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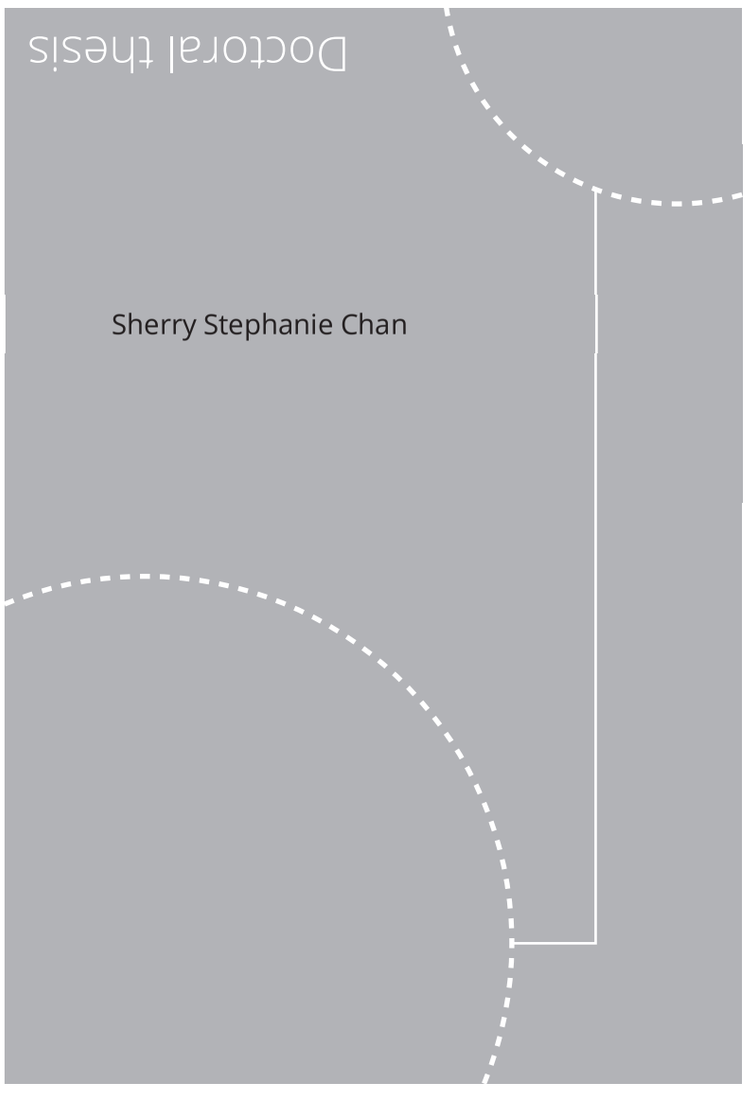
Sherry Stephanie Chan

Chilling of Atlantic salmon (*Salmo salar*) in refrigerated seawater

Its effect on water holding properties and general quality through the whole value chain



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Trondheim, December 2021

Sherry Chan

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Summary – English

Superchilling at sub-zero temperatures is known to prolong shelf life and contributes to the food safety of meat and seafood products. This technique is usually applied after the processing of fillets. The traditional method of fish slaughter is usually to pump fish and transport it on a well boat before processing on land and storing on ice. Recently, an unprecedented fish slaughter method has been introduced in the Norwegian aquaculture industry. Fish are directly slaughtered by gutting onboard a vessel by the cage and immersed in refrigerated seawater (RSW) at sub-zero temperatures for 1-4 days during transportation. RSW chilling removes the need for ice and effectively uses the seawater and the fish itself to rapidly cool down to the desired temperature. However, this method introduces a knowledge gap in understanding how water holding properties and other quality parameters (e.g. colour, texture, microbiological composition and stability, and enzyme activity) are affected.

Water holding properties, including drip loss (DL) and water holding capacity (WHC), are important quality parameters relating to sensory attributes and product yields of fresh and processed salmon. This thesis compares the water holding properties and important quality parameters of superchilling fish in RSW against the traditional method of storing whole Atlantic salmon on ice. It follows the whole value chain from whole fish to processed cold-smoked fillets and further refrigerated storage. In addition, the effect of packaging methods (vacuum skin and traditional vacuum) on the shelf-life extension was also studied, both on chilled and superchilled fish.

A weight gain of 0.7-0.9% was observed as fish was immersed in RSW. This finding was accompanied by salt uptake with a better water holding capacity and a lighter colour than ice-stored fish before filleting. Moreover, the count of hydrogen sulphide producing bacteria (HSPB) was significantly lesser in RSW-stored fish, with better gaping and blood spot scores. Fillets originating from RSW storage then kept on ice after filleting presented the lowest DL. The dry salting and cold-smoking process greatly influenced the fillet quality. Smoked fillets had a higher DL (up to 7% during storage), lower water content, darker and less reddish, and a firmer texture than the corresponding raw fillets. The various common packaging techniques tested showed that vacuum skin packaged fillets gave a higher drip loss and were lighter, less reddish and less yellowish than fillets packaged in traditional vacuum, regardless of the whole fish storage method.

A physics-based mathematical model on heat and mass transfer in cold-smoked salmon from the two whole fish storage methods was developed to predict the water and salt uptake after dry salting and cold-smoking. Water loss and salt gain were inversely related. An increase in salt uptake and a decrease in water activity as the duration of salt exposure increased were observed. Colour and drip loss were significantly affected by the processing step (salting, smoking and vacuum storage). The mathematical model successfully predicted the temperature distribution, moisture loss, and salt uptake during the dry salting process and agreed well with the experimental results and a previous empirical model. Besides, using the predicted values as input parameters for the simulation of water activity was achievable and validated with experimental values.

From the present results, it is reasonable to conclude that storing Atlantic salmon in RSW increases water and salt uptake and provides good water holding properties. This observation increases the yield of gutted whole fish, giving a comparably good quality product after processing. When RSW and ice-stored fish were processed into cold-smoked fillets, the differences between the whole fish storage method was minimal. The examined packaging techniques significantly influenced drip loss and colour, but both have specific advantages that effectively extend microbial shelf life that depend on the industries' and consumers' preferences. Furthermore, the type of whole fish storage did not influence the quality after portioning and packaging. Introducing mathematical modelling into this study also widens the possibility for strengthening and expanding this tool for other industrial applications and process optimization.

Sammendrag – Norsk

Kjøling ved temperaturer under null grader (superkjøling) gir økt holdbarhet og bidrar til bedre mattrygghet. Denne teknologien er mest studert som en ekstra behandling etter at fisken er filetert. Atlantisk laks slaktes tradisjonelt ved at fisken først pumpes fra produksjonsmerden til en brønnbåt, før den transporteres levende til slakteriet. Ved ankomst pumpes fisken enten til en ventemerd eller direkte inn til slakteriet der den blir avlivet og prosessert videre før eksport. I løpet av de siste årene har det blitt introdusert en ny slaktemetode i den norske havbruksnæringen. Med denne metoden blir fisk pumpet direkte om bord i et slaktefartøy, slaktet direkte og deretter lagret i nedkjølt sjøvann (RSW, <0 grader) ved transport. RSW kjøling fjerner behovet for is og bruker RSW systemet og selve fisken som et effektivt kjølemedia for å oppnå en rask nedkjøling til ønsket temperatur. Imidlertid mangler det kunnskap i forståelsen av hvordan fiskens vannbindende egenskaper og generelle kvalitetsparameter blir påvirket av denne prosessen.

Produktets vannbindende egenskaper, inkludert drypptapp og vannbindingskapasitet, er viktige kvalitetsparametere relatert til sensoriske egenskaper og produktets generelle oppfattelse. Denne doktorgradsavhandlingen gir et dypdykk i hvordan ulike kjøleregimer påvirker laksens vannbindende egenskaper og generelle kvalitet. I studiene ble Atlantisk laks superkjølt i RSW og videre sammenlignet med laks, tradisjonelt nedkjølt på is. Laksen ble fulgt gjennom hele verdikjeden fra hel fisk til bearbeidet kaldrøkte fileter og videre gjennom lagring. I tillegg ble også ulike emballeringsmetoder (vakuum skin og tradisjonelt vakuum) undersøkt etter porsjonering, både på kjølt og superkjølt fisk.

Når hel Atlantisk laks blir lagret i RSW, ble det observert en vektøkning på 0.7-0.9%. I tillegg var det et opptak av salt, med påfølgende forbedret vannbindingskapasitet. RSW-kjølt fisk fikk også en lysere filet farge sammenlignet med fisk som var lagret på is. Det ble også observert lavere forekomst av H_2S-produserende bakterier, mindre filetspalting og blodflekker. Ved produksjon av kaldrøkt laks påvirket både saltetrinnet og røykeprosessen kvaliteten. Gjennom prosessen ble det observert en større drypptapp (opptil 7% etter lagring), og kaldrøkte fileter hadde et lavere vanninnhold, fastere tekstur, samt at de var mørkere og mindre rødlig i fargen enn rå fileter. De ulike emballasjeteknologiene som ble testet viste at skin-pakket filet ga et høyere drypptapp og resulterte i lysere, mindre rødlige og mindre gulaktig fileter sammenlignet med filet pakket i tradisjonell vakuumemballasje, uavhengig av om fisken var lagret i RSW eller på is før prosessering.

En fysikkbasert matematisk modell basert på varme- og massetransport i produktet, ble utviklet for å forutsi vann- og saltopptaket etter tørrsalting og kaldrøking og dermed gi industriaktører et verktøy for å optimalisere prosessbetingelsen ved produksjon av kaldrøkt laks. I en tørrsaltingsprosess er tap av vann og opptak av salt omvendt korrelert. Ved eksponering for salt over tid, observeres et økt saltopptak og en reduksjon i produktets vannaktivitet. Samtidig blir produktets drypptapp og filetens farge signifikant påvirket av de ulike prosesstrinnene (salting, røyking og i vakuumemballasje). Den matematiske modellen predikerte produktets temperaturfordeling, vanntap og opptak av salt ved tørrsalting. De predikerte dataene var i overensstemmelse med de eksperimentelle verdiene og en tidligere utviklet empirisk modell. De forutsagte inngangsparameterne som ble brukt for simulering av vannaktivitet, viste et godt samsvare med de oppnådde eksperimentelle verdiene.

Basert på forsøkene i denne avhandlingen kan en konkludere med at lagring av fisk i RSW gir et produkt med gode vannbindingsegenskaper og generelt god kvalitet. Valg av emballasjeteknologi, har hver og en sine spesifikke fordeler, og må derfor velges basert på industriaktørenes og forbrukernes spesifikke preferanser. Felles for begge teknologiene er at de forlenger produktets mikrobiologiske holdbarhet. Hvorvidt fisken er kjølt i RSW eller på is før prosessering vil ikke påvirke produktets kvalitet etter porsjonering og emballering. En matematisk modell som beskriver massebalansen til salt og vann ved produksjon av kaldrøkt laks kan gi industrien direkte fordeler ved optimalisering av prosessbetingelser og produktkvalitet. Denne modellen kan være et godt verktøy for industrielle applikasjoner direkte, eller benyttes som et utgangspunkt ved utvikling av fremtidige modeller for andre industrielle applikasjoner.

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(I) **Chan, S. S.**, Roth, B., Jessen, F., Jakobsen, A. N., & Lerfall, J. (2021). Water holding properties of Atlantic salmon. *Comprehensive Reviews in Food Science and Food Safety, 2021,* 1-22. doi: 10.1111/1541-4337.12871.

(II) **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. doi:10.1016/j.aquaculture.2020.735381.

(III) **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. *Scientific Reports, 10*, 17160. doi:10.1038/s41598-020-73302-x.

(IV) Skare, M., **Chan, S. S.**, Handeland, S., 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*, 736681. doi:10.1016/j.aquaculture.2021.736681.

(V) **Chan, S. S.**, Rotabakk, B. T., Løvdal, T., Lerfall, J., & Roth, B. (2021). Skin and vacuum packaging of portioned Atlantic salmon originating from refrigerated seawater or traditional ice storage. *Food Packaging and Shelf Life, 30*, 100767. doi: 10.1016/j.fpsl.2021.100767.

(VI) **Chan, S. S.**, Feyissa, A. H., Jessen, F., Roth, B., Jakobsen, A. N., & Lerfall, J. (2022). Modelling water and salt diffusion of cold-smoked Atlantic salmon initially immersed in refrigerated seawater versus on ice. *Journal of Food Engineering, 312*, 110747. doi:10.1016/j.jfoodeng.2021.110747.

Abbreviations

a_w: Water activity

DL: Drip loss

HSPB: Hydrogen sulphide producing bacteria

LAB: Lactic acid bacteria

MAP: Modified atmospheric packaging

PDE: Partial differential equation

QI: Quality index

QIM: Quality Index Method

RSW: Refrigerated seawater

SSO: Specific spoilage organism

TPC: Total psychrotrophic counts

TMA: Trimethylamine

TMC: Total mesophilic counts

TVB-N: Total volatile base nitrogen

WHC: Water holding capacity

WPS: Water phase salt

Z&L: Zugarramurdi and Lupín

Introduction

Atlantic salmon (*Salmo salar*) is one of the most valuable and intensively farmed fish species, with an annual worldwide production of 2.7 million tonnes in 2020 (FAO, 2021). The Norwegian aquaculture industry has been expanding and is the leading producer of Atlantic salmon, occupying a global production share of 51% (FAO, 2021). Salmon is a nutritious product rich in protein, high in omega-3 fatty acids, and low in saturated fatty acids. As fish is a perishable product, quality must be maintained throughout the whole value chain, from slaughter to retail. In the past decades, extensive research has been done to improve the quality and shelf life of salmon. Temperature and water holding properties, including drip loss (DL) and water holding capacity (WHC), are important quality parameters related to yield and freshness. A low and stable storage temperature immediately after slaughter must be obtained to extend a product's shelf life and prevent food deterioration.

One of the proposed methods for shelf life extension is to apply the superchilling concept, a food preservation method where the core temperature of fish is kept between traditional chilling and freezing below 0°C (Ando et al., 2004; Banerjee & Maheswarappa, 2019). Studies involving superchilling primarily focused on the processing line on fresh fillets, but the industrial application of superchilling can be challenging. The current fish slaughter practice is to slaughter fish on land-based facilities and store whole salmon on ice. The utilization of a slaughter vessel, which allows fish to be slaughtered directly by the cage and immersed in RSW tanks at sub-zero temperature during transportation, is a novel technology that condenses several handling steps into only one. However, superchilling during the initial whole fish storage and its potential advantages have not been fully exploited. Moreover, there is a lack of research on how water holding properties and other quality parameters for freshness influences the quality of Atlantic salmon with such storage method. The work on this thesis follows fish through two whole value chains from slaughter to primary fillet processing, to 1. a secondary process producing cold-smoked salmon, and finally to packaging and cold storage; or 2. packaging of fresh salmon fillet portions and cold storage (**Figure 1.1**).

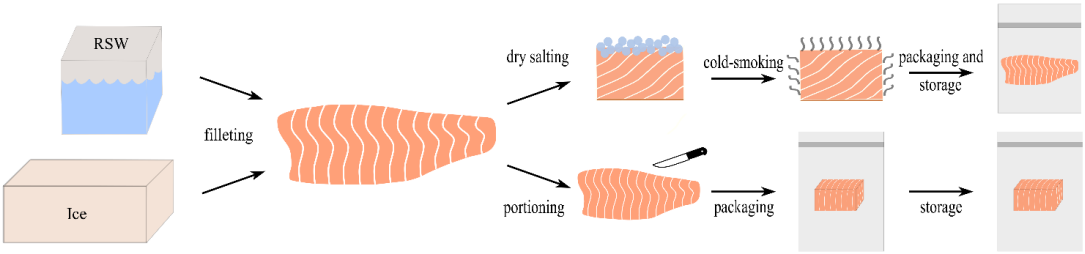

Figure 1.1: An overview of the whole value chains of Atlantic salmon studied in this thesis.

Mathematical modelling can be a powerful tool to reduce experimentation processes in relevance to the industries. Numerical modelling in the food industry can simulate the complex physical phenomena involved and aid in improvement and optimization in food processes. This is especially useful for visualizing the spatial variations and distributions within the food product that occur over time. In contrast, laboratory experiments can be laborious, costly and time-consuming. Numerical models have been widely used for heat and mass transport phenomena in food, such as the common thermal processes of heating and cooling and preservation methods like salting. Such models rely on differential equations from the first principles under valid assumptions. Validations and experiments must be carried out to create a robust model. Therefore, the final segment of this thesis uses mathematical modelling to simulate the dry salting process during cold-smoking of salmon.

