillips, A.J., Kestin, S.C., 2003. Evaluation of methods for determining the prevalence of blood spots in smoked Atlantic salmon and the effect of exsanguination method on prevalence of blood spots. Aquaculture 217 (1–4), 125–138. Retrieved from. https://doi.org/10.1016/S0044-8486(01)00901-2.

Roth, B., Moeller, D., Veland, J.O., Imsland, A., Slinde, E., 2002. The effect of stunning methods on rigor mortis and texture properties of Atlantic salmon (Salmo salar). J. Food Sci. 67 (4), 1462–1466. Retrieved from. https://doi.org/10.1111/j.1365-2621.2002.tb10306.x.

Roth, B., Torrissen, O.J., Slinde, E., 2005. The effect of slaughtering procedures on blood spotting in rainbow trout (Oncorhynchus mykiss) and Atlantic salmon (Salmo salar). Aquaculture 250 (3–4), 796–803. Retrieved from. https://doi.org/10.1016/j.aquaculture.2005.03.010.

Roth, B., Grimsbø, E., Slinde, E., Foss, A., Stien, L.H., Nortvedt, R., 2012. Crowding, pumping and stunning of Atlantic salmon, the subsequent effect on pH and rigor mortis. Aquaculture 326–329, 178–180. Retrieved from. https://doi.org/10.1016/j.aquaculture.2011.11.005.

Sivertsvik, M., Rosnes, J.T., Kleiberg, G.H., 2003. Effect of modified atmosphere packaging and superchilled storage on the microbial and sensory quality of Atlantic salmon (Salmo salar) fillets. J. Food Sci. 68 (4), 1467–1472. Retrieved from. https://doi.org/10.1111/j.1365-2621.2003.tb09668.x.

Skjervold, O., Fjaera, S.O., Østby, B., Einen, O., 2001. Live-chilling and crowding stress before slaughter ž / of Atlantic salmon Salmo salar. Aquaculture 192. Retrieved from. www.elsevier.nlrlocateraqua-online.

Southgate, P., Wall, T., 2001. Welfare of Farmed Fish at Slaughter. retrieved from. https://search-proquest-com.pva.uib.no/docview/1770110637?rfr_id=info%3Axri%2Fsid%3Aprimo.

Sveinsdottir, K., Martinsdottir, E., Hyldig, G., Jørgensen, B., Kristbergsson, K., 2002. Application of quality index method (QIM) scheme in shelf-life study of farmed Atlantic salmon (Salmo salar). J. Food Sci. 67 (4), 1570–1579. Retrieved from. https://doi.org/10.1111/j.1365-2621.2002.tb10324.x.

Aquaculture 526 (2020) 735381

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Effect of chilling technologies on water holding properties and other quality parameters throughout the whole value chain: From whole fish to cold-smoked fillets of Atlantic salmon (*Salmo salar*)

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A B S T R A C T

The effect of different chilling technologies on water holding and quality parameters was investigated on Atlantic salmon throughout the entire value chain. Chilling technologies of whole fish before filleting, included superchilling(S) by refrigerated seawater (RSW) or ice (I), followed by chilling of fillets with liquid nitrogen (SS, IS) or ice (SI, II). Superchilling by shell-freezing with liquid nitrogen (IS and SS) caused increased drip loss throughout storage for both raw and smoked fillets. Whole salmon stored in RSW followed by ice storage (SI) had the least drip loss. Moreover, fish stored in RSW had lower H_2S producing bacteria for raw fillets, lower blood spot counts and gaping after smoking. Therefore, this method is likely more feasible than storing whole fish in ice or shell-freezing of fillets. Water content, muscle pH and colour parameters were higher for raw than smoked fillets, while breaking force, firmness and water holding capacity were higher for smoked than raw fillets.

1. Introduction

Water holding capacity (WHC), the ability for raw meat to retain moisture, is known as an important quality parameter of raw and cold-smoked Atlantic salmon (*Salmo salar*). Having a high WHC is one of the major goals in food processing as it relates to the products' yield, quality and sensory attributes (Duun, 2008; Huff-Lonergan, 2002). WHC can affect weight changes during storage and transport, weight loss during thawing and cooking, and meat texture (Duun, 2008; Kaale et al., 2014). Most free water that can be easily released lies between the actin and myosin filaments of myofibrils in live or *pre-rigor* muscles. During *post mortem*, some of this water is lost as drip loss, which is closely related to WHC. This represents liquid loss during processing, storage, or thawing, and it occurs due to extrusion of tissues juices from the structural change of muscle (Huff-Lonergan and Lonergan, 2005). Water soluble compounds are also lost as drip which provides a nutritious medium for microbial growth (Wu et al., 2014). This can directly influence the producers' profitability and consumers' perception on appearance and texture.

There are several *pre-* and *post-mortem* factors which can affect the WHC in salmon, like *pre-mortem* stress (Roth et al., 2006), starvation (Mørkøre, 2008) and state of *rigor mortis* (Rotabakk et al., 2017). Muscle stiffening usually starts a few hours *post mortem* and increases to a maximum rigidity after 12–24 h. In general, fishing industries prefer a long *pre-rigor* period to give greater production flexibility. Thus, it is important to minimize the rapid onset of *rigor* through controllable methods like rapid cooling, gentle handling and proper processing.

Temperature has been an important parameter in the fish industry from farm to fork. Superchilling is a preservation method where temperature is kept between conventional chilling and freezing (Banerjee and Maheswarappa, 2019). This prolongs shelf life of foods. As traditional chilling on ice represents 20–30% of the total weight of each box of fish (Magnussen et al., 2008), this directly incurs extra costs to both producers and consumers. In contrast, superchilling reduces the need for ice during transportation and storage, effectively utilizing the fish itself as a cooling medium. This inhibits microbial activity, thereby maintaining high food freshness and quality (Magnussen et al., 2008).

Superchilling can be done using several methods, one of which is by refrigerated sea water (RSW) slurry. The RSW is a binary system consisting of water with microscopic ice crystals commonly used in fishing vessels for holding large quantities of fish and cooling the catch to −1 °C in large seawater tanks until processing. Storing fish in RSW has proven to be rapid and easy, and slurries have better heat exchange rates and causes less fish damage in contrast to flaked ice (Piñeiro et al., 2004; Wu et al., 2014). Erikson et al. (2011) reported that at least 3 h is required to chill whole salmon in RSW at −2 °C to attain core

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https://doi.org/10.1016/j.aquaculture.2020.735381
Received 20 February 2020; Received in revised form 14 April 2020; Accepted 17 April 2020
Available online 19 April 2020

temperatures of −1 to −2 °C.

Extensive research has proven superchilling on raw fillets to be effective (Claussen et al., 2017; Magnussen et al., 2008). Nevertheless, such conditions have so far been only applied to the processing line. The current practice is to store whole salmon in ice then superchill after filleting, but industrial application of superchilling fillets can still be challenging as many factors need to be considered including formation of ice crystals, rate of superchilling and accurate temperature measurements of pre-chilling and superchilling storage temperatures (Kaale et al., 2014; Magnussen et al., 2008). To our knowledge, superchilling during initial storage of whole salmon in RSW and its effect on water holding properties and other quality parameters in the whole value chain has not been explored. In collaboration with Hav Line AS, an experiment was carried out onboard their new hybrid fish slaughtering vessel, Norwegian Gannet. This vessel directly harvests fish at fish farms, slaughter and immediately superchill the fish in RSW tanks onboard. By doing so, temperature of salmon is already kept at superchilled conditions during the early stages of the value chain. The overall objective of this project was to superchill whole salmon and follow the entire process until fillets were dry salted and cold-smoked. Water holding properties like drip loss and water holding capacity, and other quality attributes were assessed throughout the experiment.

2. Materials and methods

2.1. Raw material and experimental design

On 10th of February 2019 at Bjørnholmen, Sogn and Fjordane county, Norway, approximately 210 tons of Atlantic salmon (*Salmo salar*) were crowded in their production pen and pumped onboard the slaughter vessel MS Norwegian Gannet (sea temperature: 6 °C, weight: 5.4 kg). The fish was starved for 5 days then slaughtered according to protocol, electrically stunned prior to bleeding and gutting 30 min later.

For the experiment a total of 82 fish was used for quality analysis. After 5 h of crowding, ten salmon were used to follow *rigor mortis* (Cuttinger's method) for 9 days, with quality also assessed using the quality index method (QIM) (Hyldig and Green-Petersen, 2005). pH was measured upon slaughter using a Mettler Toledo SevenGo pro pH meter (Mettler Toledo Inc., USA). Blood glucose and lactate were also measured using Epoc® blood analysis system (Siemens Healthcare Diagnostics, Norway) and Lactate Pro 2 m (Arkray Inc., The Netherlands).

A full factorial design was carried out (Fig. 1a); whole fish (chilled on wet ice *versus* RSW), fillet (stored on wet ice/superchilled with N_2) and processing method (raw/cold-smoked), resulting in 8 different groups. First, a group of head-on-gutted (HOG) salmon (n = 36) was stored in wet ice for 4 days until before filleting as control in expanded polystyrene (EPS) boxes. Another group (n = 36) was immediately superchilled in RSW with ice slurry to −0.7 °C in storage tanks onboard for around 12 h. TrackSense Pro® temperature loggers (Ellab A/S, Denmark) were inserted in the abdomen for iced fish, and at the gut area towards the tail for RSW fish. The superchilled fish were then taken out from the tanks and placed in EPS boxes, before transporting all fish from Tananger, Sola to Nofima AS, Stavanger. Upon arrival, fish stored in ice and superchilled fish (RSW) were kept at 0 °C and −1 °C respectively until filleting on day 4.

2.1.1. Filleting

Fish were mechanically filleted using a Carnitec fillet machine (Carnitec AS, Støvring, Denmark) on day 4. After filleting, half of the fish from each group (n = 18) were stored in ice or superchilled in liquid N_2 (−35 °C, 80s) with a cryogenic chest freezer equipped with a Siemens Simatic HMI panel at 1500 rpm speed fan rotation (CES group, Belgium). Each group was subjected to 2 different treatments (wet ice/superchill), resulting in 4 different fillet groups (II, IS, SI, SS). II and IS represents whole salmon in ice and then in ice or superchilled after filleting respectively, while SI and SS represents whole salmon in RSW and then in ice or superchilled after filleting respectively. II and SI fillets were stored in EPS boxes containing ice at 0 °C, while IS and SS fillets were subjected to shell freezing by superchilling using cryogenic freezer with liquid N_2, until −1 °C. IS and SS fillets were stored in EPS boxes at −1 °C. The left and right fillets were thereafter stored separately for three weeks as raw and cold-smoked fillets, respectively. Weekly sampling was done on raw fillets for quality analysis (t = 9, 16 and 23 days *post mortem*).

2.1.2. Salting and smoking

At day 9 *post mortem*, right fillets were randomized using a trolley with 11 grids and dry salted with refined salt (GC Rieber, Norway) for 18 h, 0 °C. The fillets were then rinsed briefly and gently dried. Fillet weights were recorded before and after salting. Cold-smoking was performed in a Bastramat C1500 smoking cabinet equipped with a Bastra Profi700 microprocessor (Bayha Strackbein GmbH, Arnsberg, Germany). A Bastra FR 100 smoke generator (Bayha Strackbein GmbH, Arnsberg, Germany) supplied with Reho Raucher Gold HBK 750/200 wood chips (J. Rettenmaier & Sohne GmbH, Rosenberg, Germany) was used for smoke generation. The fillets were dried in the chamber for 60 min before they were smoked and dried 5 times consecutively in alternating intervals of 45 min and 15 min at 22 °C, 75% humidity. They were then cooled, vacuumed packed with 99% vacuum and stored at 4 °C. Weekly sampling was done throughout the storage for 3 weeks (t = 17, 24 and 31 days *post mortem*).

2.2. Quality analyses

A schematic illustration where analysis was done is shown in Fig. 1b. Sensory attributes on raw fillets were first assessed using the fillet index method until day 23, giving a demerit point for each key attribute (smell, gaping, colour, consistency, surface). The criteria for smell, gaping, colour and consistency was graded by a 4-scale point (0: best, 3: worst) while surface texture was graded by a 2-scale point (0: dry, 1: loose). The total score was summed up (0: best, 13: worst).