Thesis Outline

This thesis has been an incremental process and is divided into 4 chapters. Chapter 1 forms the background and covers theoretical explanations concerning the topics covered in the thesis. Chapter 2 provides the objectives of the project and an overview of the papers, while Chapter 3 summarizes the main results obtained from the experiments, including thorough discussions. Finally, Chapter 4 presents the industrial implications, impacts and conclusion. As part of this thesis, a comprehensive review article on the core topics of water holding properties and their influence on Atlantic salmon is presented (**Paper I**). This article discusses the various methods in measuring WHC, the pre and post-mortem factors, and processing and preservation methods that influence water holding properties. In addition, 5 research articles are included (**Papers II-VI**).

Chapter 1: Background

1.1 Fish muscle

The fish skeletal muscle is an elaborate and dynamic structure. It relies on the coordinated interactions between myofibrillar, sarcoplasmic and stromal proteins for structural and functional maintenance, occupying about 50-70%, 20-50% and 3%, respectively (Hashimoto et al., 1979; Kijowski, 2001; Ochiai & Ozawa, 2020).

The slaughter yield of gutted Atlantic salmon ranges between 86-92%, while its edible yield varies from 60-68% (Bjørkli, 2002; Einen et al., 1999; Torrissen et al., 2011). This makes salmon attractive for industrialization. On the macroscopic scale, the muscle fibre is classified into red and white muscles that specialize in aerobic and anaerobic activities, respectively. The dark red muscle has a higher lipid content and can be found as a narrow strip beneath the lateral line near the skin. It is mainly used for energy-efficient swimming. On the contrary, the white muscle contributes to at least 70% of the muscle as energy storage for burst swimming (Kiessling et al., 2006).

Post-mortem degradation is a complicated process that involves a series of biochemical and autolytic reactions as the muscle tenderizes and converts to meat. Starting from exsanguination, the muscle ceases to receive nutrients and oxygen. As a result, the aerobic adenosine triphosphate (ATP) production switches to anaerobic glycolysis and produces lactic acid (Daskalova, 2019; Huff-Lonergan & Lonergan, 1999). The muscle enters rigor mortis as ATP is depleted, leading to the muscle contraction and shortening of sarcomeres until the muscle goes into full rigor. The accumulation of lactic acid lowers the muscle pH, which can change the structural properties of muscle proteins and enzyme activities. Most fish products have an isoelectric point of 5.5 for myofibrillar proteins (Tahergorabi et al., 2011; Wilson, 2007). As pH decreases close to the isoelectric point of the muscle proteins, WHC is reduced, and texture is altered (Huff-Lonergan & Lonergan, 2005; Rotabakk et al., 2017). The enzymes adapted to a neutral pH (e.g. glycolytic enzymes) become inactivated or reduce their activity while enzymes favouring acidic pH become more active (e.g. lysosomal proteases such as cathepsins). This softens the muscle and changes the organoleptic properties (Chéret et al., 2007). Therefore, converting muscle to meat creates a different cellular environment from that of a living muscle.

1.2 Fish quality

Fish quality is a broad term comprising several aspects usually related to the freshness and safety of the product. Nortvedt et al. (2007) categorized quality into two main areas. First, the primary quality of the product integrates the intrinsic quality from farming live seafood (e.g., biological and health status, season, size, species) to handling procedures and product quality when it reaches the market shelves. Next, the secondary quality is the market delivery and perceived quality by the consumers. In general, product quality includes nutritional, microbiological, technological, organoleptic, ethical, and environmental aspects (Listrat et al., 2016; Nortvedt et al., 2007).

Several methods can be used to assess fish quality. These include sensory, biochemical and chemical, physical and microbiological methods that often assess the degree of freshness and shelf life of a product. Some examples of physical properties include colour, texture, muscle pH, WHC and DL. Other quality parameters include gaping, bleeding, bloodstains, marbling, melanin deposition and fat composition (Sigurgisladottir et al., 1997).

Fish is a highly perishable product, and microbial spoilage, enzymatic reactions and lipid oxidation can occur rapidly if not handled and stored properly (Fogarty et al., 2019; Gram & Huss, 1996; Tavares et al., 2021). Microbial growth determines shelf life, while proteolytic enzymatic activity and chemical reactions result in the initial loss of freshness (Chéret et al., 2007; Gram & Huss, 1996). The predominant bacteria associated with spoilage for aerobically chilled salmon include *Pseudomonas* spp., *Shewanella* spp. and *Photobacterium* spp., which can be found in live salmon (Emborg et al., 2002; Gram & Huss, 1996; Møretrø et al., 2016). Therefore, it is important to prolong or retard spoilage mechanisms to maintain good quality. In addition, good product quality is also often affiliated with low losses of water and nutrients during processing.

1.2.1 Water and water holding properties

Water is an important component of foods. It is a partially charged dipolar molecule that binds to charged protein molecules in the muscle structure and can be classified into different morphological states based on its location and mobility. Water and lipid constitute about 80% of the fish muscle (Løje et al., 2017; Murray & Burt, 2001; Ofstad et al., 1995). This water can be categorized according to low, intermediate or high mobility, namely bound, immobilized and free water (**Figure 1.2**). As the name implies, bound water binds tightly to proteins via hydrogen bonds. It accounts for the smallest amount (1-2%) of water in muscle and is also

resistant to freezing and conventional heating. Up to 80% water is immobilized or entrapped, held either by steric effects or attraction with bound water. This water is weakly associated with proteins and can be easily lost by drying or converted to ice by freezing. It can also be lost or converted to free water and is closely associated with WHC. Lastly, most free water flows freely and is held by capillary and surface tension forces which can be easily released. This water is located between the actin and myosin filaments of myofibrils in the intracellular cell compartments in living or pre-rigor muscles (Huff-Lonergan & Lonergan, 2007). The combination of immobilized and free water accounts for up to 90% of the water in Atlantic salmon (Aursand et al., 2009).

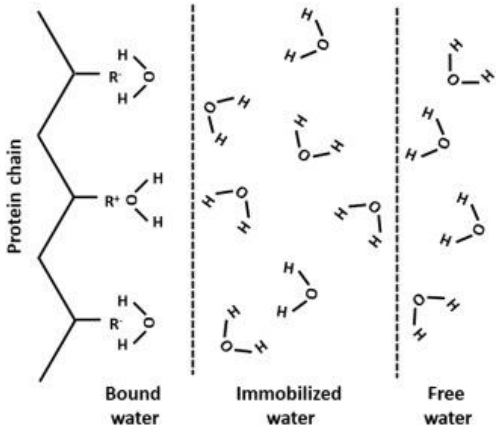

Figure 1.2: Distribution of water in the muscle (Bowker, 2017).

Water holding properties are important properties to fish quality. Drip loss, also known as "purge" or "weep", occurs when the changed capacity of the muscle structure results in the extrusion of water. The principle of WHC is based on the muscle's ability to retain water, which is the sum of bound, immobilized and free water (Bowker, 2017). Since fish is sold by weight in kg, a high DL is undesirable. It expedites hydrolytic and oxidative reactions, directly influencing the industrial producer's economic profitability and consumer's perception of appearance and texture. The presence of purge in the packaging also renders the product sensorially unappealing. WHC is correlated to textural properties such as tenderness and juiciness (Bowker, 2017; Huff-Lonergan, 2002; Kaale et al., 2014). A high WHC in fish muscle is preferred as it affects DL during thawing and weight changes during storage and cooking (Duun & Rustad, 2008; Kaale et al., 2014). Therefore, a product with low DL and high WHC is highly valued. A comprehensive review of pre and post-mortem factors and how different

processing and preservation methods can affect water holding properties in Atlantic salmon is presented in **Paper I**.

1.3 Superchilling

Various ways of food preservation techniques have been introduced to prolong shelf life. Superchilling is an efficient temperature-dependent process and maintains the internal core temperature of the fish between chilling and freezing (Banerjee & Maheswarappa, 2019). This technology can be categorized into two parts - the superchilling process and the superchilled storage (Kaale et al., 2011). Superchilling has been demonstrated effective in common fish species such as Atlantic salmon (Bahuaud et al., 2008; Claussen, 2011; Duun & Rustad, 2008; Erikson et al., 2011; Fernández et al., 2009; Gallart-Jornet et al., 2007b; Sivertsvik et al., 2003), rainbow trout *(Oncorhynchus mykiss)* (Shen et al., 2015), Atlantic mackerel *(Scomber scombrus)* (Cropotova et al., 2019), Atlantic cod *(Gadus morhua)* (Duun & Rustad, 2007; Eliasson et al., 2019; Stevik & Claussen, 2011), Nile tilapi*a (Oreochromis niloticus)* (Cyprian et al., 2013) and common carp *(Cyprinus carpio)* (Liu et al., 2014). The combined effect of superchilling together with other preservation techniques such as vacuum packaging, modified atmosphere packaging (MAP) and active releasing systems, also significantly increases the shelf life of the product (Hansen et al., 2009; Liu et al., 2010; Sivertsvik et al., 2003; Wang et al., 2008).

There are several methods of superchilling available, such as chilling in blast tunnels, contact chilling or refrigerated seawater (RSW) (Claussen, 2011; Kaale et al., 2011). Cryogenic freezing is a form of blast freezing where products are exposed to liquid N_2 (-196°C) or liquid CO_2 (-78°C) directly to the food (Kaale et al., 2011). This offers short freezing times, but operational costs can be high. In contrast, impingement freezing of salmon fillets has a lower operating cost and has the same freezing times and weight loss as cryogenic freezing (Kaale et al., 2011). Kaale et al. (2013) reported that salmon fillets rapidly superchilled with an impingement freezer maintained optimal ice crystal features on size, distribution and shape. There was also less physical damage to muscle fibres due to ice crystal formation. The concept from the abovementioned examples for superchilling fillets is through partial ice crystallization, where a thin layer of ice is formed on the surface of the product. This ice eventually seeps through the muscle leading to temperature and ice crystal equalization, freezing 5-30% of the total water content (Kaale et al., 2013; Thordarson et al., 2017) and slowing down autolytic biochemical processes, maintaining quality and freshness, and increasing the shelf life of fish by at least 1.4-5 times (Duun & Rustad, 2008; Kaale et al., 2011; Magnussen et al., 2008). In

the fish industry, superchilling fillets improve product yield and quality so that more fillets can be sold as fresh rather than frozen.

During the transportation of gutted salmon on trucks, up to 30% of the space is occupied by external ice (Bahuaud et al., 2008; Gallart-Jornet et al., 2007b; Magnussen et al., 2008). Therefore, a box of gutted fish (20-24 kg) would require 4-6 kg of ice (Nordtvedt et al., 1998). When this ice melts, the temperature fluctuation could further lead to deterioration in quality. With superchilling, the need for ice is eliminated during storage and transportation, providing logistical and economic benefits for the industries. In addition, superchilling can save 30% environmental impact than ice chilling (Claussen et al., 2011). Nevertheless, the superchilling technology does impose certain challenges. This includes the temperature and duration of freezing that can influence the degree of ice crystal formation (Banerjee & Maheswarappa, 2019; Kaale et al., 2011; Magnussen et al., 2008; Wu et al., 2014). Compared to ice storage, the temperature must be critically stable during superchilling to prevent constant freezing and thawing (Banerjee & Maheswarappa, 2019).

1.3.1 Superchilling by RSW storage

RSW systems are commonly used in fishing vessels to preserve and chill large volumes of live fish, such as pelagic species herring, tuna, mackerel and sardine through mechanical refrigeration before offloading at land and further processing (Widell & Nordtvedt, 2016). Seawater contains salt, which can be a natural preservative (Hekmatsyar et al., 2019). RSW systems onboard fishing vessels allow fish to be mechanically chilled to temperatures below that of ice. Due to the presence of salt, such units reduce the freezing point of water and cool the internal temperature of the fish rapidly as seawater is directly in contact with the fish (Venugopal, 2006). This can favour a logistical advantage for longer fishing trips or an earlier fish delivery compared to ice transport, which can be prone to delays. Chilling by slurry is another type of RSW system. The RSW slurry is a binary system consisting of water with 25-30% microscopic ice crystals and a salinity of around 3.5%. Slurries have a higher surface heat exchange rate than flake ice and give less fish damage (Piñeiro et al., 2004). Erikson et al. (2011) previously reported that it takes at least 3 hours to chill salmon of 3-5 kg in a seawater slurry of -2°C to attain a core temperature of -1 to -2°C.

Technological innovations in the aquaculture sector are constantly evolving and moving towards sustainable aquaculture. The current slaughter practice is to transport live fish from the cage to waiting pens via a well boat before pumping the fish to a land-based facility for

slaughter. In recent years, a novel concept has been introduced in the Norwegian aquaculture industry. Fish can be directly slaughtered onboard a slaughter vessel, such as the *Norwegian Gannet*, by the sea cage, condensing the handling process into one and circumvents several steps in the value chain (**Figure 1.3**).

Figure 1.3: Fish slaughter vessel *Norwegian Gannet* (source: own).

The complete slaughtering machinery available onboard, including holding tanks, electric stunning, bleeding, and gutting machines, presents the possibility for the whole slaughter process to be done manually and automatically. The gutted whole fish are then directly immersed into clean seawater in RSW tanks at superchilled conditions during transportation to land-based facilities for further primary and secondary processing. Since seawater has a high heat transfer coefficient, the fish would be chilled down quickly to the desired temperature with a more uniform cooling (**Paper II**) (Eliasson et al., 2019; Venugopal, 2006).

Despite the several advantages RSW systems bring, potential problems may arise with this storage. If fish are not gutted, pumping can be an important source of contamination as the velocity and pressure from the pump can force intestinal guts to be expelled out from wild fish, contaminating the whole catch (Svanevik & Lunestad, 2011). However, starvation, adequate bleeding and gut cleaning of fish before RSW immersion under transport, in addition to cleaning and disinfection of the factory after every harvest, could minimize the risk of contamination.

Some concerns were also reported like water and salt uptake for species with low fat content, issues with anaerobic spoilage bacteria producing hydrogen sulphide (H_2S), bleaching of gills and dulling of skin (Gokoglu & Yerlikaya, 2015; Graham et al., 1992; Roach et al., 1961). Salt uptake is probably the most significant problem as a salty taste can be detected upon consumption and render the fish unacceptable. Graham et al. (1992) stated that an undesirable

salty taste was detected in cod stored in RSW after 3 days. However, eviscerated halibut did not have this problem even after several weeks of storage. Therefore, salt uptake depends on species, size, and resistance of the skin to salt penetration. The problem with water uptake is reduced with fatty fish. Salt uptake is also relatively unimportant for salmon because of its large size and subcutaneous fat layer, hindering salt migration (Šimat & Mekinić, 2019).

1.4 Cold-smoked salmon

Cold-smoked salmon is a lightly processed ready-to-eat seafood, a popular delicacy in the European market with a salt content often ranging from 2-5% water phase salt (WPS), 63-70% water content, pH between 5.8 and 6.3, and a smoke treatment giving 0.1-0.2mg phenol/100g product (Cardinal et al., 2004; Espe et al., 2004; Hansen et al., 1995).

Smoking includes 3 main processing steps: salting, drying and smoking. Salting and drying are among the oldest food preservation methods where water activity (a_w) is lowered. As a_w measures the amount of free water available for microbial growth, reducing a_w would prolong the product's shelf life. The recommended critical limit of salt content to prevent the growth and toxin production of *Clostridium botulinum* in smoked salmon is 3.5% WPS (Huss et al., 2003; Løje, 2007).

The primary process in salting is molecular diffusion through the water phase of the muscle, causing a counter-current of water and salt transport between the salt and muscle. As a result, the highest salt concentration is on the fillet surface (Gómez-Salazar et al., 2015; Lerfall et al., 2011; Wang et al., 1998). Salting can be done in several ways, i.e. dry, brine or injection salting (Birkeland & Bjerkeng, 2005). Dry salting is a traditional method regarded as a slow, but gentle method frequently used in industries (Birkeland et al., 2004b). This is done by spreading excess crystalline NaCl on the fillet until it diffuses into the product and equilibrates before washing off the excess salt. Brine salting immerses the fish in a salt solution, reducing water diffusion. Finally, the injection method uses needles to inject brine into the fillets through pressure, distributing the brine throughout the muscle fibers (Birkeland et al., 2003).

Several factors influencing salt uptake in salmon are outlined in **Paper I**. The conformation of muscle protein changes during salting, leading to changes in WHC. For instance, a lower degree of protein denaturation occurs at low salt concentrations (<5-6% of wet weight NaCl). The muscle fibers swell from the electrostatic repulsion of Cl^- weakly adsorbed to the myofibrillar and sarcoplasmic proteins. Thus, this shifts the isoelectric point of proteins, allows water to be trapped within the lattice, and increases WHC. In contrast, the salt removes the

water molecules surrounding the protein at high salt concentrations and eventually causes protein denaturation (Gallart-Jornet et al., 2007a; Sigurgisladottir et al., 2000; Thorarinsdottir et al., 2004).

Cold-smoking usually uses a mild temperature of 20-30°C for 2-12 hours with a relative humidity of 60-75% (Birkeland et al., 2004b). The generation of phenols from the pyrolysis of wood chips generates smoke that gives the characteristic flavor and smoky taste (Birkeland, 2004; Lerfall, 2011). Wood chips from hardwoods like cherry, oak, hickory and beech are commonly used. Cold-smoked salmon are often darker and less red but more yellowish than the raw fillets (Birkeland, 2004; Birkeland et al., 2004a; Lerfall et al., 2011; Løje, 2007; Valø et al., 2020). The smoking process affects the colour through carbonyl-amino reactions of Maillard browning and protein and lipid oxidation (Hall, 2011). In addition, the texture becomes denser and more elastic due to water loss (Birkeland et al., 2004b). Therefore, cold-smoking influences the product's texture and colour and changes the conformation of muscle protein and WHC. This leads to cell shrinkage, decreased thermal stability of the myofilament actin and myosin and eventually protein denaturation.

1.4.1 Modelling the salting process

Real-time quality measurements performed in the field, laboratory or industrial setting can often be time and labor consuming. Mathematical modelling offers the possibility of reducing experimentation processes and is an alternative in the food industry for process optimization. For a model to be considered robust, validations obtained must be accurate. Empirical models rely on data collected from actual experiments and fit this with empirical correlations. The Zugarramurdi and Lupín (1980) model is a popular model that predicts the water and salt uptake phenomena during the salting process. The limitation of using empirical models is that they cannot predict beyond the experimental range, and often biological differences in raw material composition and seasonal variations are excluded. As opposed to this, numerical models provide flexibility and are not restricted to specific conditions. The varying physical properties in food (e.g. shape, specific heat capacity, thermal conductivity, density and viscosity) can be included, and spatial distributions and local variations over time can be observed. Numerical modelling based on the heat and mass transfer in food can be explained using PDEs to predict state variables like temperature and concentration under appropriate assumptions. Modelling the mass transport phenomena during dry or brine salting has previously been done on fish species, including Atlantic cod (Andrés et al., 2002; Blikra et al., 2020), Atlantic herring (*Clupea harengus*) (Laub-Ekgreen et al., 2019) and Atlantic salmon

(Martínez-López et al., 2019; Wang et al., 1998; Wang et al., 2000). These simulations were validated with experimental data.

During the dry salting process, cold air is transferred from to the product by convection, then within the product by conduction. The rate of heat transfer depends on several factors, including the thermophysical properties of salmon (specific heat capacity, thermal conductivity and density), temperature and heat transfer coefficient. In addition, heavy salting causes moisture loss and significant weight reduction from the consequential osmotic pressure of the salt on the moisture of muscle cells (Lauritzsen et al., 2004). This results in water diffusing out from the surface while salt is diffused into the muscle. **Figure 1.4** overviews the heat and mass transfer phenomena during the dry salting process in a cold room used for mathematical modelling in this thesis.

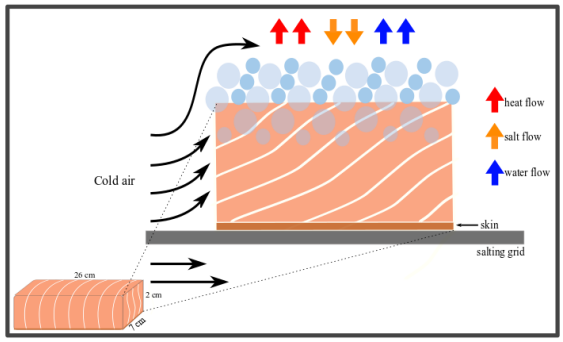

Figure 1.4: Heat and mass transfer during dry salting of salmon (Adapted from **Paper VI)**.

1.5 Packaging

The transportation of food products can require a significant amount of time. Modified atmosphere packaging plays an important role in fish preservation and contributes to the hurdle technology limiting microbiological growth (Sivertsvik et al., 2002). Effective packaging should act as a good barrier against moisture and air, keep fish moist, maintain WHC and impede bacterial growth, unwanted enzymatic processes and undesirable odors (Bindu & Sreejith, 2018). Some of the popular fish packaging methods include gas, traditional vacuum and vacuum skin packaging.

1.5.1 Gas packaging

Gas packaging utilizes a modified gas composition to replace the air in the headspace to regulate the microbial activity of the product. The gas mixtures often used are CO_2, N_2 and O_2 at varying proportions, with CO_2 being the most important for its bacteriostatic and fungistatic properties (Sivertsvik et al., 2003). For fatty fish like salmon, the CO_2 levels used for packaging are often higher with a significantly lower O_2 than lean species to reduce lipid oxidation and microbial growth (Nagarajarao, 2016; Noseda et al., 2014). As CO_2 is highly water and fat soluble, it exists as a dissolved gas and chemically dissolute to bicarbonate ions (HCO_3^-) and hydrogen ions (H^+) in the muscle tissue (Sivertsvik et al., 2002). This lessens the headspace gas in the packaging and decreases the pH of the muscle during storage (DeWitt & Oliveira, 2016; Noseda et al., 2014; Sivertsvik et al., 2002). For modified gas packaged fish, often an extension between 30-60% can be expected with increasing CO_2 levels (Sivertsvik et al., 2002). Previous studies have found that superchilling combined with MAP extends the shelf life by up to 4 times compared to air storage at refrigerated temperatures (Fernández et al., 2009; Hansen et al., 2009; Sivertsvik et al., 2003).

1.5.2 Vacuum packaging

Vacuum packaging was the first commercially developed MAP that involves removing O_2 and sealing the product in a low O_2 permeable film, providing good barrier properties against air and water (DeWitt & Oliveira, 2016; Noseda et al., 2014). This inhibits the growth of aerobic spoilage microorganisms like *Aeromonas* spp. and *Pseudomonas* spp., and at the same time maintains appearance and texture, prevents lipid rancidity and prolongs the freshness of food products by 3 to 5 times (Nagarajarao, 2016). However, the wrinkles formed during vacuum packaging may cause purge to accumulate over time and render the product unattractive (Łopacka et al., 2016; Strydom & Hope-Jones, 2014).

Vacuum skin packaging is considered an extension of traditional vacuum packaging and is an increasingly popular indication that a product is of premium quality. The product is placed on a tray before being tightly sealed with the upper lidding film. This perfectly contoured sealing prevents air pockets and wrinkles, provides a better aesthetic appearance and easier packing durability and eliminates the need for headspace gas (Lagerstedt et al., 2011; Vázquez et al., 2004). As this is a relatively new packaging technique, most studies focused on meat products and few on commercially important aquaculture species like salmon. Vacuum skin packaging was therefore studied in **Paper V**.

Chapter 2: Research Objectives

This PhD thesis aims to examine the water holding properties of Atlantic salmon and follow the RSW chilling process throughout two whole value chains: 1. from whole fish to packaged cold-smoked fillets, and 2. from whole fish to portioned and packaged fresh fillets. Quality parameters like colour, texture, microbial growth, sensory, enzyme and salt content were also included in the analyses. The final segment of the project was to develop a mathematical model using heat and mass transfer phenomena coupled with partial differential equations (PDEs) to predict the water and salt profiles during the dry salting process before cold-smoking. The specific research objectives are:

1. To present an original review on the influence of water holding properties on Atlantic salmon quality. As part of this thesis, the various methods and measurements in measuring WHC are discussed, and the effects of common preservation and processing methods on water holding properties are evaluated. (**Paper I**)
2. To investigate the different chilling technologies for whole fish (RSW, ice) and fillets (cryogenic, ice) and how these affect the water holding properties and other quality parameters throughout the whole value chain, from fresh to processed (cold-smoked) salmon fillets. (**Paper II**)
3. To study the water and salt uptake, water holding properties and other quality parameters during RSW chilling over 4 days compared to the traditional storage on ice by following the whole value chain, from whole fish to processed (cold-smoked) salmon fillets. (**Paper III**)
4. To compare the quality and microbiological shelf life between salmon first slaughtered onboard a slaughter vessel then immersed in RSW against salmon slaughtered traditionally on land then stored in ice. (**Paper IV**)
5. To study the combined effect of storing fish in RSW or ice, and packaging technique (vacuum skin, traditional vacuum), on water holding properties and quality in general. (**Paper V**)
6. To develop a mathematical model using first principles to predict temperature, water and salt profiles, and water activity during the dry salting process before cold-smoking and vacuum storage. Whole fish were initially immersed in RSW or ice, and quality attributes were compared. (**Paper VI**)

The first objective is addressed in **Paper I** as a comprehensive review of water holding properties in the Atlantic salmon industry. This paper highlights the principles, pre- and post-mortem factors and processing and preservation methods influencing DL and WHC. In addition, conventional and non-invasive methods in measuring WHC and the potential of modelling WHC are explained. **Paper II** follows the fish chilling regime and studies the new fish slaughter method implemented on *Norwegian Gannet*. Initial whole fish storage was either superchilled in RSW onboard for 12 hours or kept traditionally on ice. After filleting, the whole fish was subdivided into two groups: superchilled in liquid N_2 or stored on ice. The left fillets were kept raw while the right fillets underwent the cold-smoking procedure. Water holding properties and other quality parameters were investigated, including blood parameters, rigor index, Quality Index Method (QIM), enzyme, salt, colour, texture, and microbiological stability and shelf life for a total of 31 days. Building upon this work, **Paper III** studied the water and salt uptake of superchilling in RSW for 4 days and storage on ice, undergoing the same cold-smoking procedure. Instead of slaughtering onboard the vessel, a makeshift RSW tank was constructed in the laboratory. Since the idea of slaughtering onboard is new, an experiment described in **Paper IV** was conducted to compare the quality and shelf life against land-based slaughtering, as done today.

After 3 studies presenting that cold-smoked salmon originating from RSW and ice storage gave superior quality, the focus was shifted to packaging technologies. As vacuum skin is a relatively new technology, **Paper V** was carried out to combine whole fish storage regime and packaging on vacuum skin or traditional vacuum. Finally, the last objective implemented in **Paper VI** models the temperature, water and salt profiles, and water activity of RSW immersed and ice-stored salmon, exposed to increasing salting times during the cold-smoking process. This model was created using COMSOL Multiphysics and validated using experimental results.

Chapter 3: Main Results and Discussion

An overview of the methods and quality parameters analyzed is shown in **Table 1**. In the following section, the main results obtained from the experiments are presented and discussed.

Table 1: An overview of the methods and quality parameters analyzed from Papers II to VI.

Experimental Design	Papers				
	II	**III**	**IV**	**V**	**VI**
Chilling methods					
RSW	✓	✓	✓	✓	✓
ice	✓	✓	✓	✓	✓
Processing methods					
filleting	✓	✓	✓	✓	✓
portioning				✓	✓
cold-smoking	✓	✓	✓		✓
Packaging methods					
traditional vacuum	✓	✓		✓	✓
vacuum skin				✓	
Quality parameters					
DL	✓	✓		✓	✓
WHC	✓	✓		✓	✓
pH	✓	✓		✓	✓
salt content	✓	✓		✓	✓
QIM	✓		✓		
rigor index	✓				
fillet index	✓				
gaping/blood spot	✓	✓	✓		
colour	✓	✓	✓	✓	✓
texture	✓	✓	✓	✓	✓
enzyme activity	✓	✓			
microbial growth	✓	✓	✓	✓	
sensory profile			✓		
water activity					✓

3.1 Water holding properties and salt content

Fish is sold by weight, so a high DL is undesirable. The current work revealed that storing gutted whole fish in RSW for 4 days, then on ice for 3 days, results in a net increase in weight of 0.7% (**Paper III**) and 0.9% (**Paper V**). The differences in weight gain could be attributed to the size and/or fat content. During storage of whole fish in RSW, some salt uptake was expected from the direct contact between RSW and fish. Freshly slaughtered salmon was measured to have a salt content of 0.1% NaCl. After storage in RSW, 0.3% and 0.23% of NaCl content were measured before processing (**Paper III and V**), parallel with other studies on salmon species (Bronstein et al., 1985; Himelbloom et al., 1994). The observations for water and salt uptake show that water and salt accumulation is dependent on time. Moreover, the fish used in the experiments were gutted, so opening in the body cavity can induce water and salt uptake (Erikson et al., 2011). Since the salt concentration in RSW is higher than in the salmon flesh, the concentration difference induces salt penetration across the skin and into the flesh (Bronstein et al., 1985; DeBeer et al., 2019; Himelbloom et al., 1994). Salt diffusion is dependent on the fat content of the muscle. At higher fat contents, the muscle has more resistance to the transfer of aqueous NaCl solution (Wang et al., 2000).

Regardless of the storage condition, the DL of raw fillets increased through storage (**Papers II, III and V**). This is an established phenomenon as DL is a time-dependent process, as observed in other studies (Huff-Lonergan, 2009; Rotabakk et al., 2017). Whole fish storage in RSW then on ice after filleting demonstrated the lowest DL of 1.5% compared to shell frozen fillets or traditional chilling on ice (**Paper II**). This was consistent with the findings from **Paper III,** with a DL of around 1.5% for all treatment groups. During shell freezing of fillets, water is frozen out, forming ice crystals in the muscle and leading to a higher solute concentration, cell damage, and protein denaturation (Bahuaud et al., 2008; Duun & Rustad, 2008; Kaale et al., 2013). Nonetheless, a DL of 1-2% is not considered high (Duun & Rustad, 2008).

The highest DL was observed after dry salting and cold-smoking due to diffusion of water to the exposed muscle surface before plateauing out through cold vacuum storage. After the cold-smoking procedure, the obtained yields were 92-94%, and differences between the different chilling regimes were removed (**Papers II and III**). The salting procedure for cold-smoking was done the same way in the studies, i.e. dry salting with refined salt at 0°C. Apart from **Paper VI**, the salting time was fixed at 18h and salt contents varied from 3.4-4.9% NaCl (**Paper II**) and 3.0-3.2% NaCl (**Paper III**). In **Paper VI**, the fillets were exposed to salt at an increasing time interval from 3h to 21h. Increasing the time of fillets exposed to salt lead to a gradual

increase in DL after an incremental processing step of salting, smoking and storage. The yield obtained after smoking decreased with increasing salt exposure.

No differences in DL were observed between the sample groups in the studies, regardless they were initially stored in RSW or ice. Therefore, the choice of storage regime does not seem to affect the diffusion of salt nor DL during the cold-smoking process. Heavy salting significantly affects weight loss from the consequential osmotic pressure of the salt on the moisture of muscle cells (Lauritzsen et al., 2004). As salting is a diffusion process, salt is absorbed while water is expelled from the muscle until equilibrium is achieved. The greatest DL was seen after the smoking process. Interestingly, there was a minimal loss in drip after 2 weeks of cold vacuum storage (**Paper VI**), which may be related to the fatty acid profile (Lerfall et al., 2016). A compilation of previous studies on the influence of smoking on water holding properties is presented in **Paper I**.

Drip loss occurs from the expulsion of tissue juices from the postmortem degradation and structural changes within the muscle, increasing the amount of free water released as drip (Offer & Trinick, 1983). The drip in salmon contains mostly water, but other constituents like proteins, lipids, water-soluble vitamins and minerals are also lost during storage (Lerfall, 2011; Ofstad et al., 1995; Rotabakk et al., 2017; Strasburg et al., 2007). The effect of whole fish storage (RSW, ice) was not pronounced after portioned fillets were packaged. However, it was evident that skin packaging had a greater DL than traditional vacuum (**Paper V**). Therefore, the DL caused by packaging superseded the whole fish storage method. This may suggest that protein denaturation may be greater in skin packaged fish, although previous research presented contradicting results. Chan et al. (2021) reported no differences in DL between vacuum skin and gas packaged salmon (60%CO_2: 40%N_2) originating from ice-stored fish. Vázquez et al. (2004) found a higher DL in skin packaged beef cuts than traditional vacuum packaging, yet Strydom and Hope-Jones (2014) presented otherwise. Huff-Lonergan (2009) explained that the pressure applied during vacuum packaging might lead to more significant DL than gas packaging, rendering a negative product appearance. Therefore, more studies are recommended to examine the cause of the greater DL observed in skin packaged salmon.