Cylinders were punched (diameter 31 mm) on the anterior dorsal part of each fillet and kept at −80 °C for enzyme and salt content analysis. Muscle pH was also measured on the anterior dorsal muscle. For smoked fillets, the number of visible blood spots were counted, while the extent of muscle gaping was evaluated on a scale of 0–5 (0: no gaping, 5: severe gaping).

For salt content analysis, samples (1–1.5 g) were taken from the frozen smoked samples on day 24. Hot deionized water (30 ml) was added and homogenized (9500 r min^{-1}, 60s) by an Ultra Turrax T25 (Janke & Kunkel IKA® – Labortechnik, Staufen, Germany). The samples were heated in a water bath (100 °C, 10 min), cooled to room temperature and diluted to 100 ml before contents of chloride (mg l^{-1}) were measured on a Hach HQ40d multi Portable Meter, Hach, USA connected to an Intellical™ (Cl^-) Ion Selective Electrode (Hach, USA). The content of NaCl was calculated based on molecular weight and expressed as per cent NaCl of sample weight.

2.2.1. Drip loss and yield

Drip loss (%) was calculated as $\frac{m_0 - m_t}{m_0} \times 100\%$ where m_0 was the initial weight (g) and m_t the weight of fillet during sampling (g). Raw fillets were measured on t = 4, 9, 16, 23 days while smoked fillets were measured on t = 9, 10, 11, 17, 24, 31 days. The post-smoking yield (%) was calculated as $\frac{m_{sm}}{m_0} \times 100\%$ where m_{sm} was the weight of fillet after smoking (g) and m_0 weight of initial unprocessed fillet (g).

2.2.2. Water holding capacity and water content

Water holding capacity (WHC) and water content (WC) were measured in replicates from the dorsal back and backwards, above the lateral line on the white muscle tissue on each sampling day for both raw and smoked fillets (diameter 31 mm, height 6 mm, Fig. 1b). Two

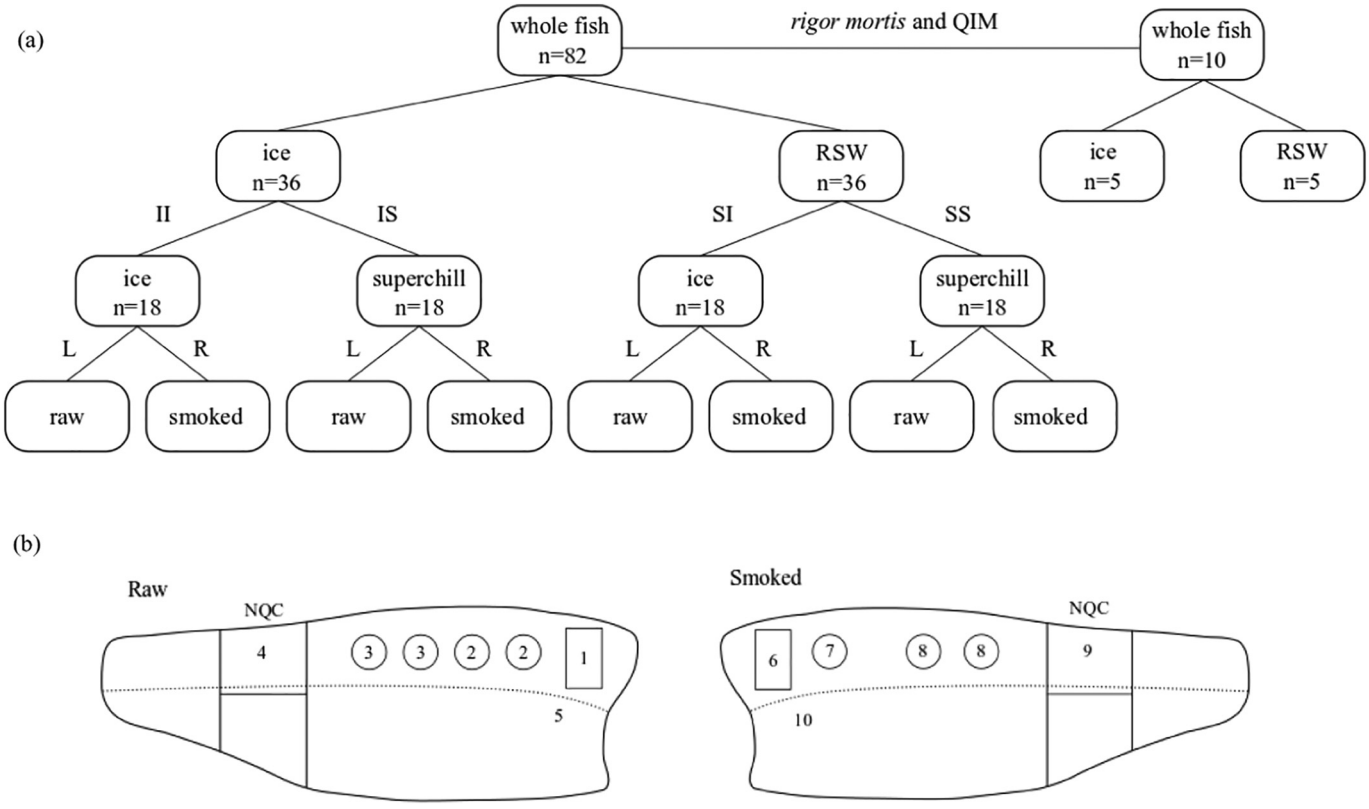

Fig. 1. (a) Experimental overview. 10 fish were used for *rigor mortis* and quality index measurements (QIM). II and IS represents whole fish on ice and stored in ice or superchilled after filleting respectively; SI and SS represents whole fish in RSW and stored in ice or superchilled after filleting respectively; L and R represents left and right fillets respectively. (b)Schematic illustration showing the areas where analysis on raw and smoked fillets were done. 1 and 6. Microbiology analysis, 2 and 7. Frozen samples for enzyme and salt content analysis for raw and smoked fillets respectively; 3 and 8. Water holding capacity and dry matter; 4 and 9. Norwegian Quality Cut (NQC) for texture analysis; 5 and 10. pH.

portions from each sample (~4 g) were punched transversally, and WHC calculated as described by Skipnes et al. (2007). Weighed samples from the top portion were placed in carriers (Part No.4750, Hettich Lab Technology, Germany) and centrifuged (Rotina 420 R, Hettich Lab Technology, Germany) using a free swing rotor at 530 ×g (15 min, 4 °C). The bottom portion was weighed and dried to analyze contents of dry matter, thereby WC, by drying at 105 °C for 16-18 h to constant weight.

WHC was calculated using $\frac{w-\Delta w}{w} \times 100\%$ where $w = \frac{m_w}{m_w + m_D} \times 100\%$ and $\Delta w = \frac{\Delta m_w}{m_w + m_D} \times 100\%$. m_w and m_D are the mass of water and dry matter in the sample respectively, and Δm_w is the mass of liquid separated from the sample during centrifugation (Skipnes et al., 2007).

2.2.3. Colour analysis

Colourimetric analysis was performed on the top loin of both raw and smoked fillets on each sampling day using a digital colour imaging system (DigiEye full system, VeriVide Ltd., Leicester, UK). The fillets were placed in a standardized light-box (daylight, 6400 K) and photographed with a calibrated digital camera (Nikon D80, 35 mm lens, Nikon Corp., Japan). The software Digipix (version 2.8, VeriVide Ltd., Leicester, UK) was used to calculate L*a*b* values from RGB values obtained from the fillet image. L* describes lightness of the sample (L* = 0 = black, L* = 100 = white), a* the redness (a* > 0) and b* the yellowness (b* > 0).

2.2.4. Texture analysis

Texture analysis was performed with a Texture Analyzer TA-XT® plus (Stable Micro Systems Ltd., UK), equipped with a 5 kg load cell. A 12.7 mm P/0.5 flat-ended cylindrical probe was used to create triplicate punctures above the mid-line of the Norwegian quality cut (NQC, NS1975) directly on both raw and smoked fillets transverse to the muscle fiber orientation. The force-time graph was recorded by a computer equipped with the Texture Exponent light software to analyze the data. The resistance force (N) was recorded with a constant speed of 2 mm s^{-1}, where the surface breaking strength (fracturability, *i.e.* force at first breaking point) was recorded. A Warner Bratzler shear test was also done to assess fillet firmness (hardness) by observing the highest recorded peak. Analysis was done in triplicates for the puncture test and in replicates for the shear test.

2.2.5. Cathepsin B + L analysis

Frozen samples of raw fillets from days 4 and 9 (II and SS group) were used. A phosphate buffer (3.38 mM Na_2HPO_4, 15 mM NaH_2PO_4, pH 7.5) was prepared. Sucrose solution (0.25 M) containing 1 mM of EDTA and 100 mM NaCl in phosphate buffer was added to the muscle at 1:5. Samples were then homogenized (13,500 rpm, 2 × 20s, 4 °C) by an Ultra Turrax T25 (Janke & Kunkel IKA® – Labortechnik, Staufen, Germany). The homogenates were centrifuged at 17000 ×*g* (20 min, 4 °C) and supernatants collected for enzymatic analysis.

Cathepsin B + L activity was measured fluorimetrically. The release of the fluorogenic reagent 7-amino-4-methylcoumarin from the substrate *Z*-Phe-Arg-Nmec was measured at its excitation and emission wavelengths, 360 nm and 460 nm respectively. Enzyme and activation buffer (340 mM sodium acetate, 60 mM 100% acetic acid, 4 mM EDTA, 0.1% Brij 35 (30%), pH 5.5 + 500 µl dithiothreitol) were mixed and heated to 40 °C. 100 µl substrate was added, mixed and incubated for 10 min at 40 °C. The reaction was stopped by the addition of 1 ml cold "stop" buffer (100 mM NaOH, 30 mM CH_3COONa, 70 mM 100% CH_3COOH, 100 mM $ClCH_2COOH$, pH 4.3). Enzyme activity was

quantified by a standard curve from 7-amino-4 methylcoumarin solutions of 0–200 nM dilution series.

2.2.6. Microbiological analysis

Total psychotropic viable plate count (TVC) were quantified in accordance to the NMKL method No. 184 using Long and Hammer (L&H) agar on the first (day 4) and last sampling day (day 23) for raw fillets, and on the last sampling day (day 31) for smoked fillets. H_2S producing bacteria was analyzed on raw fillets by counting the black colonies from iron agar (Lyngby, Oxoid, Norway) supplemented with 0.04% L-cysteine (Sigma-Aldrich, Norway).

Around 10 g of samples were cut from each fillet, placed in a stomacher bag and weighed. Sterile buffered peptone water (Merck, Germany) was added 9× the sample weight and blended using a Smasher® (AES Laboratorie, bioMérieux Industry, USA) for 120 s. Dilution series of the homogenates were prepared in Eppendorf tubes with sterile peptone water. L&H plates were incubated for 5 days at 15 °C, while iron agar plates were incubated for 72 ± 6 h at 25 °C.

2.3. Statistical analysis

Data were analyzed in MINITAB® Version 19 (Minitab Inc., State College, Pennsylvania, USA) by multivariate analysis using generalized linear model (GLM) where sample groups were considered as factors, and storage days as covariate. A two-way *t*-test was used when comparing data between raw and smoke fillets, while mood's median test was used for data on blood spot counts and gaping. One-way analysis of variance (ANOVA) was used to compare groups on their respective days for microbiological analysis. The alpha level was set to 5% ($p < .05$). All results are presented as mean ± standard deviation.

3. Results

3.1. Blood parameters, temperature, QIM and state of rigor mortis

The initial pH of fish after gutting and bleeding was 7.2 ± 0.8, while lactate content was 1.7 ± 1.5 mmol l^{-1}. The blood parameters were Na^+: 162.5 ± 1.2 mmol l^{-1}, K^+: 4.4 ± 0.9 mmol l^{-1}, Ca^{2+}: 1.7 ± 0.1 mmol l^{-1}, hematocrit: 23.9 ± 2.9% and glucose 4.1 ± 0.3 mmol l^{-1}.