As mentioned in **Paper I**, WHC is an important quality parameter and is the muscle's ability to entrap water. It is generally agreed that DL and WHC are inversely related based on the calculation of liquid loss (Huff-Lonergan & Lonergan, 2005). However, methodological differences in measuring and calculating WHC can influence the results. In this thesis, the

method used to measure WHC was based on the low-speed centrifugation method by Skipnes et al. (2007), incorporating the amount of dry matter (water content) into the calculation. Regarding WHC of whole salmon stored in RSW, **Papers III and V** observed an increase in WHC, especially on day 7, where fish were stored on ice for 3 days after storing for 4 days in RSW, instead of only storing on ice immediately after slaughter. WHC is influenced by the surrounding salt concentration. As explained, RSW can be considered a brine with a salt content of around ~3.5%, so the muscle fibers swell and entrap more water. Nevertheless, the effect of salt uptake is rendered insignificant since the skin works as a protective layer against diffusion. Also, the subcutaneous fat layer and large size of salmon prevents salt migration (Šimat & Mekinić, 2019).

The location on where sampling was taken for WHC is important. Water holding capacity is influenced by the fat content, which varies along the fillet (Mørkøre et al., 2001). The samples collected for WHC in this work were taken from the anterior part above the lateral line of the fillets. Therefore, the variations caused by sampling location should be minimized. The WHC obtained in the studies ranged between 82-92% after filleting (**Figure 3.1a**; **Papers II, III and V**). WHC of raw fillets remained relatively unchanged, but those of cold-smoked fillets packaged in vacuum decreased through storage (**Paper II and III**).

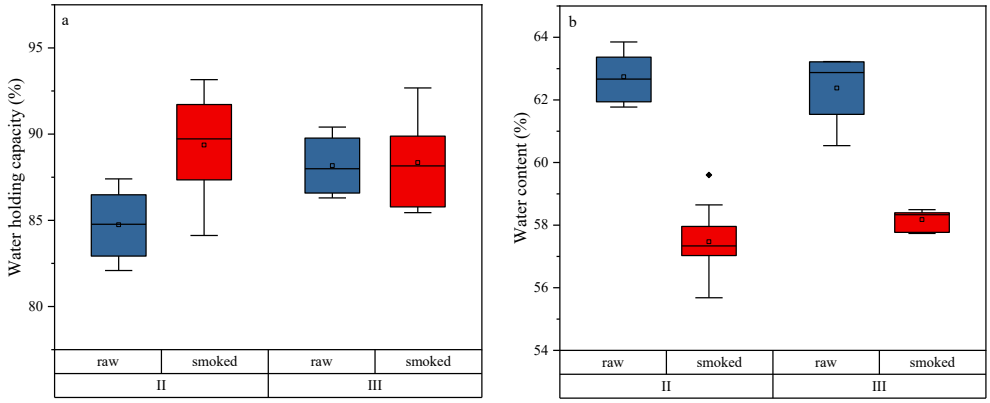

Figure 3.1: Compiled results in (a) water holding capacity (%) and (b) water content (%) of raw and smoked salmon from Papers II and III. ◆ represents outliers.

Using the calculation method by Skipnes et al. (2007), a higher WHC was observed for the smoked samples than their raw counterparts (**Figure 3.1a**; **Paper II**), where WHC of smoked fillets ranged from 84-93%. This corresponds to previous studies explaining that WHC increased considerably in smoked samples due to the added salt and lower water content (Gomez-Guillen et al., 2000; Rørå et al., 2003). Water is lost during the smoking process, so

the remaining water is more likely held tighter. However, this phenomenon was not evident in **Papers III and VI**, probably due to biological variations within and among samples that contribute to the uncertainty. The water content was predominantly lower in smoked than raw salmon due to the salting out process during smoking that causes muscle shrinkage as water and lipid leach out of the muscle (**Figure 3.1b; Papers II and III**).

The correlation between DL and WHC were more noticeable when raw fillets were portioned and packaged in traditional vacuum and vacuum skin (**Paper V**). WHC decreased through traditional vacuum and vacuum skin packaging storage, regardless of the whole fish storage method (**Paper V**). Similarly, Chan et al. (2021) presented a decrease in WHC for salmon in vacuum skin packaging, while a similar trend was seen for MAP portions until day 13 before an increase in WHC until day 20.

3.2 Surface appearance

The flesh colour of salmon is a decisive factor for consumers at the point of sale for both raw and smoked fillets. The lipid soluble carotenoid astaxanthin, supplemented into fish feed, gives the characteristic colour of fillets (Anderson, 2000; Skrede & Storebakken, 1986a, 1986b); the redder and more even the colour, the fresher and better the quality fillet is perceived to be. The flesh colour observed during whole fish storage in RSW and ice was inconsistent, indicating the complexity in visual perception. **Paper IV** presented that whole fish kept in RSW for 2 days were lighter and more reddish than iced fish when colour was measured on day 5. **Papers III and V** also suggested a lighter colour for RSW fish before filleting.

During whole fish storage of 4 days, **Paper III** noticed a decrease in lightness (L^*) and redness (a^*), and an increase in yellowness (b^*) for both RSW and iced fish. This contrasted with an observed increase in L^* and a decrease in b^* (**Paper V**). No difference was observed between the storage methods. The discrepancies seen may be explained by a variety of factors that can influence colourimetric properties. This includes processing techniques (Birkeland et al., 2004b; Cardinal et al., 2001; Lerfall, 2011), seasonal variations (Mørkøre & Rørvik, 2001), starvation and stress (Einen & Thomassen, 1998; Erikson & Misimi, 2008; Mørkøre et al., 2008), slaughtering procedures (Kiessling et al., 2004; Roth et al., 2010) and storage conditions (Erikson & Misimi, 2008; Espe et al., 2004; Regost et al., 2004; Stien et al., 2005).

The thermochromic effect, where a sample's colour depends on its temperature, is particularly sensitive to red and orange-coloured samples (Erikson & Misimi, 2008; Hiltunen et al., 2002). In the experiments, the temperature at the point of measurement may differ. This may also have

affected the colour properties when samples were removed from the cold room for analysis. A more distinct visualization was detected in the gills, where the gills of RSW fish appeared lighter and less reddish than of ice fish (**Paper III**). The bleaching of gills may be regarded as a possible disadvantage in RSW storage. This was also observed in RSW stored cod for 4 days, although its general appearance was more appealing than iced cod after 7 days (Roach et al., 1961).

Information on how different whole fish storage regimes affect the fillet colour is scarce. The storage of raw fillets in expanded polystyrene boxes exposed to air indicated a drop in L^* values after filleting for 2 weeks (**Paper II**). In contrast, the results in **Paper III** presented lighter, less reddish, and less yellowish fillets through refrigerated storage. This was in tally with the results when packaging was introduced, where the alterations in colourimetric properties became more evident. Packaging portioned fillets in traditional vacuum and vacuum skin increased L^* and decreased a^*, b^*, chroma and hue values through storage. The skin packaged fish had a greater colour change, giving a lighter, less reddish, less yellowish colour and lower chroma than traditional vacuum packaged fish (**Paper V**). During storage, changes in the muscle integrity due to autolytic enzyme activities and protein denaturation may affect the reflective properties and confound the instrumental colour assessments, as the natural colour of the fillet is lost (Erikson & Misimi, 2008; Ozbay et al., 2006). The loss of a^* and b^* could also be related to the increase in DL over time, increasing the light reflection of the fillets (Daskalova, 2019). Likewise, the role of pH caused by stress and a more rapid rigor contraction can contribute to protein conformational changes that give a lighter and more opaque product (Stien et al., 2005).

The cold-smoking process causes carbonyl-amino reactions of Maillard browning and denaturation of astaxanthin (Hall, 2011; Lund & Nielsen, 2001). This justifies the observed decrease in L^* and a^*, and changes in b^* after smoking compared to the raw counterparts (**Papers II, III and VI**), as supported by previous studies (Birkeland et al., 2004b; Bjørnevik et al., 2018; Cardinal et al., 2001; Lerfall, 2011; Løje, 2007). The smoke components also react with fatty acids in the muscle that may influence the light scattering properties of the fillets (Lerfall et al., 2016). Furthermore, the processing step can significantly affect the colour difference, ΔE (CIE, 1994), perceived by the consumer. This was apparent in **Paper VI,** where ΔE values were higher after storage than after smoking.

3.3 Texture

A firm and elastic fillet texture makes it easier for further processing and is often preferred by consumers (Rasmussen, 2001). Cathepsins are lysosomal cysteine proteases believed to contribute to post-mortem muscle tenderization as they are released from the organelles into the cytosol (Bahuaud et al., 2010b; Chéret et al., 2007). Bahuaud et al. (2010a) found that cathepsins B+L and cathepsin L were significantly higher in soft salmon fillets. A negative correlation was also seen between muscle pH and cathepsins B+L activity, texture properties, and muscle degradation. The observations from **Papers II, III and V** presented that the texture of raw fillets significantly decreased through storage, an established theory of muscle softening due to protein denaturation (Espe et al., 2004; Hultmann & Rustad, 2002). Whole fish stored in RSW had a softer texture until processing (**Paper III**). However, measuring the cathepsins B+L activity did not give a higher indication of muscle tenderization, suggesting that the softer texture observed may be due to other reasons. This was not the case in **Papers IV and V**, where there was no significant difference in texture between RSW and ice whole fish storage. Several factors may have contributed to this variation, including differences in physical and chemical factors like chemical composition, season, size and raw material (Cheng et al., 2014). When the effect of packaging on raw fillets was implemented, those from RSW fish presented a firmer texture regardless of packaging in vacuum skin or traditional vacuum (**Paper V**). However, more studies are needed to confirm the textural observations.

Textural properties are also inversely related to the water content (Birkeland et al., 2004b). This explains the increase in firmness after salting and cold-smoking (**Figure 3.2a**; **Papers II, III and VI**), where the muscle becomes denser and more elastic (Birkeland et al., 2004b; Løje, 2007). No differences were observed between the cold-smoked salmon originating from RSW or ice fish, indicating a comparable textural quality. The firmness observed in both raw and smoked fillets were lower in **Paper III** than **Paper II**, which could be attributed to the differences in fish size and the thickness of the fillets. Fillet height consistently influenced the measured texture of raw and smoked salmon (**Papers III, V and VI**).

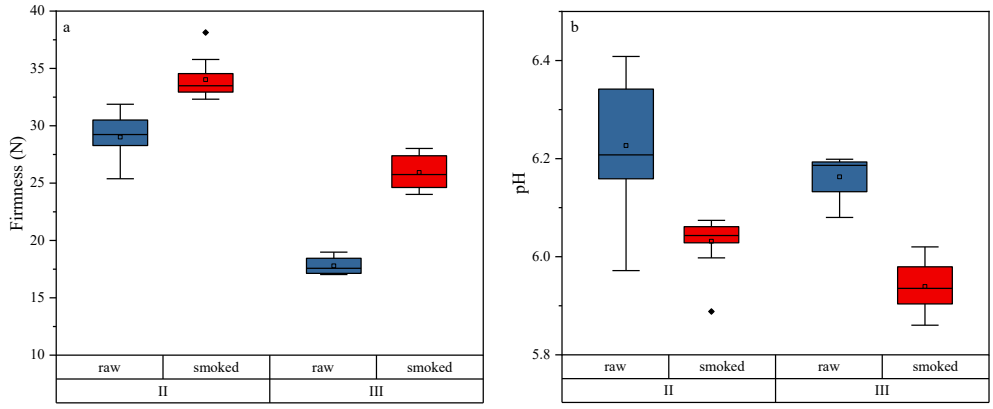

Figure 3.2: Compiled results in (a) firmness (N) and (b) pH of raw and smoked salmon from Papers II and III. ◆ represents outliers.

3.4 Gaping and blood spots

The presence of gaping and blood spots visually downgrades the quality of the product and can be sensorially rejected by the consumers that can lead to financial losses. Gaping occurs due to the disintegration of connective tissues in the muscle, which creates slits in the fillet (Jacobsen et al., 2017). The transition from CO_2 to the electrical stunning method has led to injuries in the fish vertebrae and ruptures of major aortas and veins. Hence blood spots and hemorrhages can be seen (Digre et al., 2010; Robb et al., 2003; Roth et al., 2010). Moreover, poor exsanguination from the remaining residual blood in the blood vessels results in blood spots and blood effusion on the surface and within the fillets (Olsen et al., 2006; Robb et al., 2003; Roth et al., 2009).

In general, RSW-stored fish presented a better gaping score on raw fillets (**Papers III and IV**). Espe et al. (2004) showed that fish becomes softer with more gaping during ice storage. The cause of the lower gaping scores for RSW fish is still unclear, but the lower temperature used during RSW storage may have contributed to this observation. After cold-smoking, **Paper II** reported a lower gaping and almost no blood spots for RSW-stored fish. **Paper III** presented no differences in gaping for smoked fish, and **Paper IV** presented no differences in gaping and blood spots between smoked fish slaughtered onboard the slaughter vessel or in a land-based facility. The results indicated that gaping seemed to mainly affect the unprocessed fillets. The observations also indicate that RSW preserves freshness and gives a comparable quality in gaping and blood spots as ice storage. Roth et al. (2009) reported that counting blood spots along the midline of the smoked fillet is reliable in determining how well bleeding was done.

Blood spots initially hidden within the raw fillet emerge after smoking as the blood oxidizes and becomes dark brown. Besides, Roth et al. (2009) emphasized the importance of washing and early gutting and filleting to allow blood to flow out, reducing the blood spot counts. In RSW systems, gutted fish are bled thoroughly in the tanks in addition to the recirculation of seawater, ensuring that blood remnants do not remain in the body cavity (Jacobsen et al., 2017).

3.5 Shelf life

3.5.1 Microbial growth

Fish are in constant contact with indigenous microorganisms in their aquatic environment, making them highly prone to spoilage after harvest. Hence, it is crucial to lower the fish's core temperature immediately after slaughter to delay enzymatic, biochemical and microbiological induced reactions. Differences in microbiota can be observed depending on the fish species, environment, processing and storage conditions (Gram & Dalgaard, 2002; Gram & Huss, 1996; Zhuang et al., 2021). Only a small fraction of the microbial population accounts for spoilage, known as the specific spoilage organisms (SSO). The production of volatiles like trimethylamine (TMA), ammonia and hydrogen sulphide (H_2S) by, e.g., *Photobacterium* spp. and *Shewanella* spp. are responsible for the off-odors and negative flavors in fish (Dalgaard, 1995). In addition, lactic acid bacteria (LAB) can be prevalent in smoked salmon, giving off-odors and off-flavors from producing organic acids and ethanol as fermentation products (Hansen et al., 1998; Tomé et al., 2006).

There is currently no consensus on the type of bacteria to be monitored in determining microbiological shelf life (Fogarty et al., 2019). The typical microbiota found in cold-water species is dominated by psychrotrophic gram-negative microorganisms of the genera *Shewanella*, *Pseudomonas*, *Vibrio* and *Photobacterium* (Emborg et al., 2002; Fogarty et al., 2019). These are usually quantified by total psychrotrophic counts (TPC), where a total count of 10^6-10^7 cfu/g indicates spoilage and the end of shelf life (Centre for Food Safety, 2014; Dalgaard et al., 1997; Hansen et al., 1998). The black colonies produced by the iron agar to measure total mesophilic counts (TMC) quantify for hydrogen sulphide producing bacteria (HSPB), accounting for e.g. *Shewanella* spp., *Enterobacteriaceae*, *Vibrionaceae* and LAB.

In this thesis, TPC, TMC and HSPB were analyzed on raw fillets. Microbial growth increased through storage, and there were generally minimal differences in TPC and TMC between RSW or ice-stored fish (**Papers II, IV and VI**), apart from **Paper IV,** which indicated a lower TMC

for fish slaughtered at the vessel. Furthermore, HSPB was not detectable in all studies before processing and storage, confirming that the raw materials were of high microbial quality.