Whole fish stored in RSW cooled at a faster rate than those in ice, reaching a core temperature of −0.5 °C within 4 h, and down to −0.7 °C within 6 h (Fig. 2a). Fish in ice took up to 2 days to reach 0 °C. The temperature of both groups remained quite stable throughout the entire shipping period to the laboratory.

The experimental design did not affect *rigor mortis* where maximum stiffness was observed at an average of 24 h for both groups ($p = .784$, Fig. 2b). There was an effect of processing method ($p = .048$) and storage time ($p < .001$) on QIM scores on whole fish. The fish chilled in ice had a slightly higher QIM score until day 5 (day 1: 2.2 ± 0.8, day 5: 5.0) than those stored in RSW (day 1: 1.6 ± 0.6, day 5: 4.2 ± 0.8). In contrast, RSW fish had a higher QIM score (7.0 ± 2.0) than ice (6.2 ± 0.8) on day 9.

3.2. Drip loss and yield

There was a steady increase in drip loss for all groups of raw fillets (Fig. 3a). A rapid increase in drip loss for all smoked fillets was observed after smoking on day 11, before it becomes relatively constant through storage (Fig. 3b). There was a significant effect for raw fillets on how the whole fish (ice *versus* RSW, $p = .039$) and fillets (ice *versus* superchilled with N_2, $p < .001$) were treated. In general, II and SS salmon had higher drip losses than IS and SI, with II reaching as high as 5.6 ± 1.6% on day 23. SI salmon had the lowest loss of 1.5 ± 0.6% and the drip losses of IS and SS were 3.2 ± 0.9% and 4.6 ± 0.6% on day 23, respectively.

All groups had a 4.2–4.7% decrease in weight after dry salting, with salt content measured on smoked fillets on day 24 (II: 3.4 ± 0.2%, IS: 4.8 ± 1.0%, SI: 4.9 ± 0.4% and SS: 4.7 ± 0.3%). Product yield after smoking was found to be similar among all groups, ranging from 92.5–93.3%. Moreover, the weight loss of smoked fillets was found to be significantly affected by storage time ($p < .001$) and how whole fish were stored ($p = .002$). Fillet treatment among smoked fillets were not significantly affected by the experimental design ($p = .740$).

3.3. Water holding capacity, water content and muscle pH

WHC of smoked fillets were significantly higher, while WC and muscle pH were lower than the raw counterparts (Table 1, $p < .001$, $p < .001$, $p < .001$, respectively). Raw fillets for II had the highest WHC while SS the lowest at the end of storage. A significant effect on WHC was observed among different groups ($p = .002$), but not storage days ($p = .369$). Both SI and SS raw fillets decreased by 3% and 0.9% respectively in WHC throughout the fillet storage time. In contrast, there was no difference among groups of smoked fillets ($p = .445$), but storage duration had an effect ($p < .001$). The WHC of all smoked fillets generally decreased through storage time, and SI fillets had the highest WHC on days 17 and 24.

No specific trends on WC was seen among groups of raw fillets ($p = .875$), but there was a general increasing trend for smoked fillets

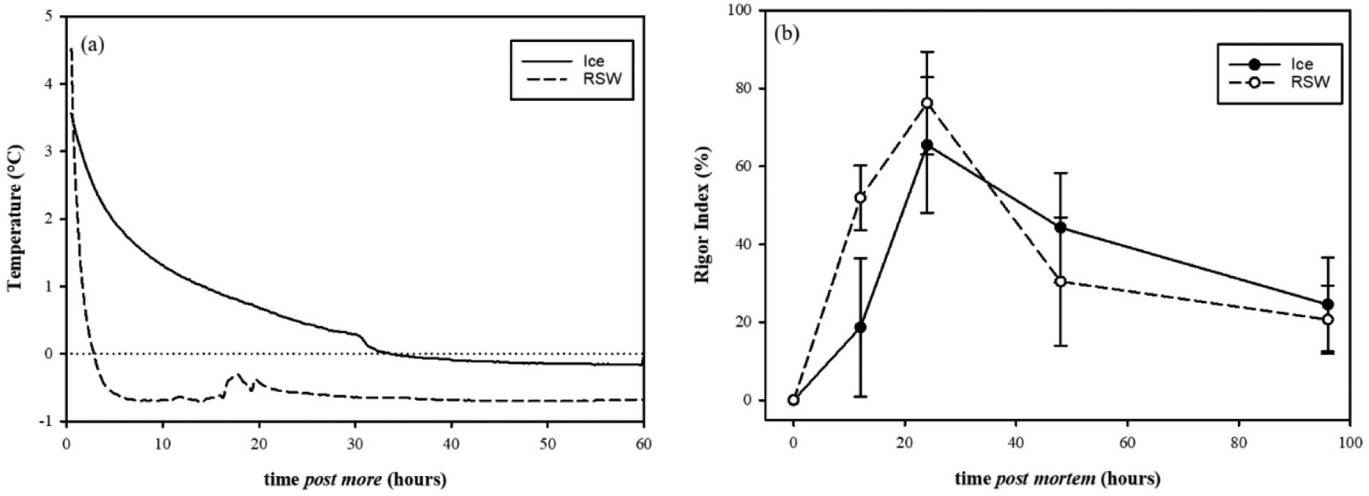

Fig. 2. (a) Temperature change and (b) rigor index of whole fish in ice and RSW (GLM, $p = .784$) over time.

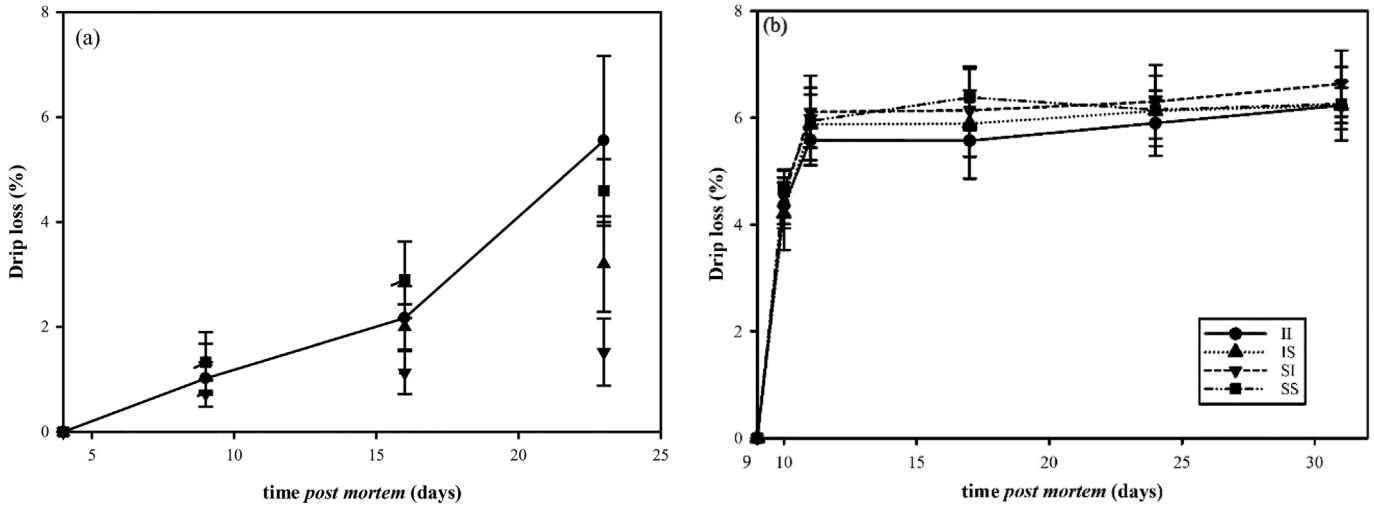

Fig. 3. (a) Drip loss of raw fillets after filleting on day 4 (GLM; storage days: $p < .001$; whole fish: $p = .039$; fillets: $p < .001$); and (b) smoked fillets processed at day 9 as a function of time after processing (GLM; storage days: $p < .001$; whole fish: $p = .002$; fillets: $p = .740$).

through storage ($p = .001$). At the end of storage, II smoked fillets had the highest water content while IS the lowest. pH was found to be similar among all groups of raw and smoked fillets. Only storage time influenced pH ($p = .029$) for smoked fillets.

3.4. Surface appearance

Fillet index showed that storage days had an effect ($p < .001$), but not on treatment groups of raw fillets ($p = .692$). There were minimal differences on fillet index during the first 2 weeks of storage in all groups, ranging from an average score of 0.9 ± 0.9 to 1.5 ± 1.0 on day 4, then to 1.5 ± 1.4 to 2.8 ± 1.7 on day 16. However, a considerable increase in score was seen on day 23 where all groups ranged from 5.2 ± 1.0 to 5.8 ± 2.3.

The lightness, redness and yellowness of raw fillets were significantly higher than those smoked ($p < .001$, $p < .001$, $p = .026$, respectively). A significant effect of storage duration was also found on raw fillets' translucence (L*, $p < .001$) and redness (a*, $p < .001$), but not on yellowness (b*, $p = .178$) (Table 2). The lightness value was found to decrease with an increasing storage duration until day 16. In addition, treatment groups were different in L*($p = .001$), a* ($p < .001$) and b* ($p = .007$). In general, a* decreased ($p = .008$) in all groups of smoked fillets through time, whereas no effect was observed regarding yellowness ($p = .158$) and lightness ($p = .057$). It was further observed that II smoked fillets were significantly darker ($p = .024$) and less yellowish ($p = .021$) than the other groups. SS smoked fillets showed the highest a*-value, although this was insignificant ($p = .104$). In contrast, SI fillets were lighter and more yellowish and greenish in colour.

There was a significant difference in the number of blood spots ($p = .001$) and fillet gaping score ($p < .001$) among the cold-smoked groups. Whole fish stored in RSW (SI on average: 0.0 ± 0.3, SS on average: 0.0 ± 0.2) had almost no blood spots on day 31 compared to those initially stored on ice (II on average: 3.0 ± 3.4, IS on average: 2.5 ± 1.2, day 31). Likewise, cold-smoked SI and SS fillets showed lower gaping scores throughout the storage period (on average: 1.0 ± 0.5 and 1.5 ± 0.9, respectively) as compared to II and IS (2.0 ± 0.9 and 2.5 ± 0.5, respectively).

Table 1
Water holding capacity, water content and pH of raw and smoked fillets throughout storage.

Group	Raw fillets					Smoked fillets				
	Day	WHC (%)	WC (%)	pH	n	Day	WHC (%)	WC (%)	pH	n
II	9	86.8 ± 2.7	61.8 ± 2.6	6.2 ± 0.0	6	17	91.9 ± 1.8	57.2 ± 2.2	6.1 ± 0.0	6
	16	87.0 ± 3.7	63.9 ± 1.8	6.4 ± 0.2	6	24	90.0 ± 3.7	57.1 ± 3.1	6.1 ± 0.1	6
	23	86.2 ± 5.1	63.4 ± 1.9	6.0 ± 0.1	6	31	87.0 ± 3.0	59.6 ± 2.1	6.0 ± 0.1	7
IS	9	82.6 ± 5.8	61.9 ± 1.9	6.2 ± 0.1	5	17	91.7 ± 2.1	56.5 ± 2.5	6.1 ± 0.0	6
	16	85.2 ± 3.7	62.0 ± 1.7	6.4 ± 0.2	6	24	87.7 ± 3.7	57.6 ± 1.9	6.0 ± 0.1	6
	23	83.7 ± 4.6	62.5 ± 4.6	6.3 ± 0.2	5	31	87.9 ± 3.9	57.0 ± 2.3	5.9 ± 0.1	6
SI	9	87.4 ± 3.2	63.2 ± 3.9	6.1 ± 0.0	6	17	93.2 ± 2.3	57.1 ± 3.0	6.0 ± 0.0	6
	16	86.1 ± 5.2	62.7 ± 1.1	6.3 ± 0.0	6	24	91.8 ± 2.9	57.5 ± 1.3	6.0 ± 0.0	6
	23	84.4 ± 4.6	63.3 ± 1.8	6.3 ± 0.1	6	31	84.1 ± 6.1	58.4 ± 1.9	6.1 ± 0.0	6
SS	9	83.2 ± 5.1	61.8 ± 3.0	6.2 ± 0.0	6	17	91.3 ± 3.1	55.7 ± 1.4	6.1 ± 0.0	6
	16	82.1 ± 6.9	63.8 ± 1.6	6.4 ± 0.1	6	24	89.4 ± 3.1	58.6 ± 2.1	6.1 ± 0.1	6
	23	82.3 ± 7.3	62.7 ± 1.7	6.2 ± 0.1	6	31	86.5 ± 3.9	57.5 ± 1.9	6.0 ± 0.0	6
GLM[a]	P_D	0.369	0.730	0.624		P_D	< 0.001*	0.001*	0.029*	
	P_G	0.002*	0.875	0.274		P_G	0.445	0.295	0.095	
t-test[b]	P_R	< 0.001*	< 0.001*	< 0.001*						

[a] General Linear Model (GLM) analyses of variance with fillet groups as factors and storage days as covariance. P_D and P_G are the significant levels for the effects of the storage days and groups, respectively.
[b] Two-way *t*-test comparing fresh and smoked fillets as factors. P_R is the significant level for effects of raw *vs* smoked fillets.
* Significant levels with less than 0.05.