Paper III detected higher microbial counts on RSW-stored fish throughout storage, but this was attributed to various reasons, including handling and contamination, highlighting the importance of proper hygiene and risks of using a makeshift RSW tank. As better hygiene in the RSW tank was maintained in **Paper VI**, a distinct difference was found in the salmon's HSPB counts between the chilling regimes. The growth kinetics estimations obtained from the Baranyi and Roberts model illustrated a longer lag phase in HSPB counts of 1-2d with a lower maximum population density for RSW-stored fish, regardless of the packaging method. This trend was similar to **Paper II**, which reported the lowest HSPB counts for fish in RSW then stored on ice after filleting. **Paper IV** also found a higher HSPB count for fish slaughtered on a land-based facility than those from the vessel immersed in RSW. The lower storage temperature may have played a role in the lower HSPB growth for the RSW-fish. As seen in **Figure 3.3**, fish were kept rather stable in RSW at -0.9°C for 4 days by adding pre-frozen seawater ice. When the RSW-fish were drained and kept on ice for 3 more days, the temperature increased slightly but was stable at -0.7°C. The temperature for the iced-fish was maintained rather stable during the 7-day storage at around 0°C.

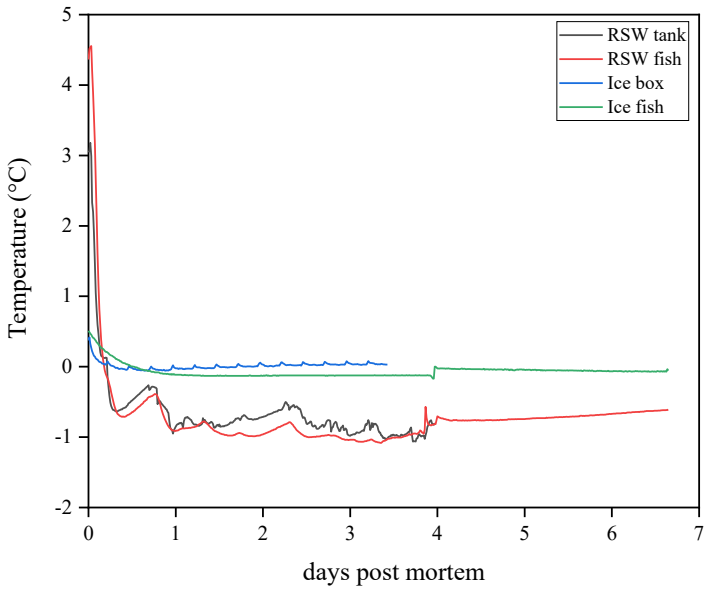

Figure 3.3: Temperature profile of whole fish in RSW and ice for 4 days, and on ice for another 3 days before processing (unpublished data from **Paper V**).

For smoked salmon, TPC was similar to all storage groups ranging from 4.6 to 5.2 log cfu/g (**Paper II**) and 3.8 to 4.9 log cfu/g (**Paper III**). After 29 days of storage, a significantly higher LAB count was found from the control fish, where they were initially wrapped in plastic and chilled in boxes containing ice but without ice contact (**Paper III**).

3.5.2 Sensory methods

Sensory methods such as QIM can be used as objective tools to determine the product's freshness and remaining shelf life (Hyldig & Green-Petersen, 2004). Quality Index Method is a well-defined rapid assessment based on characteristics of raw fish where demerit points are given and summed. The quality index (QI) increased with storage time and indicated the relative freshness and remaining shelf life (**Papers II and IV**). Fish slaughtered in a land-based facility had a significantly higher QIM score than fish slaughtered onboard the vessel and stored in RSW for 2 days. The main difference in the measured attributes was observed on the mucus from the gills and skin and the gills' smell (**Paper IV**).

In terms of other sensory characteristics, **Paper II** revealed no differences in fillet index scores among fish either stored in RSW or ice and superchilled or kept on ice after filleting. However, in correlation with the microbial spoilage, the scores increased through storage until day 16 post-mortem. Sensory assessment from a trained panel also affirmed minimal differences between RSW stored fish onboard the vessel against fish slaughtered on a land-based facility then kept on ice, apart from higher protein precipitation from the iced fish (**Paper IV**).

3.6 Other observations

Results from **Paper VI** indicated that RSW-stored fish had a lower a_w value after smoking and storage, which may be attributed to the greater salt gain and water loss. The onset of rigor mortis was similar at 24h for RSW and ice-stored fish, suggesting that superchilling in RSW likely does not accelerate the rigor process from rapid chilling (**Paper II**). The pH ranged between 6.0-6.4 for raw salmon and 5.9-6.1 for the cold-smoked product (**Figure 3.2b; Papers II and III**), which correlated to the observations of Løje (2007). A higher pH is normally related to lower DL and higher WHC, but it is also influenced by the salt concentration and temperature (Ofstad et al., 1995). The decreased pH seen in raw fillets through storage (**Papers II, III and V**) was likely due to the depletion of glycogen reserves that induces lactic acid formation. However, the later increase in pH observed indicated a possibility of bacterial contamination as metabolic activity from bacteria produces basic compounds like TMA and ammonia (**Papers II and III)**.

3.7 Application of modelling

Modelling the salting process can be applied to give a realistic representation of the preservation of many fish products. This is an important tool for determining the mass kinetics of water and salt mobility within the muscle under different conditions and reducing labour and experimental costs. In this thesis, the mathematical model developed in COMSOL Multiphysics was based on the first principles of heat and mass transfer kinetics and provides a fundamental understanding of the water loss and salt uptake during the cold-smoking process (**Paper VI**). The predictions on heat, water and salt changes from the numerical model agreed well with the experimental results from the low RMSE values. This model also agrees well with the empirical model of Z&L and gave good predictions on a_w values, as validated with the experimental results. Overall, the empirical model is suitable and can serve as a rapid solution in calculating the water and salt contents. However, it does not outline the process but rather gives the outcome of the final product. On the other hand, the numerical model can visualize the spatial changes within the muscle, as seen in the differences in salt and water concentration within the muscle (**Figure 3.4**). In this experiment, constant values fixed at one temperature were assumed for the calculation of thermophysical properties (specific heat capacity, thermal conductivity, density) to simplify the model. The change in temperature during the cooling process in this experiment was small, and no freezing was involved (6 to 0°C), so the change in thermophysical properties does not significantly affect the model prediction. If the temperature difference is greater, these varying parameters can be included in the model as a function of temperature.

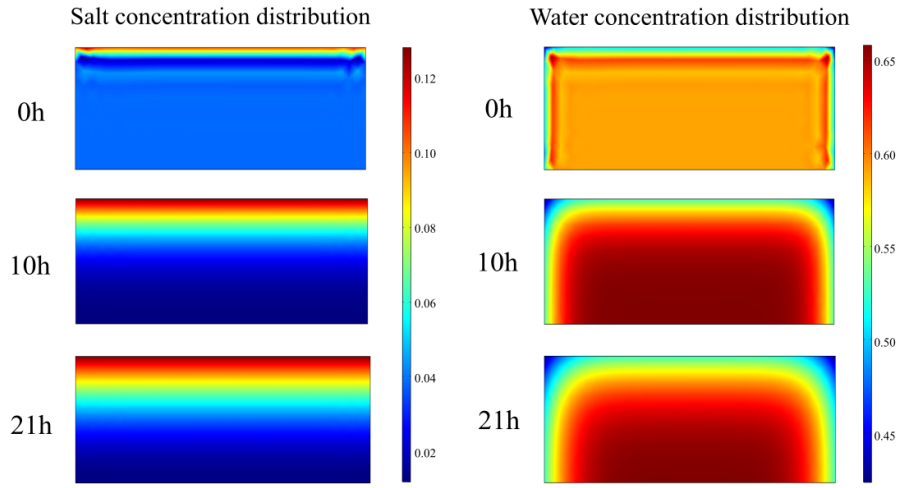

Figure 3.4: Spatial changes of salt and water concentration from RSW-stored whole fish at 0h, 10h and 21h (**Paper VI**). The legend represents the concentration in kg/kg.

It is generally acknowledged that salt migration occurs by diffusion due to the differences in concentration gradient. The diffusion model based on Fick's second law is often used to describe the salting process in several food products (Akköse & Aktaş, 2016; Andreetta-Gorelkina et al., 2016; Gómez-Salazar et al., 2015; Wang et al., 2000; Zhang et al., 2011). In **Paper VI**, a constant salt and water diffusivity was assumed when equilibrium is attained after 2 weeks of vacuum storage. The influence of temperature on the effective diffusion coefficient can be calculated using the Arrhenius equation so that the Fick model can be transformed to include temperature dependence. Wang et al. (1998) argued that there is a diffusion anisotropy of salt in fish muscle, as salt diffusivity is dependent on the salt concentration and is, therefore, time-dependent. As salting time increases, the gradual denaturation of protein results in less resistance for salt diffusion at higher salt concentrations. Furthermore, Barat et al. (2011) found that the diffusivity was higher at the beginning of the salting process resulting from the rapid increase of WHC of the surface in contact with the salt. Therefore, future models can include more parameters to improve the robustness and aid in industrial optimization.

3.8 Summary of results

The main results on quality traits from **Papers II to VI** are summarized in **Table 2**.

Table 2: Summary of the main results on raw and cold-smoked salmon quality and packaged fillet portions with different packaging types, from RSW and ice-stored whole fish.

Quality Parameters	Main Observations
Drip loss	• Storing gutted whole fish in RSW induced a weight gain of 0.7-0.9%. • Whole fish storage in RSW then on ice after filleting presented low DL. • The DL of raw, cold-smoked and packaged fillets increased through storage duration. • Skin packaged portions, regardless of the whole fish storage method, gave a higher DL. • A greater DL was observed after the cold-smoking process.
Water holding capacity	• The WHC of RSW-stored fish was higher than ice-stored fish before filleting. • The WHC of cold-smoked fillets decreased through storage. • The WHC of skin and vacuum packaged from RSW and ice-stored fish decreased through storage.
Salt content	• Storing gutted whole fish in RSW led to a salt uptake of up to 0.2%. • The salt content of cold-smoked salmon increased as dry salting duration increased.
Colour	• The colour of raw fillets and packaged portions increased in L^* and decreased in a^* and b^* through storage. • Regardless of the whole fish storage method, skin packaged portions gave the highest L^* and lowest a^* and b^* values. • Cold-smoking led to a decrease in L^*, a^* and changes in b^*. • The processing step (dry salting, cold-smoking and vacuum storage) affected the ΔE.
Texture	• RSW-stored fish presented a firmer texture after vacuum or skin packaging. • The texture of raw fillets and packaged portions decreased through storage. • The cold-smoking process led to a firmer texture. • Fillet height influenced textural properties.
Gaping and blood spots	• RSW-stored fish gave the best gaping scores on raw and cold-smoked fillets. • RSW-stored fish had the best blood spot counts on cold-smoked fillets.
Shelf life	• The HSPB growth was lowest for RSW-stored fish. • Microbial counts on whole fish, raw fillets and packaged portions increased through storage. • The RSW-fish had a better QI score than ice-stored fish.

Chapter 4: Industrial Implications and Conclusion

Improving the quality and shelf life of fish and incorporating sustainable development goals have been the objectives of modern-day research and development in aquaculture. This thesis presents an overall notion that RSW storage of whole gutted salmon is a feasible method for fish transportation as it gives good quality salmon compared to those traditionally stored on ice. Furthermore, RSW-stored gutted salmon also presents several industrial advantages like quicker cooling, faster transportation time and the ability to store large volumes of fish.

It must be noted that the magnitude of a laboratory-scale experiment can introduce differences in an industrial setting. The RSW systems outlined in **Papers II and IV** were performed from an industrial production onboard the fishing vessel, while **Papers III, V and VI** utilized a makeshift RSW tank under laboratory conditions. The experimental setup in **Paper III** shows an example of the potential risks that may arise concerning hygiene. Storing fish in fishing vessels and closed fish bins may introduce anaerobic bacteria, specifically, HSPB producing hydrogen sulphide (H_2S) from decomposed fish and negatively affect the whole catch (FAO, 1986). Still, the general finding from the experiments indicated a lower HSPB growth. The quick chilling to sub-zero temperatures during storage may have contributed to the lower microbial growth. This is supported by the findings of Eliasson et al. (2019), who reported that onboard superchilling slows the growth of HSPB with a lower total volatile base nitrogen (TVB-N) content on whole gutted cod stored in RSW at -1°C.

Moreover, strict regulations are implemented regarding processing and hygiene in industrial settings. Temperature control is an important aspect of quality maintenance and experimental control. In the experiments, the temperature was kept as stable as possible. Nevertheless, during processing and on sampling days, it was inevitable for the fish to be removed from the storage room, which may have disrupted the cold chain. In industrial settings, the cold chain is expected to be kept stable with strict hygienic conditions.

A good packaging method increases the microbial shelf life of fish products. The manuscripts on packaging methods (**Paper V**) described the quality differences between vacuum skin and traditional vacuum packaging. These differences lie mainly in DL and colour. Otherwise, the methods present an extended microbial shelf life.

The results obtained in this thesis supports the following conclusions:

- Several pre- and post-mortem factors and processing and preservation methods can influence the water holding properties of Atlantic salmon. The presented review can help understand the factors that minimize DL with a better WHC and introduce emerging technologies in quality improvement. Several methods, both conventional and non-invasive, are available in measuring WHC. Although these methods are not directly comparable, the information obtained can give a general idea of the water holding properties for the product of interest.
- Superchilling whole gutted fish in RSW results in water and salt uptake and retains water better than chilling on ice. As fish is sold by weight, the weight gain observed during whole fish RSW storage introduces logistical and economic benefits. Muscle tenderization, as measured by cathepsins B+L, was not higher in RSW fish. Storage of these fish on ice after filleting and cold-smoking provided better gaping and blood spot counts with a quality that can be considered similar to whole fish storage on ice.
- Storing fish onboard RSW in a slaughter vessel and the makeshift RSW tank presented a lower HSPB growth and better QIM scores than storage on ice from fish slaughtered in a land-based facility. Therefore, RSW storage at sub-zero temperatures may potentially lengthen microbiological shelf life.
- The cold-smoking process gives significantly different quality parameters than the raw fillets. It eliminates the main differences between whole fish stored in RSW and on ice, such as water holding properties and texture.
- The study comparing vacuum skin and traditional vacuum packaging illustrated that the packaging method superseded the differences between RSW and iced storage. Vacuum skin packaging presented a higher DL, with a lighter, less reddish and less yellowish colour than traditional vacuum.
- Numerical modelling can be a useful predictive tool to estimate the temperature, water and salt migration pattern during the dry salting process. In addition, this model can be further extended to simulate the a_w of the product. The comparison with the empirical model provides advantages for a quick and reliable way of calculating water and salt content. Overall, this work can serve as a foundation for further improvements to the model.

Future Perspectives

This thesis has demonstrated that chilling Atlantic salmon and storing it in RSW provide good quality products through the whole value chain. With the global increase in demand for food to meet the rising population, more food will come from aquaculture, which calls for better logistics in storage and transport. Fishing vessels using RSW systems is a common storage method currently practiced for fisheries. Nonetheless, the slaughter vessel introduced in this thesis is by far the first of its kind. Other similar vessels are also simultaneously under planning and construction, suggesting the potential of this slaughter method and a possible shift in logistics from land to the storage and transport of gutted fish in RSW tanks.

More research can be done to better understand the RSW storage system and the new slaughter method. For example, more focus can be directed to fish welfare and biosecurity. In this thesis, the maximum storage time for fish in RSW was kept up to 4 days to simulate a practical scenario of sea transport within and to nearby countries. The optimal duration and temperature of RSW storage before quality deterioration and how this affects the water holding properties and general quality would be interesting to follow as this extends the possibility of a longer transportation time. Furthermore, since minimal differences were observed between the chilling methods after filleting, it would be interesting to investigate the appropriate storage duration of fillets before they start losing water. In addition, whether the fillets can be kept without the need for ice and perhaps in superchilled storage can be better understood. As quality encompasses many aspects, other parameters that were not studied can be included. Muscle samples can be observed at a cellular level, and biological variations in terms of fat content within the population and the effect of seasonal changes can be evaluated.