Table 2
L*, a*, b* of raw and smoked fillets throughout storage.

Group	Raw fillets					Smoked fillets				
	Day	L*	a*	b*	n	Day	L*	a*	b*	n
II	4	52.0 ± 2.1	50.4 ± 1.1	26.3 ± 1.3	20	10	42.8 ± 1.1	41.0 ± 1.0	24.9 ± 1.1	19
	9	50.2 ± 2.1	52.1 ± 1.7	27.9 ± 1.2	18	17	41.9 ± 0.9	41.8 ± 1.2	25.2 ± 1.2	6
	16	48.1 ± 0.8	49.7 ± 0.7	28.9 ± 1.3	6	24	42.9 ± 1.3	40.7 ± 1.1	25.9 ± 1.5	6
	23	51.0 ± 2.1	47.7 ± 1.9	25.8 ± 1.4	7	31	–	–	–	–
IS	4	56.4 ± 2.8	52.8 ± 1.7	26.5 ± 1.7	22	10	43.7 ± 1.0	41.3 ± 0.8	26.1 ± 1.2	18
	9	51.4 ± 2.8	51.6 ± 2.7	27.8 ± 1.3	14	17	43.4 ± 1.5	41.1 ± 0.5	26.8 ± 1.5	6
	16	51.2 ± 2.2	49.2 ± 1.8	29.7 ± 1.3	6	24	43.6 ± 0.9	40.6 ± 0.9	26.5 ± 0.5	6
	23	52.3 ± 2.0	49.4 ± 1.2	26.9 ± 1.6	6	31	–	–	–	–
SI	4	54.6 ± 2.5	51.2 ± 1.8	28.0 ± 1.5	20	10	44.4 ± 1.3	41.2 ± 0.9	27.0 ± 1.5	17
	9	51.7 ± 3.1	51.4 ± 2.9	28.7 ± 1.6	18	17	43.0 ± 1.1	40.8 ± 0.7	26.2 ± 1.1	6
	16	49.9 ± 1.4	49.2 ± 1.4	29.2 ± 1.4	6	24	44.6 ± 1.3	40.5 ± 1.1	28.4 ± 2.0	6
	23	53.1 ± 2.0	50.7 ± 2.0	28.0 ± 1.4	6	31	–	–	–	–
SS	4	54.5 ± 2.5	53.8 ± 1.2	28.2 ± 1.9	19	10	44.6 ± 1.5	42.0 ± 1.1	26.8 ± 1.5	18
	9	50.4 ± 3.6	51.3 ± 3.6	28.2 ± 1.4	18	17	42.4 ± 2.3	41.9 ± 1.0	26.0 ± 2.5	6
	16	48.9 ± 3.3	48.6 ± 0.6	28.9 ± 2.1	6	24	43.2 ± 1.4	40.9 ± 1.0	26.3 ± 1.1	6
	23	50.9 ± 1.7	48.6 ± 1.5	27.9 ± 1.6	6	31	–	–	–	–
GLM[a]	P_D	< 0.001*	< 0.001*	0.178		P_D	0.057	0.008*	0.158	
	P_G	0.001*	< 0.001*	0.007*		P_G	0.024*	0.104	0.021*	
t-test[b]	P_R	< 0.001*	< 0.001*	0.026*						

[a] General Linear Model (GLM) analyses of variance with fillet groups as factors and storage days as covariance. P_D and P_G are the significant levels for the effects of the storage days and groups, respectively.
[b] Two-way t-test comparing fresh and smoked fillets as factors. P_R is the significant level for effects of raw *vs* smoked fillets.
* Significant levels with less than 0.05.

3.5. Texture and cathepsins B + L

The breaking force ($p < .001$) and firmness ($p < .001$) of smoked groups were significantly higher than raw fillets (Table 3). Results from the compression test on raw fillets further showed a general decrease in breaking force in all groups through time. There was an effect of storage days on texture of raw fillets ($p < .001$), but not chilling method ($p = .832$). II had the firmest texture until day 16, while SI had the firmest texture on day 23 ($p = .047$).

A significant difference in breaking force was also observed among the chilling methods of smoked groups ($p = .005$) and storage days ($p = .001$). II, SI and IS groups increased in fracturability based on its breaking force until day 24, with SI having the highest force. On the last storage day, II and IS continued to increase in breaking force while SI and SS decreased. Based on shear test, smoked groups ($p = .031$) and storage duration ($p = .001$) differed significantly in firmness. SI group were highest in firmness on day 17, while SI and SS were both higher than the iced group (II and IS) on day 24.

Muscle cathepsin activity of II and SS groups were analyzed on days 4 and 9. Overall, storage time did not affect the total cathepsins B + L activity ($p = .170$), but there was a significant difference between the two groups ($p = .002$). SS group had a significantly higher enzyme activity ($p = .005$) on day 4 (1.4 ± 0.2 mU g^{-1} muscle) than II (1.0 ± 0.2 mU g^{-1} muscle). In contrast, II (1.2 ± 0.3 mU g^{-1}

Table 3
Texture analysis of raw and smoked fillets throughout storage.

Group	Raw fillets				Smoked fillets			
	Day	Breaking force (N)	Firmness (N)	n	Day	Breaking force (N)	Firmness (N)	n
II	4	8.8 ± 1.3	13.3 ± 3.5	6	10	–	–	–
	9	8.6 ± 2.2	12.5 ± 4.2	6	17	16.9 ± 2.4	19.6 ± 2.9	6
	16	7.3 ± 1.0	15.2 ± 2.8	6	24	17.9 ± 3.1	17.0 ± 1.8	6
	23	7.6 ± 0.8	13.1 ± 3.2	6	31	20.2 ± 3.6	17.2 ± 1.9	7
IS	4	8.8 ± 1.3	13.3 ± 3.5	6	10	–	–	–
	9	7.4 ± 1.1	10.8 ± 2.2	5	17	16.6 ± 1.9	17.8 ± 3.1	6
	16	7.6 ± 0.9	14.4 ± 3.6	6	24	18.5 ± 3.5	16.9 ± 2.9	6
	23	7.4 ± 0.7	11.6 ± 3.1	5	31	19.0 ± 3.2	17.9 ± 2.9	6
SI	4	9.1 ± 1.3	11.4 ± 2.0	6	10	–	–	–
	9	7.5 ± 0.9	11.3 ± 3.7	6	17	17.9 ± 2.1	22.7 ± 3.2	6
	16	7.1 ± 1.1	12.8 ± 2.8	6	24	20.6 ± 3.3	18.6 ± 2.0	6
	23	7.5 ± 1.0	15.0 ± 3.7	6	31	19.5 ± 2.6	19.0 ± 2.3	6
SS	4	9.1 ± 1.3	11.4 ± 2.0	6	10	–	–	–
	9	7.7 ± 1.4	11.0 ± 2.7	6	17	20.1 ± 3.5	16.8 ± 2.2	6
	16	8.8 ± 2.3	12.8 ± 2.4	6	24	18.8 ± 2.8	18.7 ± 2.4	6
	23	7.6 ± 1.1	14.3 ± 2.6	6	31	19.4 ± 2.7	15.3 ± 1.8	6
GLM[a]	P_D	< 0.001*	0.005*		P_D	0.001*	0.001*	
	P_G	0.832	0.047*		P_G	0.005*	0.031*	
t-test[b]	P_R	< 0.001*	< 0.001*					

[a] General Linear Model (GLM) analyses of variance with fillet groups as factors and storage days as covariance. P_D and P_G are the significant levels for the effects of the storage days and groups, respectively.
[b] Two-way t-test comparing fresh and smoked fillets as factors. P_R is the significant level for effects of raw *vs* smoked fillets.
* Significant levels with less than 0.05.

muscle) had a slightly higher activity than SS (0.9 ± 0.2 mU g^{-1} muscle, p = .138) on day 9.

3.6. Microbiology

TVC for whole fish on ice and RSW on day 4 were both 2.7 ± 0.3 log cfu g^{-1}. II and IS had the lowest (8.0 ± 0.7 log cfu g^{-1}) and highest (9.0 ± 0.1 log cfu g^{-1}) psychrotropic counts on day 23 (p = .003), respectively. No H_2S producing bacteria were detected on day 4 in all groups. After 23 days, IS had the highest counts of H_2S producing bacteria (7.5 ± 0.2 log cfu g^{-1}), while SI the lowest (6.3 ± 0.6 log cfu g^{-1}, p < .001). For smoked salmon, TVC was measured on the last sampling day. There was a similar bacterial development for all storage groups (II: 4.6 ± 0.5; IS: 4.6 ± 0.6; SI: 4.8 ± 0.7; SS: 5.2 ± 0.4 log cfu g^{-1}, p = .304).

4. Discussion

As demonstrated in this study, superchilling whole fish in RSW, followed by storage on ice after filleting, resulted in lesser drip loss as compared to the traditional storage method on ice. In addition, superchilling resulted in better gaping scores, lower blood spot counts and higher firmness and toughness after smoking.

The blood and lactate content measured after slaughter were physiologically within the baseline level of unstressed and healthy fish (Einarsdóttir and Nilssen, 1996; Lerfall et al., 2015). In this experiment, fish were slaughtered on-site. The high capacity of pumps used in the vessel reduces the crowding density and gives a positive effect of stress during crowding. Therefore, this method gently handles and lessens stress in fish by condensing 3 handling processes, where fish are traditionally pumped into well-boats and waiting cages before slaughter, into only one handling process. The initial pH of 7.22 was also close to previous reported values of unstressed fish (Lerfall et al., 2015). The decline in pH to 6.13–6.22 on day 9 for raw fillets indicated a high glycogen reserves in the unstressed fish slaughtered on-site, which was converted to lactic acid during *post mortem* glycolysis. However, a small increase of pH was observed on day 16 which may be caused by bacterial contamination from metabolic activity in bacteria, decomposing nitrogen compounds to form basic compounds like ammonia and trimethylamine, thereby increasing the pH (Castro et al., 2017). This could also explain why the higher QIM score observed for salmon in RSW than in ice on day 9, which was likely due to frequent handling during measurement days.

Rigor mortis in unstressed salmon normally reaches a maximum between 24 and 30 h (Wang et al., 1998). In this study, fish stored both in ice and in RSW went into maximum rigor at around the same time. This illustrates that superchilling of whole fish in RSW did not accelerate the progression of the rigor process as seen in cold shortening on winter acclimatized salmon due to rapid chilling immediately after slaughter. It is important to note that temperature variations during storage should be minimal as this can affect ice melting and recrystallisation, which changes the ice distribution and size within the fish (Wu et al., 2014). In this study, the temperature was kept rather stable during transportation of whole fish. Fish in RSW was observed to cool down at a faster rate than in ice, which was expected since the recirculating water has a higher convective heat transfer coefficient, consequently a better heat exchange rate as compared to ice. A greater surface area of fish is also exposed to seawater, providing a more even temperature distribution.

4.1. Water holding properties

In the study, drip loss of II raw fillets was considered high. Drip loss may be attributed to various factors such as fat content (Mørkøre et al., 2001), starvation (Mørkøre et al., 2008), stress prior to slaughtering (Roth et al., 2006) and storage conditions (Huff-Lonergan, 2002). Increasing the storage temperature could also significantly increase drip loss (Huff-Lonergan, 2002). Therefore, the temperature rise from superchilled to chilled conditions in the early stages of the value chain could justify why II fillets had the greatest drip loss. Furthermore, the drop in pH for II fillets may lead to a higher degree of protein denaturation which could also cause an increase in drip loss. In salmon, the main drip loss is water, but lipids, proteins and carotenoids are also lost during storage of smoked fillets (Lerfall, 2011). It could be an interesting aspect to observe the possible loss of water-soluble constituents contained in drip loss in future experiments.

The effect of superchilling on drip loss in salmon has been controversial. The observed drip loss for IS and SS raw fillets were likely due to freezing out of water during superchilling of fillets which forms ice crystals in the muscle, leading to a higher solute concentration, cell damage and protein denaturation (Bahuaud et al., 2008; Duun, 2008). This was also observed by Duun (2008) and Kaale et al. (2014), who recorded that drip loss in raw superchilled salmon fillets stored at −1.4 and − 1.7 °C respectively, were usually 1–2% lower than the chilled reference. Claussen et al. (2017) however showed that superchilled fillets at −1.5 °C using an impingement freezer, with filleting done in a pre-rigor state, had a slightly increase in drip loss of 5% at the beginning of the storage period, but towards the end this loss remained stable. In the present experiment, drip loss was also found to be significantly affected by how the whole fish was stored and the storage duration for both raw and smoked fillets. This especially applies for superchilling whole fish in RSW then storing fillets in ice (SI) which gave a lower drip loss than traditional chilling on ice.

WHC of raw fillets observed in this study (82.1–87.4%) was found to be reasonably comparable to previous studies (Hultmann and Rustad, 2002; Løje, 2007; Rotabakk et al., 2017). Kaale et al. (2014) reported that WHC of superchilled salmon fillets increased with storage time, but in the present study this was not seen in IS and SS fillets. Samples with higher drip loss are also more likely to retain the remaining water during the centrifugation process of water holding analysis (Duun, 2008). This phenomenon was only observed for II raw fillets, having a higher WHC. The results observed for raw fillets were more in agreement with Hultmann and Rustad (2002), who observed that WHC of salmon was not affected by storage time, likely due to the high within and among sample group variations. As the calculation of WHC is dependent on the WC, samples may be slightly inconsistent in size when being placed in the oven for WC analysis. The filleting machine used may also induce micro-ruptures in the muscle, affecting its WHC and WC (Rotabakk et al., 2017).

Cold-smoked salmon is a lightly preserved fish product with 3.5–6% salt content (Hansen et al., 1996) which were within the reported range from this study. Drip loss of the groups of smoked salmon were affected by storage duration and how the whole fish was treated. Since SI had the least drip loss of raw fillets, they retained more loosely bound water than II fillets. This water could have evaporated during salting and smoking, explaining why SI had the highest drip loss in smoked fillets.

All groups of smoked salmon had a weight reduction of 4.2–4.7% after dry salting, coinciding with other studies reporting a 3.6–7.4% decrease in fillet weight (Birkeland et al., 2004; Lerfall and Rotabakk, 2015). The product yields obtained after smoking for all groups were slightly higher than reported values of 86–92% (Birkeland et al., 2004; Cardinal et al., 2001; Lerfall and Rotabakk, 2015; Sigurgisladottir et al., 2000). This is economically beneficial but may be due to biological variations such as differences in fat content, as a higher fat content is known to give better yield after processing (Cardinal et al., 2001).

WHC of smoked fillets were significantly higher, while WC lower, than their raw counterparts. Weight loss and lower WC of smoked fillets were mainly due to salting-out process from drying during the process and lipids leaching out from the muscle, causing muscle shrinkage (Sigurgisladottir et al., 2000). This process is diffusion-driven involving two fluxes, where water diffused out while salt diffused in, until equilibrium is reached between the ambient and fish concentration. In this

experiment, there was no difference between smoked groups on WHC, which may be due to variation in the salt and lipid contents of samples. However, WHC in all group of smoked fillets significantly decreased through time in all groups as also observed in other studies (Løje, 2007). This is probably caused by the denaturation of muscle proteins through storage especially with the influence of low thermal processing and salt. Birkeland et al. (2004) stated that accumulated leakage over time in vacuum packed smoked salmon negatively influences the product appearance. This means that smoked fillets are more prone to liquid leakage which explains the increase in drip loss. As water retention after smoking is an important factor for the industry, it is stressed that smoked fillets should not be stored too long. There were only small changes observed in WC during storage despite the increase in drip loss on all raw and smoked groups. This was supported by Jørpeland et al. (2015) in raw Atlantic cod fillets, who explained that WC is measured by relative differences instead of the absolute difference as samples were taken on the same fillet locations throughout storage.

The pH of meat is inversely related to drip loss and greatly affects WHC and flesh softening due to changes in protein net charge. Conversion of muscle to meat lowers the initial pH to 6.1–6.2, as seen in this study. The variation of pH for raw and smoked fillets were similar to Løje (2007), who also observed that pH did not change despite the decrease in WHC for smoked fillets.

4.2. Surface, enzymatic and microbiological indicators

Results from fillet index scores deduced that the sensory quality of raw fillets is acceptable for 16 days, regardless of treatment method. Colour relates to consumers' perception and is a key parameter on both raw and smoked salmon products. However, information on how superchilling influences fillet colour are still limited (Erikson et al., 2011). This study observed a darker, lesser red but more yellowish colour in all groups of raw fillets until day 16. Erikson et al. (2011) reported decreased fillet lightness and redness in ice storage. In contrast, Espe et al. (2004) indicated that ice storage of raw fillets gave paler and more reddish colour. One factor that could have contributed to the darker colour observed may be the pH increase during fillet storage from day 9 to 16. Roth et al. (2009) stated that L* is negatively correlated with muscle pH in Atlantic halibut. Therefore, the end pH at the point of changes according to factors like season, glycogen levels, dietary intake and starvation period are important to control. Fish size and the variation in fat content are also known to affect colour. L* and b* values are reported to increase with an increasing fat content for both raw and smoked fillets, while a* increases only in smoked fillets (Mørkøre et al., 2001). The observed increase in lightness and decrease in yellowness in the present study after day 16 could be a spoilage indication for raw fillets, in correlation to the fillet index measurements.

A decrease in lightness and redness was observed in this study after smoking, confirming with previous studies (Birkeland et al., 2004; Cardinal et al., 2001; Lerfall, 2011; Lerfall and Rotabakk, 2015). This is due to the smoking step causing carbonyl-amino reactions of Maillard browning (Hall, 2011), and denaturation of astaxanthin from alterations in the protein composition (Lund and Nielsen, 2001). Nonetheless, although statistical analysis in this study demonstrated that colour affected treatment groups, this difference was not discriminated by visual observation.

Texture of fish is also an important quality parameter known to decrease throughout storage. Textural properties in fish is influenced by several factors including species, age and size, fat content and distribution, and proteases (Huff-Lonergan and Lonergan, 2005). The fillet thickness can likewise be considered as a source of variation when the probe was directly applied. Therefore, the comparison became more uncertain and its textural properties varied. Texture may be further affected by seasonal variations. Espe et al. (2004) reported that fillets after 14 days of storage on ice were softest when fish were harvested in February, the same period this study was conducted. In this study, all smoked fillet groups gave a lower WC yet higher WHC as compared to raw fillets. The force required to shear smoked fillets were also significantly higher than the raw fillets, which was expected as fish loses moisture and becomes denser and more elastic during smoking. Therefore, WC is negatively correlated with textural breaking force and fillet firmness (Birkeland et al., 2004), while breaking force is positively correlated with WHC (Hultmann and Rustad, 2002).

The breaking force obtained in all groups of raw fillets throughout storage (7.3–9.1 N) were close to the acceptable level of 8-11 N. Less than 7 N implies a soft fillet as measured from a compression test using a cylindrical probe (Mørkøre, 2008). There was an effect seen on breaking force and firmness through storage on raw and smoked fillets. The reduction in breaking force on raw fillets was likely due to the myofiber-myofiber detachments which increases through time (Taylor et al., 2002).

Gaping negatively affects texture caused by the loss of strength in the connective tissue due to increasing amount of collagenases (Espe et al., 2004) and endogenous proteases that detaches muscle fibers from the myocommata (Hultmann and Rustad, 2002). From the results, firmness of SI and SS were higher than II and IS smoked fillets on day 24. This suggests that the connective tissue for SI and SS fillets are more intact. Blood counts and gaping score were also found low in RSW fish (SI and SS smoked fillets), likely due to sufficient cleaning in the RSW tanks. Jacobsen et al. (2017) explained that a higher score is strongly correlated to improper cleaning of fish where remnants like blood and fluids are left in the belly cavity. In this study, fish onboard the vessel were thoroughly gutted, bled and inspected by trained personnel before storage in RSW tanks. Moreover, the recirculation of seawater in the tanks removed traces of blood and fluids from the fish. To detect texture differences more accurately, Guillerm-Regost et al. (2006) suggested that a sensory panel can be considered, especially when liquid loss occurs on the fillet surface. This could be considered for further experiments to correlate texture with sensorial characteristics.

Cathepsins B + L are lysosomal cysteine proteases that degrades fish muscle *post mortem*. These enzymes play an important role in explaining muscle softening in salmonids due to proteolysis of muscle structural proteins (Bahuaud et al., 2008). Gaarder et al. (2012) presented that superchilling at −1.5 °C stimulates calpain and cathepsin activity which leads to softer fillets, but it is still challenging to fully relate enzyme activity to texture. The cathepsin activity of SS in this study was significantly higher than II on day 4, which may explain why its firmness was lower. Thereafter, enzyme activity of SS decreases in contrast to II, suggesting that the rate of proteolysis in SS may be faster than II fillets.

A total microbiological concentration of $> 10^6$ cfu g^{-1} is considered spoiled and the product is sensory rejected by consumers (Dalgaard et al., 1997). Based on the TVC data, all smoked fillets were still consumable after 31 days while all groups of raw fillets were spoiled after 23 days of storage. SI raw fillets produced the least H_2S producing bacteria, which are typical spoilage microorganisms. Therefore, superchilling whole fish in RSW and storing them on ice after filleting can potentially prolong shelf life, but more studies need to be done to confirm this. Previous studies also showed that superchilled fillets delayed growth rate of all bacterial groups in salmon, extending its shelf life (Duun, 2008; Kaale et al., 2011). This was not observed in IS or SS raw fillets, possibly due to technical difficulties in keeping the cold chain stable for superchilled storage. Therefore, future experiments should ensure that temperature is kept stable especially when using slurries as bacterial growth can occur when fish are being transferred from one medium to another (Erikson et al., 2011). Further research could also focus on a wider analysis of microbial activity in for example *Enterobacteriaceae*, *Photobacterium* spp., *Pseudomonas* spp. and anaerobes.

Industries aim to minimize drip loss in fish. Although the commercial use of superchilling can be challenging and requires substantial efforts, it seems more feasible to adopt the method of superchilling and

storing gutted whole fish in RSW. This method can store fish in bulk catches and deliver already superchilled fish to customers, greatly lessening ice demand and providing a better quality than the traditional method on ice. Storing the fish on ice after filleting from RSW fish also lessens drip loss and the need to monitor factors that can affect superchilling like the formations of ice crystals in fillets. As temperature is a critical aspect in superchilling in RSW, this must be monitored closely and kept constant throughout the whole storage period. Adequate cleaning and proper recirculation of RSW systems is also necessary for good hygiene and prevention of microbial growth.