Further developments can be done to include more parameters to simulate a more realistic setting in the industry. As an extension and improvement to the developed mathematical model, future work can include parameters that vary within and among the salmon populations (e.g. raw material composition, rigor status, seasonal variation, heterogeneity and structural changes). For instance, the fat content can influence the salting process as a higher fat content will increase salt uptake resistance. In addition, during the cold-smoking process, the drying procedure also causes the muscle to shrink, so moving boundaries can be considered when modelling.

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Paper I

Water holding properties of Atlantic salmon.

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Abstract

With global seafood production increasing to feed the rising population, there is a need to produce fish and fishery products of high quality and freshness. Water holding properties, including drip loss (DL) and water holding capacity (WHC), are important parameters in determining fish quality as they affect functional properties of muscles like juiciness and texture. This review focuses on the water holding properties of Atlantic salmon and evaluates the methods used to measure them. The pre- and post-mortem factors and how processing and preservation methods influence water holding properties and their correlations to other quality parameters are reviewed. In addition, the possibility of using modelling is explained. Several methods are available to measure WHC. The most prevalent method is the centrifugation method, but other non-invasive and cost-effective approaches are increasingly preferred. The advantages and disadvantages of these methods and future trends are evaluated. Due to the diversity of methods, results from previous research are relative and cannot be directly compared unless the same method is used with the same conditions.

1. Introduction

The quality of seafood is increasingly important and influences the production cost and consumer preference. Salmon is a dominating species in aquaculture with a worldwide total production of 2.5 million tonnes and is also an important seafood commodity with a high value (Ernst and Young, 2019). Norway is currently the world's largest producer of Atlantic salmon, with a total production of 1.4 million tonnes in 2019 (SSB, 2020). As an export commodity, Atlantic salmon represents around 93% of the Norwegian aquaculture production, and these fish are exported for further processing. They have a high calorie and protein retention of 25 and 28%, respectively (Fry et al., 2018). As salmon production becomes more lucrative, more countries are using innovative technologies to explore the possibilities of producing salmon on sea-based and land-based farms. Therefore, as one of the leading countries with a proven aquaculture industry, Norway is in a good position to strengthen its standing in the globally competitive aquaculture market and produce fish of high quality. This applies throughout the entire value chain, from production, harvesting, primary and secondary processing and finally storage and consumption.

Water is the predominant component in fish. It supports a series of biochemical, microbiological and physical reactions that affect the sensory, nutritional and functional properties during fish processing and storage (Jepsen et al., 1999). Water holding properties include drip loss (DL) and water holding capacity (WHC), two representative indicators for freshness considering the affinity between fish muscle and water. WHC, the ability of muscle protein to prevent water from being released from their three-dimensional structure against external forces, is a property that contributes significantly to both meat and fish quality (Duun, 2008; Huff-Lonergan & Lonergan, 2005; Kaale et al., 2014; Warner, 2014). WHC is also defined as the ability to retain inherent water within the muscle (Bowker, 2017; Cheng & Sun, 2008; Zhang et al., 1995). Water released without any additional force is referred to DL, sometimes called purge or weep. This is the extrusion of tissue juices from the muscle protein networks and is closely related to WHC (Huff-Lonergan & Lonergan, 2005; Szmańko et al., 2021).

A high DL is undesirable due to oxidative and hydrolytic processes from microorganisms and is intensified by the purge, resulting in lower quality. Improved WHC as a reflection of limited DL became more desired by the producers for higher net weight and better acceptable appearance to the consumers. It affects weight changes during storage and transport, DL during thawing, weight loss during cooking as muscle texture changes, and thereby consumer preferences and costs (Duun, 2008; Kaale et al., 2014). For producers, a high WHC results in lower DL and greater protein functionality, influencing profitability. It also reflects a better appearance and improved juiciness and texture. Some reports refer to liquid holding capacity (LHC) as an interchangeable term for WHC (Ofstad et al., 1996). Others differentiate the LHC into water and lipid lost during processing, especially for fatty fish (Løje, 2007; Rørå & Regost, 2003). Ofstad et al. (1995) reported that the primary liquid loss in fatty fish like salmon and rainbow trout is mostly water, and fat loss can be considered negligible. A better understanding of WHC in salmonid species could help prevent fluid loss, potentially also nutrient loss, and increase product yield through the whole value chain, leading to better quality.

The composition and muscle structure can differ between mammalian and avian meat and fish. In contrast to meat, fish has less connective tissues with shorter muscle fibres. In salmon, these muscle fibres are separated into distinct red and white muscles (Kiessling et al., 2006; Listrat et al., 2016). Two of the quality defects faced by the meat industry are pale, soft, exudative (PSE) and dark, firm and dry (DFD) meat. PSE meat results in a loss of WHC while DFD meat has a high WHC, but both give visual defects rejected by consumers (Listrat et al., 2016; Strasburg et al., 2007). Consumer research indicated a preference for tenderness for meat when making purchasing decisions, while the preferred quality for fish is a firm texture with a good WHC (Listrat et al., 2016; Maltin et al., 2003).

Several reviews have described WHC in food. Still, so far, the focus has been mainly on meat products like beef, pork and lamb (Cheng & Sun, 2008; Fennema, 1990; Forrest et al., 2000; Huff-Lonergan & Lonergan, 2005; Oswell et al., 2021) and rarely on aquaculture species. This article follows the majority of research referring to WHC as the ability of the muscle to hold water and DL as weight loss from mainly water and

includes other minor constituents like the loss of water-soluble vitamins, minerals and proteins (Kamruzzaman et al., 2012; Ofstad et al., 1995; Strasburg et al., 2007). By understanding the mechanisms and processes that influence water holding properties, products can aim to have a good WHC or lessen DL. Therefore, this review presents an overview of water holding properties and how this affects the Atlantic salmon in the value chain.

2. Measuring water holding properties

Water is an essential constitution that, together with lipids, make up 80% of fish muscle (Murray & Burt, 2001). Water is closely correlated to physical and chemical changes within the fish, including pH, textural properties, protein denaturation, enzyme activity, fatty acid hydrolysis and rheological property (Dawson et al., 2018; Wang et al., 2018). The three primary states of water are bound, immobilized and free water. These are located in different compartments within the muscle. Immobilized and free water are mainly responsible for DL, accounting for up to 90% of the total water (Aursand et al., 2009). Furthermore, the immobilized water, which accounts for most of the water (up to 80%), is correlated to texture (Bowker, 2017). This is explained by the microstructural observations of the protein-water interactions, which shows that the decrease in immobilized water content is related to quality deterioration over time (Sun et al., 2018).

The lack of a standardized method to measure WHC makes it challenging to compare the same parameters with previous literatures (Oswell et al., 2021; Szmańko et al., 2021). Moreover, WHC could differ after a product is processed or cooked. Therefore, the choice of the measurement method and its calculation is distinguished based on the experimental purpose (Hamm, 1986). It is also impossible to measure the same sampling point at different times, resulting in a certain degree of uncertainty. Therefore, it is essential to acknowledge the differences between the methods and choose one that suits the objective best. A summary of the methods is shown in Figure 1.

2.1 Conventional approaches

Established methods based on the amount of force applied to remove loosely bound or unbound water have been reported in determining the WHC of muscle. These are “no force”, “applied external mechanical force”, or “applied thermal force” (Fennema, 1990; Honikel & Hamm, 1995). Applying no force is equivalent to measuring DL, where the only force involved is the gravitational force (Cheng & Sun, 2008; Fennema, 1990; Honikel & Hamm, 1995). This is a simple but often more time-consuming technique as the samples are hung and left sitting for days while drip is collected. The amount of time is also another variable. The DL is then calculated as the % of the collected drip against the original weight.

Applying an external mechanical force includes centrifugation and compression, where pressure is applied to remove the liquid. The filter paper wetness (FPW) involves pressing the sample between filter papers and is one of the simplest and quickest methods that highly correlates to DL (Mallikarjunan, 2016). The centrifugation method involves applying centrifugal force either low-speed (200-800×g) using 2-15g of samples, or high-speed centrifugation (5000-40000×g) using 1-20g of samples to measure the ability of the sample to retain water by measuring the liquid lost after centrifugation (Varmbo et al., 2000). Applying a thermal force involves cooking and measuring the cook loss of the sample. This primarily represents the loss of intra- and extracellular water from the muscle due to protein denaturation and cell membrane disintegration. Finally, other methods also include measuring thaw loss after freezing (Bowker, 2017).

The centrifugation method, especially the low-speed centrifugation method that largely retains the microstructure of the muscle, is the most preferred way to measure WHC in fish species (Varmbo et al., 2000). A summary of selected literatures that used the centrifugation method on Atlantic salmon is shown in Table 1. WHC is calculated from liquid loss and can be expressed in %. Most studies present it in % and calculate WHC by measuring the differences in weight from the sample as the liquid is collected through a filter after centrifugation, as shown using Equation 1 (Aursand et al., 2009; Erikson et al., 2011; Gomez-

Guillen et al., 2000; Kaale et al., 2014; Løje, 2007; Ofstad et al., 1996; Ofstad et al., 1995; Rørå & Regost, 2003; Sun et al., 2018; Thorarinsdottir et al., 2004):

$$\text{WHC (\%)} = \frac{W_T - LL}{W_T} \text{x}100\% \quad (1)$$

w_T refers to the total sample weight, and LL refers to the liquid loss.

These results, however, only give the relative WHC values, and such results can only be compared with those that use the exact same method (Skipnes et al., 2007; Varmbo et al., 2000). Since most frozen foods are usually cooked and consumed after thawing, to incorporate cooking loss, Skipnes et al. (2007) developed a method that includes water content and cook loss to determine WHC of whole and comminuted samples in both raw and cooked fish (Equations 2 to 4). This method calculates the dry matter, where liquid is lost by drying the sample gravimetrically at 105°C, representing the moisture that includes the loss of bound water and has been used by several studies with Atlantic salmon and Atlantic cod (Blikra et al., 2019; Chan et al., 2020a; Chan et al., 2020b; Chan et al., 2021b; Fidalgo et al., 2020; Lerfall & Rotabakk, 2016; Rotabakk et al., 2017). In addition, the total WHC changes from raw to cooked product can also be determined.

Raw samples:

$$\text{WHC (\%)} = \frac{W_0 - \Delta W}{W_0} \text{x}100\% \quad (2)$$

where:

$$W_0 = \frac{V_0}{m_0} \text{x}100\% \quad (2a)$$

$$\Delta W = \frac{\Delta V_0}{m_0} \text{x}100\% \quad (2b)$$

V_0 represents the initial water content, m_0 is the initial sample weight, and ΔV_0 is the liquid separated after centrifugation of the raw material.

Cooked samples:

$$\mathrm{WHC_1}\ (\%) = \frac{V_1 - \Delta V_1}{V_1} \mathrm{x100\%} \quad (3)$$

where V_1 represents the water content and ΔV_1 the liquid separated after centrifugation of the cooked material.

The equation describing the total changes in WHC from raw to cooked samples is:

$$\mathrm{WHC_{TOT}}\ (\%) = \frac{V_0 - (\Delta V_1 - C_1)}{V_0} \mathrm{x100\%} \quad (4)$$

where C_1 represents the cook loss.

To compare samples with different water contents before centrifugation, WHC can also be expressed relative to the fat-free dry matter content as the amount of water retained based on the mass fraction of final to initial weight (Løje, 2007):

$$\mathrm{WHC}\ (\%) = \frac{100 - t - \Delta r}{100 - t}\ \mathrm{x100\%} \quad (5)$$

where:

$$\Delta r\ = \frac{m_0 - m_1}{m_0}\ \mathrm{x}\ 100\% \quad (5a)$$

m_0 and m_1 refer to the initial sample weight and sample weight after centrifugation, respectively. t refers to the % of initial dry matter.

The methods mentioned above are considered conventional approaches involving a certain extent of sample destruction. The centrifugal force and duration both affect water extrusion. The rotor geometry and centrifuge also need to be considered as this can affect the centrifugal force. Zhang et al. (1995) evaluated the impact of centrifugal force (959, 8630 and 34 500×g), duration (7.5, 15 and 22.5 min), sample temperature (2, 10 and 20 °C) and salt concentration (0, 0.3, 0.6 mol/l) on lean beef muscle. WHC decreased when the centrifugal duration increased from 7.5 to 15 min, but the decrease was minimal afterwards.

Likewise, the WHC decreased with a higher centrifugal force and temperature since more water was expelled. It is, therefore, crucial to measure WHC with the same test conditions to prevent misinterpretation of results.

The increasing demand for quality assurance in fish also led to the introduction of rapid, non-destructive and cost-efficient techniques for measuring WHC in fish. These include low field nuclear magnetic resonance (LF-NMR), magnetic resonance imaging (MRI) and near-infrared (NIR) spectroscopy, which can be used to measure water properties in both processed and unprocessed fish (Aursand et al., 2010; Aursand et al., 2009; Gallart-Jornet et al., 2007a; Gudjonsdottir et al., 2010; Jepsen et al., 1999; Løje, 2007).

2.2 Non-invasive approaches

LF-NMR uses a proton resonance frequency as low as 60MHz using pulse sequences such as the Carr-Purcell-Meiboom-Gill (CPMG) sequence and has been successfully implemented to study different water populations or "pools" in fish (Aursand et al., 2010; Gallart-Jornet et al., 2007b; Jepsen et al., 1999). This rapid, non-invasive method is based on T_1 (longitudinal) and T_2 (transverse) constant relaxation times and provides valuable information regarding the state of water, compartmentalization and changes in water location, and by extension the WHC in the fish muscle. Aursand et al. (2010) found that T_2 relaxation analysis can distinguish differences in water distribution in salmon muscle according to antemortem handling, fillet location and brine salting. From the exponential fitting of transversal relaxation (T_2) measurements, the three water components can be separated based on their location within the myofibrillar protein structures. T_{2b} represents strongly bound water with the shortest relaxation time at 1-10 ms relaxation, T_{21} and T_{22} have relaxation times at 10-100 and 100-400 ms, representing immobilized and free water between the muscle fibres, respectively (Aursand et al., 2008; Wang et al., 2018). LF-NMR can also be combined with other analytical methods like ^{23}Na NMR and MRI to optimize processing methods such as fish salting by analyzing water and salt distributions (Gudjónsdóttir et al., 2015; Veliyulin & Aursand, 2007). T_{21} relaxation times correlate to WHC during salting. A longer relaxation time indicates increased water mobility due to salt-induced muscle swelling, thereby increasing WHC (Aursand et al., 2008;

Gudjónsdóttir et al., 2015). As storage time increases, the greater protein denaturation causes water to flow more freely. Some bound water then becomes immobilized, while some immobilized water becomes free water, increasing DL (Sun et al., 2018). LF-NMR can therefore describe the water pools and predict WHC in fish muscle (Andersen & Jørgensen, 2004; Jepsen et al., 1999).

MRI can be considered an extension of NMR and gives the spatial and morphological observations of the molecular water, salt and fat distribution within the muscle. This system can be applied to different processing methods such as salting, freezing and thawing, and allows for time-related analysis of water mobility (Aursand et al., 2009; Wang et al., 2018). Only a few studies have been done using MRI as a tool to analyze water properties in fish (Aursand et al., 2010; Nott et al., 1999; Veliyulin et al., 2006; Wang et al., 2018). Due to high equipment costs, this method is more suited for laboratory research. It is also advantageous to measure salt content in muscle directly instead of chemical methods to prevent sample destruction (Aursand et al., 2010).

Chemical compositions are heterogeneous in the salmon fillet. For example, fat content decreases from head to tail and belly to back (Katikou et al., 2001; Zhu et al., 2014). Conventional approaches to measuring WHC can be challenging to account for the overall spatial distribution and variation of WHC in the fillet (Wu & Sun, 2013). Near-infrared (NIR) spectroscopy can be used alone or combined with imaging. Hyperspectral imaging is a promising on-line quality detection tool increasingly used industrially (Cheng & Sun, 2014; He et al., 2013). This online, non-invasive, rapid method integrates spectroscopy and computer imaging into one technique. It collects images at varying wavelengths in the same spatial area, providing detailed information simultaneously of the spectral and spatial assessment for quality analysis and food control. This includes physicochemical attributes, microbial quality, and contamination in fish and seafood products (Cheng & Sun, 2014; Cheng & Sun, 2015). The major constituents of fish like fat, water and protein have absorption peaks in the NIR region of 760-1100nm (Heia et al., 2016). Hyperspectral imaging has been used for several quality measurements related to water holding properties in Atlantic salmon. These include ice fraction after superchilling (Stevik et al., 2010), water content (He et al., 2014),

WHC (Wu & Sun, 2013), DL and pH (He et al., 2014). Therefore, hyperspectral imaging can determine DL and WHC and provide a spatial distribution of WHC within salmon fillets at the pixel level (He et al., 2014; Wu & Sun, 2013). With the wide range of traits that this imaging technique can measure, individual and multiple rapid quality assessments can be obtained.

3. Factors influencing water holding properties

3.1 pH

Post-mortem pH and protein denaturation are critical determinants of DL and WHC in fish and meat (Duun, 2008; Huff-Lonergan & Lonergan, 2007; Kaale et al., 2014; Rotabakk et al., 2017). Other pre- and post-mortem factors that influence DL and WHC in Atlantic salmon have also been reported (Figure 2), such as pre-mortem stress (Lerfall et al., 2015; Roth et al., 2006), starvation (Mørkøre et al., 2008) and the state of rigor mortis (Ofstad et al., 1996; Rotabakk et al., 2017).

There are three main proteins in fish muscle classified according to solubility, i.e., sarcoplasmic, stromal and myofibrillar proteins. The latter accounts for >50% of muscle proteins (Kijowski, 2001). Myosin and actin comprise the major share of the total myofibrillar protein content at ~65% of myofibrillar protein (Strasburg et al., 2007). Post-mortem glycolysis leads to the accumulation of lactic acid and the decline of muscle pH. At the overall isoelectric point (pI) of myofibrillar proteins (~5.5), the strong protein-protein attraction destabilizes the protein matrix and limits the space between the peptide chains for water to penetrate (Ofstad et al., 1995; Strasburg et al., 2007). The protein-water interaction is at its minimal, resulting in the shrinkage of myofibrils and loss of WHC. At pH below or above the global pI, the overall charge becomes positive or negative, causing the peptide chains to repel and create more space to bind with water molecules. Ofstad et al. (1995) studied the effects of pH, salt and temperature on WHC in comminuted salmon. A combination of low pH (6.0), low NaCl concentration (0.17 mol/l) and high temperature (70°C) gave the most significant interaction effect on liquid loss (i.e. lowest WHC), as compared to high pH (7.0),

high NaCl concentration (0.34 mol/l) and low temperature (30 °C). More mincing of salmon muscle with NaCl (0.34 mol/l) led to microstructural changes and gave a higher WHC. The higher DL seen at low salt concentrations (0.17 mol/l) may indicate inadequate swelling of the protein matrix. As more salt is added to the salmon mince, the myofibrillar proteins solubilize with salt and become a homogeneous paste in the matrix, thus holding water (Ofstad et al., 1995).

3.2 Rigor status

The pre-rigor period of salmon varies and can range from two h to over a day post-mortem. The immobilized water is the water most affected by the structural changes within the sarcomere. During the conversion of muscle to meat, the muscle goes into rigor as the myosin and actin filaments become bound. The shortening of the sarcomere without changing the filament length causes water to be lost within the myofibrils and relocated to the extracellular space, eventually released as drip (Huff-Lonergan & Lonergan, 2005; Hughes et al., 2014; Wong, 2018).

Rotabakk et al. (2017) reported that the season (spring, autumn) or locality (northern, southern Norway) on the Norwegian coast did not affect the WHC of Atlantic salmon after slaughter. Moreover, salmon slaughtered in spring (May) had a higher DL by 0.3% than in autumn (November). The difference in sea temperature and photoperiod along the coastline explained this observation, where the temperature is lower, but daylight is longer in the north. In addition, the filleting method and state of rigor had a significant effect. Fish that were filleted, instead of kept as head-on gutted (HOG), had a lower WHC, while pre-rigor salmon after slaughter had better water holding properties than post-rigor salmon kept in ice for 4 days. Therefore, the study described the potentiality of filleting fish pre-rigor. As DL is a time- and temperature-dependent phenomenon, chilled products should be stored at low temperatures (e.g. -1 to 4°C) with short storage duration. It is also important to minimize the quick onset of rigor through controllable methods like gentle handling and proper chilling processes immediately after slaughter (Chan et al., 2020b).

3.3 Temperature and species

WHC of fresh and cold-smoked salmon fillets does not seem to be affected by the muscle temperature at the point of filleting (Lerfall & Rotabakk, 2016). The pH and chemical composition in fish muscle differ among individuals, and there are also chemical variations depending on where the analysis is done on the fillet (Ofstad et al., 1993).

It was mentioned that DL increases in the cranial-caudal direction for fresh and frozen rainbow trout, but after ice storage, these variations became minimal among the fillet portions (Mørkøre et al., 2002). The species of interest and killing process also influence WHC. Farmed salmon was shown to have a higher WHC than lean species like wild and farmed cod, which was related to species-specific features and the higher stability of their actin and myosin (Duun, 2008; Ofstad et al., 1996). This was also consistent with the results of Duun (2008), who concluded that Atlantic salmon has better WHC than cod with a similar muscle pH. Interestingly, the comparison of WHC in farmed salmon and rainbow trout by Løje et al. (2017) found that the species with the higher fat content (salmon) was less able to hold water in the muscle, thereby lowering the WHC.

3.4 Diseases and ploidy

Diseases and ploidy can influence DL and WHC. Salmon containing the salmonid alphavirus (SAV) and those from a fish farm with repeated pancreatic disease (PD) outbreaks showed a higher DL than salmon with no records of PD and from farms diagnosed with PD 5-7 and 11-12 months before slaughter (Lerfall, 2011). In fish farms, triploid salmons were introduced to prevent breeding between wild and farmed fish that might escape from a cage. Lerfall et al. (2017) did a study to distinguish the quality differences between diploid and triploid salmon farmed at 5, 10 and 15°C. DL was significantly affected by the rearing temperature and ploidy, whereas ploidy did not influence WHC. Increasing the rearing temperature from 5 to 15°C also led to a larger increase in DL for both ploidies. DL was generally higher in triploid salmon,

with the most significant differences observed at 10°C. This was related to the larger cellular volume to accommodate the extra chromosome (Benfey, 1999; Bjørnevik et al., 2004).

3.5 Stress and slaughter conditions

Roth et al. (2008) showed that fillets exposed to electrical stunning after a percussive blow to the head during slaughter led to a higher DL than fillets without electrical stimulation. In a follow-up study, Roth et al. (2010) further observed that fillets exposed to 12 or 180 s of electrical stunning had a higher DL and lowered WHC than those exposed for 6 s after 16 days of storage at 3.8°C. The pre-slaughter crowding method, where fish are crowded in net pens before slaughter, induces significant stress responses, accelerates rigor mortis in fish and negatively affects the quality (Bahuaud et al., 2010). Few studies, however, have analyzed the crowding effect on water holding properties in salmonid species. Gatica et al. (2010) concluded that crowding and reduced oxygen levels increased the DL of salmon fillets. Disparities may be observed among various species attributed to the different crowding densities and the duration to which they were confined.

4. Effects of processing and preservation methods

4.1 Salting and smoking

Various processing and preservation methods are available to prolong fish shelf life. In Europe, a substantial amount of the fish produced for human consumption are smoked (Birkeland & Akse, 2010; Cardinal et al., 2004; European Commission, 2016). The smoking process involves either soaking in brine, injection or dry salting, then smoking and drying. During lightly processed procedures such as gentle salting and cold-smoking, protein denaturation in the muscle shifts the water distribution within the salmon. As measured using NMR, the population of water with the relaxation time T_{21} (immobilized water) decreases while the T_{22} population (free water) increases (Aursand et al., 2008; Gudjónsdóttir et al., 2015; Løje, 2007). This indicates an increase in water mobility (Aursand et al., 2008). As a result, the water that remained in the

muscle would be more tightly bound (Gudjónsdóttir et al., 2015; Wang et al., 2018), resulting in a higher WHC as observed in previous studies with cold-smoked salmon (Chan et al., 2020b; Gomez-Guillen et al., 2000; Lakshmanan et al., 2007; Løje, 2007). An overview of previous research done on DL and WHC for the standard salting techniques combined with cold-smoking is shown in Table 2.

Rørå et al. (2003) studied the effect of diets containing fish oil (control) or soybean oil on WHC in salmon after dry salting and cold-smoking. Neither diet influenced the WHC. However, the rigor status before secondary processing and temperature during cold-smoking affected the WHC. After vacuum storage, pre-rigor brine injected (25% brine (w/w)) fillets had a slightly higher exudate of 0.3% than those processed post-rigor (Birkeland & Akse, 2010). This was explained by the osmotic pressure that forces moisture out of the muscle during the vacuum packaging of pre-rigor fillets. Rørå and Regost (2003) studied the effect of WHC on smoking salmon packed in plastic bags from 5 to 40°C in a water bath or heating chamber. WHC was better for those cold-smoked below 30°C, but there was no difference between heating methods.

The degree of muscle swelling and WHC are dependent on factors like salting procedure, salt concentration and smoking conditions. Salt and smoking temperatures denature actin and myosin, as confirmed using differential scanning calorimetry (DSC) (Schubring, 2006). Myosin is typically sensitive and undergoes structural denaturation quickly during basic procedures such as processing involving salt. When fish is immersed in brines lower brine concentrations, a lower degree of protein denaturation occurs (Gallart-Jornet et al., 2007b). The Cl^- ions from salt weakly attach to the protein. These repulsive electrostatic forces cause the protein to entrap water and induce swelling of muscle fibres, thereby increasing WHC (Offer & Trinick, 1983; Thorarinsdottir et al., 2004). This is also known as the “salting in” effect and was observed by Chan et al. (2020a) on the immersion of whole salmon in refrigerated seawater (salinity 3.5%).

Better processing yields were obtained for brine and injection salting than dry salting of salmon fillets (Birkeland et al., 2004; Birkeland et al., 2003; Bjørnevik et al., 2018; Cardinal et al., 2001). Compared to injection salting, dry salting induces a lower WHC (Birkeland et al., 2004; Bjørnevik et al., 2018). Maximum swelling and maximum WHC is usually obtained at 1M (5.8% NaCl) (Fennema, 1990; Gallart-

Jornet et al., 2007b; Thorarinsdottir et al., 2004). Gallart-Jornet et al. (2007b) found that the weight of salmon fillets increased as brine concentration decreased and brine concentrations with <18% NaCl (w/w) decreased protein denaturation and increased WHC. The maximum weight increase was at 4% NaCl (w/w). When salt concentration increases (e.g. 25% NaCl (w/w) and dry salting), proteins denature and the myofibrils dehydrate, leading to muscle shrinkage, lower WHC and higher yield loss (Gallart-Jornet et al., 2007b; Thorarinsdottir et al., 2004).

A higher fat content gives greater resistance to salt uptake. The relevance of fat content and fillet shape on WHC of raw and cold-smoked fillets was studied by Mørkøre et al. (2001). A decrease in weight loss (i.e. greater yield) with increasing fat content was observed during the salting and smoking process, as less water is available for osmotic dehydration. The WHC in cold-smoked salmon was reduced as fat content increased, measured by centrifugation and expressed as water loss. A significant amount of the fat in the white muscle is found in the connective tissue surrounding the muscle fibres (Stien et al., 2007). Ofstad et al. (1993) explained that myofibres severely shrinks at 45°C, likely due to myosin denaturation. Therefore, this facilitates the fluid release and may explain the correlation between WHC and fat content.

4.1.1 Salt and smoke replacers

High consumption of NaCl is associated with hypertension and cardiovascular diseases. In Norway, a salt content of 3g NaCl/100g product for cold-smoked salmon is voluntarily encouraged by permitting the display of "The Keyhole" label on food packages, representing healthier products (Ministry of Health and Care Services, 2015). Alternatives have been introduced to replace NaCl, but the salt replacers should have similar functional properties and not compromise the overall sensory profile, safety, and quality of the food. KCl is considered a good substitute for NaCl based on its similar physical and chemical properties. The comparison of using 50% KCl/50% NaCl with 100% NaCl on vacuum packaged smoked salmon after 42 days of storage, using water vapour permeable bags during the salting-smoking process, showed no differences in weight loss nor the formation of exudates (Rizo et al., 2018). Lerfall (2011) studied the influence on quality using nitrite salt (99.4% NaCl, 0.6% $NaNO_2$) on cold-smoked salmon and found no

difference in weight loss compared to 100% NaCl. Nevertheless, the food industry remains skeptical about using KCl as a replacement due to the undesirable after-taste and possibility of health risks such as hyperkalemia (Cepanec et al., 2017). More research needs to be done to identify the quality changes with using salt replacers on smoked salmon.

The use of liquid smoke can be a healthier alternative than the traditional smoking method of using wood chips. It contains lesser amounts of polycyclic aromatic hydrocarbons (PAH), which are undesirable for human health. Birkeland and Skåra (2008) indicated no difference in DL between the application of smoke condensate or wood chips after vacuum packaged storage. Valø et al. (2020) used purified condensed smoke (PCS) and found that smoke from the atomization of PCS successfully inhibited microbial growth in salmon. Throughout storage, DL was significantly higher for PCS processed salmon.

4.2 Chilling

Temperature is a critical factor in food preservation, and this should be lowered as early as possible. The internal temperature of fish is usually aimed to be 0-2°C (Bantle et al., 2015). The most common method of fish chilling is by using ice, but other methods, such as superchilling and ice slurry, are also used. These various chilling methods could influence the amount of DL and WHC. However, industrial and laboratory chilling may vary due to the more significant variations and process differences with large scale industrial chilling.

4.2.1 Superchilling

Superchilling is a preservation method where the core temperature of the fish is lowered between conventional chilling and freezing (Banerjee & Maheswarappa, 2019). As Magnussen et al. (2008) described, superchilling is also defined as where a thin layer of ice forms on the fillet surface. This ice eventually absorbs heat from the internal reservoir to achieve equilibrium. The use of fish as a cooling medium eliminates the need for external ice, which usually takes up to 30% of space during transportation (Bahuaud et al., 2008; Magnussen et al., 2008). Extensive research has shown that superchilled Atlantic

salmon introduces several benefits, including reducing enzymatic reactions and microbiological growth, improving quality and extended shelf life compared to traditional chilling (Claussen et al., 2017; Duun, 2008; Kaale et al., 2011; Magnussen et al., 2008).

Determining the freezing time and temperature measurement during superchilling remains challenging (Banerjee & Maheswarappa, 2019; Magnussen et al., 2008). The freezing time, and thereby the amounts and distribution of the ice fraction, significantly affect the water holding properties and processing yield (Magnussen et al., 2008; Stevik et al., 2010). Kaale et al. (2013) studied the effect of cooling rates (153 and 227 W m^{-2} K^{-1}) and superchilling temperature (-20 and -30°C) on salmon fillets. A faster freezing rate (227 W m^{-2} K^{-1}) with a low temperature (-30°C) produced small crystals evenly distributed in and out of muscle cells and can reduce DL during thawing more than larger crystals formed at slow freezing rates. However, this advantage can be diminished during superchilled storage as the small crystals can collectively form large crystals. Consequently, cell membranes rupture and cell components are disrupted, leading to negative consequences for texture, DL and WHC (Bahuaud et al., 2008; Kaale et al., 2013). An earlier study by Duun and Rustad (2008) showed that salmon fillets stored superchilled at -1.4°C had a significantly higher DL (1.6%) than those at -3.6°C (0.3%). Nonetheless, a DL of <2% is considered low. The WHC, as measured by liquid loss, was similar for both groups and increased until 16 days of storage. Kaale et al. (2014) also stated that WHC increased with 21 days of superchilled storage at -1.7°C for salmon fillets, while a decrease in WHC was observed for chilled fillets at 4°C during the first 7 days, followed by an increase.

The optimal degree of superchilling was suggested to be freezing 5-30% of the free water (Kaale & Eikevik, 2014). This range of ice fractions was investigated on salmon by Stevik et al. (2010), where superchilled salmon with 30% ice level gave a consistently lower WHC than 10 and 15% ice levels and chilled samples stored at 0 and 2°C. Claussen et al. (2017) further found that superchilled storage of organic salmon at -1.5°C (with about 15% ice fraction) led to a slightly greater DL during the first 7 days than those chilled at 3°C, which may be due to damage from partial freezing. These differences disappeared afterwards.

Therefore, attention must be given to the temperature fluctuations and development of ice crystals within the muscle during the superchilling process.