5. Conclusion

The present study showed that superchilling by RSW of whole fish leads to lower drip loss and H_2S producing bacterial counts than traditional methods using wet-ice, along with better blood spot counts and gaping after cold-smoking. Compared to superchilling fillets in liquid N_2, it is more feasible to store fillets from RSW fish chilled on ice due to lesser drip loss and better WHC. Smoking of fillets significantly changed WHC, WC, texture and colour of all raw fillets. In this experiment, the uptake of water and salt from whole fish, and how this affects water holding properties through the whole value chain were not examined. This could be an interesting aspect to explore for further work in addition to shelf life and sensory studies including a taste panel.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Norwegian University of Science and Technology (NTNU) and the project “Hybrid slaktefartøy – Fremtidens slakterier” (project ES612950), co-financed by Regional Research Fund (RFF-Vest), funded this project. The authors would like to thank Hav Line AS for allowing us to work onboard Norwegian Gannet. We would also like to thank the team at Nofima AS, Stavanger namely Karin S Tranøy, Laila Budal, Sigurd Øines, and Bente Wiig Husebø for their technical work, as well as Dagbjørn Skipnes in helping with the liquid N_2 equipment for superchilling. The authors declare that there is no conflict of interest. Sherry Stephanie Chan was supported by a Ph.D. grant from NTNU, as part of the OPTiMAT project.

References

Bahuaud, D., Mørkøre, T., Langsrud, Ø., Sinnes, K., Veiseth, E., Ofstad, R., Thomassen, M.S., 2008. Effects of −1.5°C super-chilling on quality of Atlantic salmon (*Salmo salar*) pre-rigor fillets: cathepsin activity, muscle histology, texture and liquid leakage. Food Chem. 111, 329–339. https://doi.org/10.1016/j.foodchem.2008.03.075.

Banerjee, R., Maheswarappa, N.B., 2019. Superchilling of muscle foods: potential alternative for chilling and freezing. Crit. Rev. Food Sci. Nutr. 59 (8), 1256–1263. https://doi.org/10.1080/10408398.2017.1401975.

Birkeland, S., R., A.M.B., Skåra, T., Bjerkeng, B., 2004. Effects of cold smoking procedures and raw material characteristics on product yield and quality parameters of cold smoked Atlantic salmon (*Salmo salar* L.) fillets. Food Res. Int. 37, 273–286. https://doi.org/10.1016/j.foodres.2003.12.004.

Cardinal, M., Knockaert, C., Torrissen, O., Sigurgisladottir, S., Mørkøre, T., Thomassen, M., Luc Vallet, J., 2001. Relation of smoking parameters to the yield, colour and sensory quality of smoked Atlantic salmon (*Salmo salar*). Food Res. Int. 34 (6), 537–550. https://doi.org/10.1016/S0963-9969(01)00069-2.

Castro, P.L.D., Lewandowski, V., Souza, M.L.R.D., Coradini, M.F., Alexandre, A.A.D.C., Sary, C., Ribeiro, R.P., 2017. Effect of different periods of pre-slaughter stress on the quality of the Nile tilapia meat. Food Sci. Technol. 37, 52–58. https://doi.org/10.1590/1678-457x.05616.

Claussen, I.C., Gullsvåg, P.E., Bantle, M., Tolstorebrov, I., Kvalsvik, K., 2017. Superchilling of Organic Food. Part 2: Storage Test With Superchilled Organic Salmon and Pork Chops. Retrieved from. http://hdl.handle.net/11250/2488509/ (web archive link, 03 November 2019) Accessed 3 November 2019.

Dalgaard, P., Mejlholm, O., Christiansen, T.J., Huss, H.H., 1997. Importance of *Photobacterium phosphoreum* in relation to spoilage of modified atmosphere-packed fish products. Lett. Appl. Microbiol. 24 (5), 373–378. https://doi.org/10.1046/j.1472-765X.1997.00152.x.

Duun, A.S., 2008. Superchilling of Muscle Food. Storage Stability and Quality Aspects of Salmon (*Salmo salar*), cod (*Gadus morhua*) and Pork (PhD Thesis). Norwegian University of Science and Technology, Trondheim, Norway.

Einarsdóttir, I.E., Nilssen, K.J., 1996. Stress responses of Atlantic salmon (*Salmo salar* L.) elicited by water level reduction in rearing tanks. Fish Physiol. Biochem. 15 (5), 395–400. https://doi.org/10.1007/bf01875582.

Erikson, U., Misimi, E., Gallart-Jornet, L., 2011. Superchilling of rested Atlantic salmon: different chilling strategies and effects of fish and fillet quality. Food Chem. 127, 1427–1437. https://doi.org/10.1016/j.foodchem.2011.01.036.

Espe, M., Ruohonen, K., Bjørnevik, M., Frøyland, L., Nortvedt, R., Kiessling, A., 2004. Interactions between ice storage time, collagen composition, gaping and textural properties in farmed salmon muscle harvested at different times of the year. Aquaculture 240 (1), 489–504. https://doi.org/10.1016/j.aquaculture.2004.04.023.

Gaarder, M.Ø., Bahuaud, D., Veiseth-Kent, E., Mørkøre, T., Thomassen, M.S., 2012. Relevance of calpain and calpastatin activity for texture in super-chilled and ice-stored Atlantic salmon (*Salmo salar* L.) fillets. Food Chem. 132 (1), 9–17. https://doi.org/10.1016/j.foodchem.2011.09.139.

Guillerm-Regost, C., Haugen, T., Nortvedt, T., Carlehog, M., Lunestad, B.T., Kiessling, A., Rørå, M.B., 2006. Quality characterization of farmed Atlantic halibut during ice storage. J. Food Sci. 71, 83–90. https://doi.org/10.1111/j.1365-2621.2006.tb08926.x.

Hall, G.M., 2011. Preservation by curing (drying, salting and smoking). In: Hall, G.M. (Ed.), Fish Processing – Sustainability and New Opportunities. Blackwell Publishing, UK, pp. 51–74.

Hansen, L.T., Gill, T., Røntved, S.D., Huss, H.H., 1996. Importance of autolysis and microbiological activity on quality of cold-smoked salmon. Food Res. Int. 29 (2), 181–188. https://doi.org/10.1016/0963-9969(96)00003-8.

Huff-Lonergan, E., 2002. Water Holding Capacity of Fresh Meat. Retrieved from. http://porkgateway.org/wp-content/uploads/2015/07/water-holding-capacity-of-fresh-meat1.pdf/ Accessed 3 November 2019.

Huff-Lonergan, E., Lonergan, S.M., 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci. 71, 194–204. https://doi.org/10.1016/j.meatsci.2005.04.022.

Hultmann, L., Rustad, T., 2002. Textural changes during iced storage of salmon (*Salmo salar*) and cod (*Gadus morhua*). J. Aquat. Food Product Technol. 11 (3–4), 105–123. https://doi.org/10.1300/J030v11n03_09.

Hyldig, G., Green-Petersen, D.M.B., 2005. Quality Index Method—an objective tool for determination of sensory quality. J. Aquat. Food Product Technol. 13 (4), 71–80. https://doi.org/10.1300/J030v13n04_06.

Jacobsen, Á., Joensen, H., Eysturskarð, J., 2017. Gaping and loss of fillet firmness in farmed salmon (*Salmo salar* L.) closely correlated with post-slaughter cleaning of the abdominal cavity. Aquac. Res. 48 (1), 321–331. https://doi.org/10.1111/are.12884.

Jørpeland, G., Imsland, A., Stien, L.H., Bleie, H., Roth, B., 2015. Effects of filleting method, stress, storage and season on the quality of farmed Atlantic cod (*Gadus morhua L.*). Aquac. Res. 46 (7), 1597–1607. https://doi.org/10.1111/are.12312.

Kaale, L.D., Eikevik, T.M., Rustad, T., Kolsaker, K., 2011. Superchilling of food, A review. J. Food Eng. 107, 141–146. https://doi.org/10.1016/j.jfoodeng.2011.06.004.

Kaale, L.D., Eikevik, T.M., Rustad, T., Nordtvedt, T.S., 2014. Changes in water holding capacity and drip loss of Atlantic salmon (*Salmo salar*) muscle during superchilled storage. LWT Food Sci. Technol. 55 (2), 528–535. https://doi.org/10.1016/j.lwt.2013.10.021.

Lerfall, J., 2011. The Effect of Pancreas Disease and Salting Conditions on the Quality of Raw and Cold-smoked Atlantic Salmon (*Salmo salar* L.) (PhD Thesis). Norwegian University of Life Sciences, Ås, Norway.

Lerfall, J., Rotabakk, B.T., 2015. Muscle temperature at the point of filleting - subsequent effect on storage quality of prerigor filleted raw- and cold-smoked Atlantic salmon. Food Sci. Technol. Int. 22 (2), 153–163. https://doi.org/10.1177/1082013215577737.

Lerfall, J., Roth, B., Skare, E.F., Henriksen, A., Betten, T., Dziatkowiak-Stefaniak, M.A., Rotabakk, B.T., 2015. Pre-mortem stress and the subsequent effect on flesh quality of pre-rigor filleted Atlantic salmon (*Salmo salar* L.) during ice storage. Food Chem. 175, 157–165. https://doi.org/10.1016/j.foodchem.2014.11.111.

Løje, H., 2007. The Quality of Cold Smoked Salmon - Influence of Raw Material and Technological Parameters (PhD Thesis). Technical University of Denmark, Denmark.

Lund, K.E., Nielsen, H.H., 2001. Proteolysis in salmon (*Salmo salar*) during cold storage; effects of storage time and smoking process. J. Food Biochem. 25 (5), 379–395. https://doi.org/10.1111/j.1745-4514.2001.tb00747.x.

Magnussen, O.M., Haugland, A., Torstveit Hemmingsen, A.K., Johansen, S., Nordtvedt, T.S., 2008. Advances in superchilling of food – process characteristics and product quality. Trends Food Sci. Technol. 19 (8), 418–424. https://doi.org/10.1016/j.tifs.2008.04.005.

Mørkøre, T., 2008. Tekstur i oppdrettslaks- Kunnskapsstatus og forhold som bidrar til fastere filet. Retrieved from Ås: Nofima Marin: https://nofima.no/pub/1217063/ Accessed 3 November 2019.

Mørkøre, T., Vallet, J.L., Cardinal, M., 2001. Fat content and fillet shape of Atlantic salmon: relevance fo processing yield and quality of raw and smoked products. J. Food Sci. 66, 1348–1354. https://doi.org/10.1111/j.1365-2621.2001.tb15213.x.

Mørkøre, T., Mazo, T., I., P., Tahirovic, V., Einen, O., 2008. Impact of starvation and handling stress on rigor development and quality of Atlantic salmon (*Salmon salar* L.). Aquaculture 277 (3), 231–238. https://doi.org/10.1016/j.aquaculture.2008.02.036.

Piñeiro, C., Barros-Velázquez, J., Aubourg, S.P., 2004. Effects of newer slurry ice systems on the quality of aquatic food products: a comparative review versus flake-ice chilling

methods. Trends Food Sci. Technol. 15 (12), 575–582. https://doi.org/10.1016/j.tifs.2004.09.005.

Rotabakk, B.T., Melberg, G.L., Lerfall, J., 2017. Effect of season, location, filleting regime and storage on water-holding properties of farmed Atlantic salmon (*Salmo salar* L.). Food Technol. Biotechnol. 56 (2), 238–246. https://doi.org/10.17113/ftb.56.02.18.5346.

Roth, B., Slinde, E., Arildsen, J., 2006. Pre or post mortem muscle activity in Atlantic salmon (*Salmo salar*). The effect on rigor mortis and the physical properties of flesh. Aquaculture 257 (1), 504–510. https://doi.org/10.1016/j.aquaculture.2005.10.021.

Roth, B., Foss, A., Imsland, A.K., 2009. Relationship between muscle pH and flesh color of Atlantic halibut. J. Food Sci. 74 (3), 123–125. https://doi.org/10.1111/j.1750-3841.2009.01089.x.

Sigurgisladottir, S., Sigurdardottir, M.S., Torrissen, O., Vallet, J.L., Hafsteinsson, H., 2000. Effects of different salting and smoking processes on the microstructure, the texture and yield of Atlantic salmon (*Salmo salar*) fillets. Food Res. Int. 33 (10), 847–855. https://doi.org/10.1016/S0963-9969(00)00104-6.

Skipnes, D., Østby, M.L., Hendrickx, M.E., 2007. A method for characterising cook loss and water holding capacity in heat treated cod (*Gadus morhua*) muscle. J. Food Eng. 80 (4), 1078–1085. https://doi.org/10.1016/j.jfoodeng.2006.08.015.

Taylor, R.G., Fjaera, S.O., Skjervold, P.O., 2002. Salmon fillet texture is determined by myofiber-myofiber and myofiber-myocommata attachment. J. Food Sci. 67 (6), 2067–2071. https://doi.org/10.1111/j.1365-2621.2002.tb09502.x.

Wang, D., Tang, J., Correia, L.R., Gill, T.A., 1998. Postmortem changes of cultivated Atlantic salmon and their effects on salt uptake. J. Food Sci. 63 (4), 634–637. https://doi.org/10.1111/j.1365-2621.1998.tb15801.x.

Wu, C.-H., Yuan, C.-H., Ye, X.-Q., Hu, Y.-Q., Chen, S.-G., Liu, D.-H., 2014. A critical review on superchilling preservation technology in aquatic product. J. Integr. Agric. 13 (12), 2788–2806. https://doi.org/10.1016/S2095-3119(14)60841-8.

Appendix 2 – Statistics
Paper 1

Table A1 – Median, abs median and average of blood spots and gaping on fresh salmon from slaughtered on vessel

Group	Fish #	gaping	median	median absolute value	average	blood spot	median	median absolute value	average
Vessel	1	1	0	1	0,38	0	0	0	0,03
Vessel	2	1	0	1		0	0	0	
Vessel	3	0	0	0		0	0	0	
Vessel	4	1	0	1		0	0	0	
Vessel	5	0	0	0		0	0	0	
Vessel	6	0	0	0		0	0	0	
Vessel	7	0	0	0		0	0	0	
Vessel	8	0	0	0		0	0	0	
Vessel	9	0	0	0		0	0	0	
Vessel	10	0	0	0		0	0	0	
Vessel	11	2	0	2		0	0	0	
Vessel	12	0	0	0		0	0	0	
Vessel	13	1	0	1		1	0	1	
Vessel	14	0	0	0		0	0	0	
Vessel	15	0	0	0		0	0	0	
Vessel	16	0	0	0		0	0	0	
Vessel	17	1	0	1		0	0	0	
Vessel	18	0	0	0		0	0	0	
Vessel	19	1	0	1		0	0	0	
Vessel	20	1	0	1		0	0	0	
Vessel	21	2	0	2		0	0	0	
Vessel	22	1	0	1		0	0	0	
Vessel	23	0	0	0		0	0	0	
Vessel	24	0	0	0		0	0	0	
Vessel	25	1	0	1		0	0	0	
Vessel	26	0	0	0		0	0	0	
Vessel	27	0	0	0		0	0	0	
Vessel	28	0	0	0		0	0	0	
Vessel	29	0	0	0		0	0	0	
Vessel	30	0	0	0		0	0	0	
Vessel	31	0	0	0		0	0	0	
Vessel	32	0	0	0		0	0	0	
Vessel	33	0	0	0		0	0	0	
Vessel	34	0	0	0		0	0	0	

Table A2 – Median, abs median and average of blood spots and gaping on fresh salmon from slaughtered on land

Group	Fish #	gaping	median	median absolute value	average	blood spot	median	median absolute value	average
Facillity	1	0	1	1	1,04	0	0	0	0,14
Facillity	2	0	1	0		0	0	0	
Facillity	3	1	1	1		0	0	0	
Facillity	4	1	1	1		2	0	2	
Facillity	5	0	1	0		0	0	0	
Facillity	6	0	1	0		0	0	0	
Facillity	7	1	1	1		0	0	0	
Facillity	8	2	1	2		0	0	0	
Facillity	9	1	1	1		0	0	0	
Facillity	10	1	1	1		0	0	0	
Facillity	11	1	1	1		0	0	0	
Facillity	12	1	1	1		0	0	0	
Facillity	13	2	1	2		0	0	0	
Facillity	14	2	1	2		0	0	0	
Facillity	15	2	1	2		0	0	0	
Facillity	16	1	1	1		0	0	0	
Facillity	17	1	1	1		1	0	1	
Facillity	18	1	1	1		0	0	0	
Facillity	19	2	1	2		0	0	0	
Facillity	20	3	1	3		0	0	0	
Facillity	21	2	1	2		0	0	0	
Facillity	22	0	1	0		0	0	0	
Facillity	23	1	1	1		0	0	0	
Facillity	24	1	1	1		0	0	0	
Facillity	25	1	1	1		0	0	0	
Facillity	26	1	1	1		0	0	0	
Facillity	27	0	1	0		0	0	0	
Facillity	28	0	1	0		1	0	1	

Table A3 – Median, abs median and average of blood spots and gaping on smoked salmon on day 28

D28 (22/11)								
Fish #	gaping	median	median absolute value	average	blood spot	median	median absolute value	average
H1	0	0	0,666667	0,74	3	1	1,888889	1,12
H2	0		0,666667		0		1,111111	
H3	0		0,666667		0		1,111111	
H4	1		0,333333		2		0,888889	
H5	0		0,666667		0		1,111111	
H101	2		1,333333		3		1,888889	
H102	2		1,333333		1		0,111111	
H104	0		0,666667		1		0,111111	
H105	1		0,333333		3		1,888889	
I1	2	2	1,25	1,19	0	0	0,75	1,13
I2	1		0,25		0		0,75	
I3	2		1,25		4		3,25	
I4	3		2,25		0		0,75	
I5	1		0,25		0		0,75	
I101	0		0,75		2		1,25	
I102	3		2,25		0		0,75	
I104	2		1,25		0		0,75	

Table A4 – Levenes test Gaping smoked salmon

Gaping smoked salmon	MS effect	MS error	F	p
Gaping	2,06	0,24	8,43	0,01
Day	0,12	0,28	0,41	0,53
Gaping Fresh salmon	**MS effect**	**MS error**	**F**	**p**
Gaping	0,020	0,189	0,107	0,745
Dag	0,000	0,000		

Table A5 – Levenes test Blood spots smoked salmon

Blood spots smoked salmon	MS effect	MS error	F	p
Blood spots	1,48	0,63	2,34	0,14
Dag	0,12	0,28	0,41	0,53
Blood spots fresh salmon	**MS effect**	**MS error**	**F**	**p**
Blood spots	0,62	0,08	8,21	0,01
DAG	0,00	0,00		

Table A6 – Kolmogorov Smirnov test of blood spots and gaping on fresh salmon

	Max Neg Differnc	Max Pos Differnc	p-value	Mean Vessel	Mean Facility	Std.Dev. Facility	Std.Dev.Facility	Valid N Vessel	Valid N Facility
Bloodspots	-0,08	0	p > .10	0,03	0,15	0,17	0,46	33	27
Gaping	-0,43	0	p < .01	0,38	1,04	0,60	0,79	34	28

Table A7 – Levenes test texture

Texture	MS effect	MS error	F	p
Topp Force	304,10	89,67	3,39	0,07
Auto test height	0,89	2,04	0,43	0,51
Break	1,07	9,14	0,12	0,73
Auto test height	0,89	2,04	0,43	0,51
80% Force%	269,67	87,57	3,08	0,09
Auto test height	0,89	2,04	0,43	0,51
60% Force	1,23	15,96	0,08	0,78
Auto test height	0,89	2,04	0,43	0,51

Table A8 - Texture analysis on Fresh Salmon

Group	Topp force	Topp force	Topp force	Topp force	N	p-value
	Mean	Std.Err.	-95,00 %	95,00 %		
Vessel	60,65	1,92	56,67	64,63	24	p<0.89
Facility	60,08	3,57	52,71	67,46	25	
Group	Break	Break	Break	Break	N	p-value
	Mean	Std.Err.	-95,00 %	95,00 %		
Vessel	21,49	0,66	20,12	22,86	24	p<0.35
Facility	20,36	1,00	18,28	22,43	25	
Group	Force 80%	Force 80%	Force 80%	Force 80%	N	p-value
	Mean	Std.Err.	-95,00 %	95,00 %		
Vessel	58,48	1,96	54,43	62,53	24	p<0.98
Facility	58,41	3,51	51,17	65,65	25	
Group	Force 60%	Force 60%	Force 60%	Force 60%	N	p-value
	Mean	Std.Err.	-95,00 %	95,00 %		
Vessel	24,93	1,00	22,85	27,00	24	p<0.40
Facility	23,52	1,31	20,81	26,23	25	

Table A9 – Colour analysis on fresh salmon

Group	Mean L*	Mean a*	Mean b*	STDV L*	STDV a*	STDV b*
Vessel	46,40	46,57	25,40	2,04	0,75	1,36
Facility	43,76	44,36	24,75	1,59	0,95	1,28
T-test	0,00	0,00	0,13			

Table A10 – Levenes test QIM

QIM	MS effect	MS error	F	p
Total score	1,50	3,43	0,44	0,51
Day	0,00	9,89	0,00	1,00

Table A11 – Nested ANOVA analysis of the attributes of QIM

		SS	Degree of freedome	MS	F	p-verdi
Colour skin	Group	0,04	1	0,04	1,247	0,269
	Day(Group)	0,821	8	0,103	3,197	,005*
Slime skin	Group	0,264	1	0,264	6,159	,016*
	Day(Group)	3,299	8	0,412	9,621	,000*
Smell skin	Group	0,014	1	0,014	0,347	0,558
	Day(Group)	2,348	8	0,293	7,379	,000*
Texture skin	Group	0,002	1	0,002	0,176	0,676
	Day(Group)	0,051	8	0,006	0,618	0,759
Pupils eye	Group	0	1	0	0,005	0,943
	Day(Group)	3,623	8	0,453	4,907	,000*
Form eye	Group	0,154	1	0,154	1,799	0,186
	Day(Group)	2,876	8	0,359	4,2	,001*
color gills	Group	0,072	1	0,072	0,314	0,578
	Day(Group)	7,17	8	0,896	3,898	,001*
Slime gills	Group	1,984	1	1,984	17,86	,000*
	Day(Group)	4,542	8	0,568	5,11	,000*
Smell gills	Group	1,02	1	1,022	10,78	,002*
	Day(Group)	16,02	8	2,002	21,13	,000*
Blood gut	Group	0	1	0	0	0,983
	Day(Group)	1,32	8	0,165	2,783	,013*
Smell gut	Group	0,11	1	0,112	0,9	0,349
	Day(Group)	22,39	8	2,799	22,42	,000*
Total score	Group	14,7	1	14,7	5,19	,027*
	Day(Group)	437,7	8	54,71	19,3	,000*

Table A12 – Post-hoc test of QIM

Group	Day	1	2	3	4	5	6	7	8	9	10
Vessel	4		0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00
Vessel	10	0,00		1,00	1,00	1,00	0,00	1,00	0,11	0,74	1,00
Vessel	14	0,00	1,00		1,00	1,00	0,00	1,00	1,00	1,00	1,00
Vessel	17	0,00	1,00	1,00		0,10	0,00	1,00	1,00	1,00	1,00
Vessel	21	0,00	1,00	1,00	0,10		0,01	1,00	0,01	0,07	1,00
Facility	4	1,00	0,00	0,00	0,00	0,01		0,00	0,00	0,00	0,00
Facility	10	0,00	1,00	1,00	1,00	1,00	0,00		1,00	1,00	1,00
Facility	14	0,00	0,11	1,00	1,00	0,01	0,00	1,00		1,00	0,41
Facility	17	0,00	0,74	1,00	1,00	0,07	0,00	1,00	1,00		1,00
Facility	21	0,00	1,00	1,00	1,00	1,00	0,00	1,00	0,41	1,00	

Table A13 – Average, median and standard deviation of the QIM scores on each day.

Average					
Day	4	10	14	18	21
Vessel	3,89	9,17	10,44	11,46	13,56
Facility	4,39	10,33	12,28	11,58	13,56

Median					
Day	4	10	14	18	21
Vessel	3,50	9,67	10,83	10,88	13,50
Facility	4,50	11,17	12,50	11,38	14,33

SD					
Day	4	10	14	18	21
Vessel	1,11	1,70	1,61	1,83	1,61
Facility	1,27	1,99	2,28	2,04	2,38

Table A14 – Median and t-test of Vessel and Facility on day 4 of each QIM attribute

Day 4	Total score
T-test	0,24
Median V	3,50
Median F	4,50

Day 4	Textur(Skin)
T-test	0,17
Median V	1,00
Median F	1,00

Day 4	Colour (gills)
T-test	0,31
Median V	0,17
Median F	0,33

Day 4	Colour(Skin)
T-test	0,25
Median V	0,67
Median F	0,33

Day 4	Pupils (Eyes)
T-test	0,08
Median V	0,67
Median F	0,50

Day 4	Slime (gills)
T-test	0,11
Median V	0,17
Median F	0,67

Day 4	Smell (Gut)
T-test	0,02
Median V	0,00
Median F	0,17

Day 4	Smell (Skin)
T-test	0,50
Median V	0,17
Median F	0,17

Day 4	Shape (Eyes)
T-test	0,50
Median V	0,83
Median F	1,00

Day 4	Smell (gills)
T-test	0,09
Median V	0,00
Median F	0,33

Table A15 – Median and t-test of Vessel and Facility on day 10 of each QIM attribute

Day 10	Total score
T-test	0,15
Median V	9,67
Median F	11,17

Day 10	Smell (skin)
T-test	0,23
Median V	0,67
Median F	0,67

Day 10	Shape (Eyes)
T-test	0,05
Median V	1,17
Median F	1,33

Day 10	Smell (gills)
T-test	0,03
Median V	0,67
Median F	1,33

Day 10	Colour(Skin)
T-test	0,30
Median V	0,67
Median F	0,67

Day 10	Textur (skinn)
T-test	0,30
Median V	1,00
Median F	1,00

Day 10	Colour (Gills)
T-test	0,34
Median V	1,00
Median F	1,33

Day 10	Blood (bgut)
T-test	0,29
Median V	0,33
Median F	0,33

Day 10	Slime (Skin)
T-test	0,27
Median V	0,67
Median F	0,67

Day 10	Pupils (Eyes)
T-test	0,39
Median V	1,17
Median F	1,00

Day 10	Slime (gills)
T-test	0,01
Median V	0,67
Median F	1,50

Day 10	Smell (gut)
T-test	0,22
Median V	1,33
Median F	0,83

Table A16 – Median and t-test of Vessel and Facility on day 14 of each QIM attribute

Day 14	Total score
T-test	0,07
Median V	10,83
Median F	12,50

Day 14	Smell (skin)
T-test	0,30
Median V	0,67
Median F	0,67

Day 14	Shape (eyes)
T-test	0,12
Median V	1,17
Median F	1,67

Day 14	Smell (gills)
T-test	0,06
Median V	1,50
Median F	1,83

Day 14	Colour (Skin)
T-test	0,50
Median V	0,67
Median F	0,67

Day 14	Texture (skin)
T-test	0,17
Median V	1,00
Median F	1,00

Day 14	Colour (Gills)
T-test	0,24
Median V	1,00
Median F	1,17

Day 14	Blood (gut)
T-test	0,39
Median V	0,33
Median F	0,50

Day 14	Slime (Skin)
T-test	0,03
Median V	0,33
Median F	0,67

Day 14	Pupills (Eyes)
T-test	0,39
Median V	1,17
Median F	1,17

Day 14	Slime (gills)
T-test	0,00
Median V	0,67
Median F	1,33

Day 14	Smell (gut)
T-test	0,50
Median V	1,67
Median F	2,00

Table A17 – Median and t-test of Vessel and Facility on day 17 of each QIM attribute

Day 17	Total score
T-test	0,46
Median V	10,88
Median F	11,38

Day 17	Smell (skin)
T-test	0,09
Median V	0,50
Median F	0,75

Day 17	Shape (eyes)
T-test	0,43
Median V	1,50
Median F	1,38

Day 17	Smell (gills)
T-test	0,50
Median V	1,75
Median F	1,75

Day 17	Colour (Skin)
T-test	0,04
Median V	1,00
Median F	0,75

Day 17	Textur (skin)
T-test	
Median V	1,00
Median F	1,00

Day 17	Colour (gills)
T-test	0,18
Median V	1,75
Median F	1,25

Day 17	Blood (gut)
T-test	0,38
Median V	0,25
Median F	0,50

Day 17	Slime (Skin)
T-test	0,05
Median V	0,50
Median F	0,75

Day 17	Pupils (Eyes)
T-test	0,34
Median V	1,13
Median F	1,25

Day 17	Slime (gills)
T-test	0,10
Median V	0,63
Median F	0,88

Day 17	Smell (gut)
T-test	0,36
Median V	2,00
Median F	1,75

Table A18 – Median and t-test of Vessel and Facility on day 21 of each QIM attribute

Day 21	Total score
T-test	0,50
Median V	13,50
Median F	14,33

Day 21	Smell (skin)
T-test	0,20
Median V	1,00
Median F	1,17

Day 21	Shape (eyes)
T-test	0,29
Median V	1,50
Median F	1,67

Day 21	Smell (gills)
T-test	0,16
Median V	2,17
Median F	2,17

Day 21	Colour (Skin)
T-test	0,50
Median V	1,00
Median F	1,00

Day 21	Texture (skin)
T-test	0,04
Median V	1,17
Median F	1,00

Day 21	Colour (gills)
T-test	0,11
Median V	2,00
Median F	1,67

Day 21	Blood (gut)
T-test	0,02
Median V	0,67
Median F	0,33

Day 21	Slime (Skin)
T-test	0,33
Median V	0,67
Median F	0,67

Day 21	Pupils (eyes)
T-test	0,40
Median V	1,33
Median F	1,33

Day 21	Slime (gills)
T-test	0,18
Median V	0,00
Median F	0,50

Day 21	Smell (gut)
T-test	0,01
Median V	1,83
Median F	2,50

Table A19 – Post hoc test of microbiology, Ironagar

Gruppe	Dag	1	2	3	4	5	6	7	8	9	10
Vessel	4		0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00
Vessel	10	**0,00**		0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Vessel	14	0,00	0,00		0,96	1,00	0,00	0,02	1,00	1,00	0,00
Vessel	18	0,00	0,00	0,96		0,02	0,00	1,00	1,00	0,01	0,00
Vessel	21	**0,00**	**0,00**	1,00	0,02		0,00	0,00	0,49	1,00	0,00
Facility	4	1,00	0,00	0,00	0,00	0,00		0,00	0,00	0,00	0,00
Facility	10	0,00	0,00	0,02	1,00	0,00	0,00		0,76	0,00	0,00
Facility	14	**0,00**	**0,00**	1,00	1,00	0,49	0,00	0,76		0,31	0,00
Facility	18	0,00	0,00	1,00	0,01	1,00	0,00	0,00	0,31		0,00
Facility	21	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	

Table A20 – Post hoc test of microbiology, Ironagar, H_2S

Grupper	Dag	1	2	3	4	5	6	7	8	9	10
Vessel	4		0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00
Vessel	10	0,00		0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Vessel	14	0,00	0,00		0,96	1,00	0,00	0,02	1,00	1,00	0,00
Vessel	18	0,00	0,00	0,96		0,02	0,00	1,00	1,00	0,01	0,00
Vessel	21	0,00	0,00	1,00	0,02		0,00	0,00	0,49	1,00	0,00
Facility	4	1,00	0,00	0,00	0,00	0,00		0,00	0,00	0,00	0,00
Facility	10	0,00	0,00	0,02	1,00	0,00	0,00		0,76	0,00	0,00
Facility	14	0,00	0,00	1,00	1,00	0,49	0,00	0,76		0,31	0,00
Facility	18	0,00	0,00	1,00	0,01	1,00	0,00	0,00	0,31		0,00
Facility	21	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	

Table A21 - Correlation analysis of microbiology

	Levenes test	MS effect	MS error	F	p
Jernagar	Weighted results	0,24	0,32	0,74	0,39
	Day	0,03	3,67	0,01	0,93
L&H smoked	Weighted results	3,57	0,18	19,78	0,00
	Day	0,00	0,00		
L&H	Weighted results	0,34	1,30	0,26	0,61
	Day	0,00	9,96	0,00	1,00
H2S	Weighted results	0,36	0,42	0,85	0,36
	Day	0,07	3,29	0,02	0,89

Table A22 – A two-way ANOVA on sensory analysis

	Vessel (A)	Facility (B)
Protein precipitation	5.75	6.8[A']
Rancid odour	1.1	1.1
Off odour	1	1
Fresh odour	5.55	5.8
Colour intensity	6	5.9
Discolouration	1	1
Fresh flavour	5.75	5.65
Rancid flavour	1.05	1.05
Off flavour	1	1
Hadness	6.45	6.3
Juiciness	6.05	5.75
Adhesivness	3.75	3.9

Table A23 – An overview of mean, median and variance of all the attributes of fish slaughtered on vessel

	N	Missing	Min	Max	Mean	SD	Median	Variance
Protein precipitation	20	0	4	8	5.75	1.02	6	1.04
Rancid odour	20	0	1	3	1.1	0.45	1	0.2
Off odour	20	0	1	1	1	0	1	0
Fresh odour	20	0	3	7	5.55	1.15	5.5	1.31
Colour intensity	20	0	1	8	6	1.38	6	1.89
Discolouration	20	0	1	1	1	0	1	0
Fresh flavour	20	0	4	7	5.75	1.02	6	1.04
Rancid flavour	20	0	1	2	1.05	0.22	1	0.05
Off flavour	20	0	1	1	1	0	1	0
Hadness	20	0	4	8	6.45	1	7	1
Juiciness	20	0	5	8	6.05	0.94	6	0.89
Adhesivness	20	0	3	5	3.75	0.64	4	0.41

Table A24 – An overview of mean, median and variance of all the attributes of fish slaughtered at facility

	N	Missing	Min	Max	Mean	SD	Median	Variance
Protein precipitation	20	0	5	9	6.8	1.24	7	1.54
Rancid odour	20	0	1	2	1.1	0.31	1	0.09
Off odour	20	0	1	1	1	0	1	0
Fresh odour	20	0	4	8	5.8	1.06	6	1.12
Colour intensity	20	0	1	7	5.9	1.29	6	1.67
Discolouration	20	0	1	1	1	0	1	0
Fresh flavour	20	0	3	7	5.65	1.04	6	1.08
Rancid flavour	20	0	1	2	1.05	0.22	1	0.05
Off flavour	20	0	1	1	1	0	1	0
Hadness	20	0	5	7	6.3	0.66	6	0.43
Juiciness	20	0	5	7	5.75	0.72	6	0.51
Adhesivness	20	0	3	5	3.9	0.85	4	0.73

Table A25 – QIM scheme for farmed salmon (www.QIM-eurofish.com (2001))

Ferskhets-/kvalitets-parametre		Beskrivelse	QIM poeng
Skinn	Farge/ Utseende	*Perlemorsskinn over hele skinnet*	0
		Redusert perlemorsskinn	1
		Fisken er gulaktig, særlig ved bukhulen	2
	Slim	*Klart, ikke klumpet*	0
		Melkeaktig, klumpet	1
		Gult og klumpet	2
	Lukt	*Frisk tang, nøytral*	0
		Agurk, metallisk, høy	1
		Sur, kjøkkenklut	2
		Råtten	3
	Tekstur	*I rigor*	0
		Merke etter fingertrykk forsvinner hurtig	1
		Fingertrykk etterlater merke i over 3 sekunder	2
Øyne	Pupiller	*Klar og sort, metallisk skinnende*	0
		Mørk grå	1
		Matt, grå	2
	Form	*Utstående*	0
		Flat	1
		Innsunken	2
Gjeller	Farge/Utseende	*Rød/mørk brun*	0
		Lys rød, rosa/lysebrun	1
		Gråbrun, brun, grå, grønn	2
	Slim	*Transparent*	0
		Melket, klumpet	1
		Brun, klumpet	2
	Lukt	*Frisk, tangaktig*	0
		Metallisk, agurk	1
		Sur, muggen	2
		Råtten	3
Bukhule	Blod i bukhule	*Blodet er rødt/ikke blod*	0
		Blod er mere brunt, gulaktig	1
	Lukt	*Nøytral*	0
		Agurk, melon	1
		Sur, minner om fermentering	2
		Råtten/råtten kål	3
Total QIM score			**0-24**