A practical superchilling approach beneficial for storing large volumes of fish is using refrigerated seawater (RSW) tanks, which are often used with pelagic fish. Chan et al. (2020b) studied the effect of superchilling of salmon in RSW at sub-zero temperatures with a new slaughtering method in a fishing vessel against the conventional ice storage method. This concept slaughters fish by the sea cage immediately onboard the vessel, where fish are pumped, electrically stunned, bled and gutted. Then the gutted whole fish is superchilled in RSW tanks during transportation (Chan et al., 2020b). Fish stored in RSW and then on ice after filleting had the lowest DL, but those stored in RSW and then superchilled in liquid N_2 after filleting gave the lowest WHC. However, these differences disappeared after smoking. Another similar study showed that whole gutted salmon in RSW had a significantly better WHC than those on ice (Chan et al., 2020a). This difference also disappeared after filleting and cold-smoking. Immersing whole fish in RSW is a brining method that leads to weight gain from the concentration gradient differences. Chan et al. (2020a) and Chan et al. (2022) found an overall weight gain of 0.7 and 0.9%, respectively, for salmon stored in -1°C RSW for 4 days followed by 3 days on ice. Erikson et al. (2011) also showed that salmon stored in -2°C seawater (SW) slurry led to a weight gain of 6% at 11 days. On the other hand, storing fish for a day in slurry then 3 days on ice brought about a loss in weight, like traditional ice storage, yet the WHC was better than only storing on ice during the 4-day storage. This was likely because the fish were stored for only a day in slurry, so the observable differences were minor. Therefore, storage in RSW could be advantageous in water retention and may improve cook loss. The RSW tanks also provide a high heat transfer coefficient that allows the fish's internal temperature to cool to the desired temperature in a relatively shorter time.

4.3 Freezing and thawing

Freezing and frozen fish storage is a food preservation method that significantly prolongs the product's shelf life. However, biochemical reactions like myofibrillar protein denaturation may still occur, negatively affecting functional properties, including WHC, juiciness and texture. This leads to a dry texture, reduced quality, and impacts DL. In terms of water mobility, freezing can change the immobilized water in intracellular locations of muscle tissues into free water that can be easily lost as drip (Dawson et al., 2018). Like superchilling, the freezing rate also affects the sizes and uniformity of crystals formed at the intra- and extracellular muscle structures. Faster freezing rates are better at maintaining the physical and chemical attributes of products, as ice nucleation within the intracellular tissues forms smaller and more uniform ice crystals within the structure. Einen et al. (2002) studied the effect of freezing on both pre- and post-rigor fillets of Atlantic salmon. They observed that the frozen-thawed fillets had considerably higher DL than the unfrozen counterparts, and those post-rigor had the highest DL after 10 days of cold storage. Muscle fibre shrinkage and cell damage occur during freezing, especially at slow freezing rates. This led to an increase in DL, lowering fish quality.

Decreasing the frozen storage temperature from -22 to -40°C was found to greatly improve the quality and shelf life of salmon (Haugland, 2002). All free water is frozen at -40°C, so only bound water remains in the muscle, reducing water mobility and inhibiting biochemical reactions (Bøgh-Sørensen, 2006). Indergård et al. (2014) tested various quality parameters during long term frozen storage of salmon at -25, -45 and -60°C for up to 375 days. Storage at -60°C had the lowest DL of 2%, calculated by the weight difference between the raw material before frozen storage and after thawing. At ultra-low temperatures (< -45°C), the freezing rate increases due to the high heat transfer and low temperature (Wu et al., 2017). Therefore, smaller ice crystals may be formed within the tissue, preventing tissue damage and reducing DL during thawing. The study of Zhu et al. (2004) further showed that plate freezing of salmon at -38°C resulted in a lower DL than conventional air freezing. On the other hand, an ultra-rapid freezing process in liquid nitrogen (-195°C) had the highest DL, probably due to mechanical cracking.

The thawing method after frozen storage also greatly influences water holding properties. For example, thawing in heated air at 25°C led to a significantly higher DL than in a 5°C water bath regardless of storage duration and temperature (Haugland, 2002). Thawing should be done quickly to prevent water in the muscle from shifting its position, which leads to increased DL (Cai et al., 2019). A low temperature is also recommended to prevent the acceleration of microbial and enzymatic reactions. Various food thawing technologies can assist the thawing process, such as high pressure, ultrasound, high voltage electrostatic field and radiofrequency (Wu et al., 2017). Studies of ohmic heating of beef (Llave et al., 2018) and high pressure thawing of chicken breast (Li et al., 2014) showed a reduced thawing loss. So far, few studies have focused on the effect of innovative technologies on freezing and thawing salmon (Li et al., 2020). This introduces a knowledge gap for further research and process optimization.

4.4 Thermal processes

Various cooking methods commonly used in food production, including boiling, baking, frying, steaming, sous-vide and broiling, result in a change in quality attributes. Thermal processing applies time and temperature to inactivate microorganisms and enzymes, ensuring safe consumption of the product. Fresh fish can rapidly undergo chemical, biochemical and microbial processes. Hence thermal processing should take place before these processes deteriorate the quality (Skipnes, 2014). Since the chemical and morphological composition differs within the fish muscle, this can affect the cook loss. For example, Kong et al. (2007b) described that the cook loss with pink salmon was significantly lower from the middle section close to the dorsal fin than those closer to the head and tail. So, it can be assumed that location can affect Atlantic salmon as well. When comparing oven baking and pan-frying to an internal temperature of 45-63°C, Brookmire et al. (2013) reported that pressed juice for the oven-baked and pan-fried salmon was reduced to 27% at 55°C and 25% at 60°C, respectively.

During thermal processing, the shrinkage of myofibrillar proteins caused by protein denaturation and aggregation decreases the WHC and leads to a firmer and harder texture (Ofstad et al., 1993; Skipnes, 2014; Sun et al., 2018). Also, the lightness of the muscle increases while its distinct red color is lost. For Atlantic

salmon, proteins denature around 45, 65 and 78°C for myosin, sarcoplasmic protein and actin, respectively (Ofstad et al., 1996). Cook loss increases with temperature and storage time. The majority of cook loss occurs within the first few min and reaches a maximum at 50°C in salmon due to the denaturation of myosin. Above 50°C, DL is probably reduced because of sarcoplasmic protein aggregation (Ofstad et al., 1993). The rate of quality deterioration can be expressed using an integration of the kinetic equation $\frac{dC}{dt} = -k(C)^n$, where k is the rate constant, C is the quantitative indicator of a quality parameter at time t, and n is the reaction order (Kong et al., 2007a). Ovissipour et al. (2017) examined the cook loss and kinetics of protein denaturation during heat pasteurization of salmon from 55 to 95°C. They found that cook loss follows a first-order reaction, where most cook losses occurred during the first few mins in heating and eventually slowed down. Area shrinkage also occurs from the decrease in the sarcomere length which shrunk along with the muscle fibres.

Sous-vide is a cooking method popular with ready-to-eat foods. The product is sealed in vacuum pouches, treated at a controlled time and temperature, and then rapidly cooled. A mild temperature of 60-80°C for 20-40 min is recommended for fish (González-Fandos et al., 2005), but in reality, 40-60°C is often used for optimal texture and flavor (Głuchowski et al., 2019). Lerfall et al. (2018) combined different CO_2 treatments and microwave or conventional pasteurization (62°C/12 min). They found that DL was not affected by the pasteurization method. Salmon packaged with CO_2 emitters, that allowed CO_2 to be released after pasteurization, had the lowest DL compared to the control group (unexposed to CO_2) or those that underwent soluble gas stabilization (SGS). SGS is a technology that can improve shelf-life where CO_2 is driven into the flesh before pasteurization (Abel et al., 2019; Lerfall et al., 2018). Abel et al. (2019) found no correlation between DL and WHC with packaging technology when modified atmosphere (MA) and SGS packaged salmon fillets were compared after mild heat treatment. Głuchowski et al. (2019) compared the effect of sous-vide (57°C/20 min, 63°C/80 min) with roasting (180°C/23 min) and steaming (100°C/16 min) on salmon fillet portions. The highest (94%) and lowest yield obtained (84%) were salmon treated at 57°C/20 min and roasted, respectively. The salmon treated at 63°C/80 min had the best overall sensory

scores, which were the recommended conditions for sous-vide treatment without significantly affecting yield (91%).

4.5 Non-thermal treatments

Conventional food processing technologies often use thermal methods, but this could impact nutritional values, texture and freshness. High-pressure processing (HPP) is an innovative preservation technique that extends the microbiological shelf life of seafood without incorporating heat nor loss of the organoleptic and nutritional characteristics (Campus, 2010; Christensen et al., 2017; Yagiz et al., 2009). With HPP, the packaged product is placed inside a pressure vessel, water pressure (100-900MPa) is applied, and the adiabatic heating is ~3°C/100MPa (Aymerich et al., 2008). An advantage of this technology is that it is a mild process done at room temperature, eliminating the need for heat and subsequent cooling processes. Thus, this could be an alternative to conventional heating processes in preparing ready-to-eat food with minimal change in sensory attributes while inactivating microorganisms.

The effect of HPP on the quality parameters of raw, cold-smoked, or sous-vide treated salmon (Christensen et al., 2017; Lakshmanan et al., 2005; Lakshmanan et al., 2007; Ojagh et al., 2011; Yagiz et al., 2009) has been studied. The hydrostatic pressure is important to control to reduce the DL. Hedges and Goodband (2003) found that HPP in frozen cod fillets could selectively denature the structure of myosin molecules and was correlated to WHC. An application of a pressure of up to 100MPa before freezing seemed to reduce cook loss significantly. Simultaneously, structural denaturation of actin occurs at 200MPa, which impairs the myofibrillar structure and decreases the WHC. Lakshmanan et al. (2007) reported that HPP decreases the WHC of raw salmon regardless of processing time and pressure, while there was a 2% increase in WHC for cold-smoked salmon exposed to 150MPa for 10 mins. Increasing the pressure to higher levels also seemed to give a lighter product (Lakshmanan et al., 2005).

Similarly, Christensen et al. (2017) observed that the WHC of salmon fillets decreased when exposed to 200MPa, followed by storage for 18 days. The storage method, pressure and processing time are essential

factors to optimize for the HPP method. It is also important to avoid severe treatment as high pressure may cause gaping (Gudmundsson & Hafsteinsson, 2001). Nevertheless, there is a potential value to exploring this technique further.

Another alternative for non-thermal treatment in food is the use of pulsed electric field (PEF). This is a method where short electric pulses with a high electrical field strength are applied to food between two electrodes to induce cell electroporation (i.e., holes in the cell membrane), making it accessible for the next processing step. The application of PEF could enhance heat and mass transfer processes (Toepfl et al., 2014). As muscle cells are partially disrupted, absorption rates could be improved, and the concentration of common preservatives used in food like salt, nitrate and spices could be reduced (Gómez et al., 2019). Although PEF is being used in the food industry for various plant and meat-based products, there are currently only a few studies regarding the effect of PEF on the quality attributes of fish products. A recent study of PEF treatment on the freeze-thaw quality of Atlantic salmon showed that applying PEF decreased the thawing time with better preservation of muscle fibre, leading to a lower DL and better WHC (Li et al., 2020). Klonowski et al. (2006) also showed that fish muscle becomes more porous. There is a potential to use PEF technology to increase water uptake and water holding properties, but more research needs to be done. On the other hand, Gudmundsson and Hafsteinsson (2001) found that mild PEF treatment is unsuitable for preservation as it impacted the microstructure and texture and induced gaping. Therefore, it is important to consider factors that might affect quality, such as electric potential and pulse duration.

4.6 Packaging

Various modern packaging technologies such as gas packaging, traditional vacuum and vacuum skin packaging are available to prolong the shelf life of fish. A comparison of water holding properties by Chan et al. (2021b) showed that salmon fillet portions kept in refrigerated storage in modified atmospheric packaging (MAP) with 60% CO_2:40% N_2 had similar DL, WHC and microbial shelf life duration as with vacuum skin packaging. Interestingly, vacuum skin packaged salmon produced a significantly greater DL yet similar WHC than traditional vacuum packaged fillets (Chan et al., 2021a).

The effect of combining superchilling and MAP extends the shelf life of salmon (Fernández et al., 2009; Hansen et al., 2009; Sivertsvik et al., 2003). Sivertsvik et al. (2003) found that DL was about the same in fillets stored in MAP (60% CO_2:40% N_2), either superchilled at -2°C or chilled at 4°C. On the other hand, Hansen et al. (2009) found DL for fillets superchilled in a freezing tunnel, then packaged in MAP (60% CO_2:40% N_2) and stored at 0.1°C, was significantly higher than the chilled samples. The differences observed from both studies could be attributed to the different storage temperatures. Therefore, the synergistic effect of superchilling combined with MAP can increase shelf life, but this method needs to be optimized to minimize DL. Rotabakk and Skuland (2017) established that portion size, freezing regime and packaging method influenced DL of cold-smoked salmon after freezing and thawing. Sliced salmon in vacuum packaging produced more drip than whole fillets since muscle integrity is disintegrated and more surface area is exposed. Freezing in bulk also increased DL as the freezing time is lengthened. Smoked fillets in MAP also had a significantly lower amount of DL after thawing than those vacuum packaged, as explained by the cushioning effect of the headspace gas. However, during thawed storage, DL in MAP fillets was significantly higher.

An additional step that can be introduced before packaging in MAP is SGS. As mentioned, SGS is a process that adds CO_2 into the product. This allows CO_2 to dissolve into the product before packaging and prevents package collapse (Abel et al., 2019). Hurdle technology explains how several combinations of fish preservation and packaging methods can ensure good quality and extended shelf life (Leistner, 2000). Since SGS has only been implemented on a laboratory scale, following this with a scale-up would be interesting. In addition, future research may consider combining this technology with other packaging and processing methods like vacuum packaging and HPP to observe how the quality would be affected.

Hyperbaric storage of food products has been attracting interest in the food preservation field. This method stores the product above atmospheric pressure at moderate pressures (<100MPa) and gives a better shelf life and comparable quality to conventional refrigeration (Fidalgo et al., 2020). Fidalgo et al. (2019) did a study that optimized the conditions using different pressures and storage temperatures for the shelf-life

extension of Atlantic salmon. The optimal condition was found to be 60MPa/10°C. Following up, Fidalgo et al. (2020) found that DL was relatively stable with 3-4% for hyperbaric storage/low temperature (HS/LT 60MPa/10°C) of vacuum packaged salmon throughout 30 days of storage. On the other hand, those stored under normal atmospheric pressure (0.1MPa) at 5 and 10°C gave a DL of 7% at 30 days and 15 days of storage, respectively. WHC decreased after the first 5 days before increasing after 30 days for all samples. This latter increase in WHC was probably due to the remaining water being tightly retained within the muscle. The latest study with 75MPa/25°C at room temperature showed that DL gradually increased for 30 days until 13% and was consistently higher than those at 0.1MPa/5°C, reaching 7% (Fidalgo et al., 2021). 0.1MPa/25°C had the highest DL after 5 days at 10%. This suggests that temperature could be a critical factor in quantifying DL, although several advantages have been suggested using hyperbaric storage, such as better energy efficiency.

The usage of water vapour permeable (WP) bags was introduced to reduce the processing steps during salting and smoking (Rizo et al., 2015). Salmon portions were sprayed with liquid smoke with a specified salt dosage, then packed in WP bags for 24 h in a cold room with fixed relative humidity. These bags allowed drying simultaneously with salting and smoking and gave similar sensory quality to the commercial smoked salmon. Weight loss was higher for salmon in WP than high barrier vacuum bags due to the higher dehydration rate (Rizo et al., 2015). The use of these bags presents interesting opportunities to reduce processing steps and brine wastes produced during salting. More studies could be done to optimize the conditions and possibilities to reduce weight loss.

5. Modelling water holding properties

Quality measurements can often be costly and labour intensive. Mathematical modelling has been gaining popularity and can serve as an alternative for many purposes in the food industry, reducing experimental needs. Adequate validations must be done to check the accuracy of the model. Numerical models have

been proposed based on the first principles of heat and mass transfer that studies the salting kinetics of salmon to predict state variables as a function of time and space (Martínez-López et al., 2019; Wang et al., 1998; Wang et al., 2000). Empirical models, such as Peleg's or Zugarramurdi and Lupin's models, can also be applied to predict salt and water concentrations based on mathematical equations obtained from experimental data. Besides, reaction rates for quality degradation of physical properties, like colour and texture, during thermal processing can be expressed using kinetic order equations (Kong et al., 2007a; Ovissipour et al., 2017).

Modelling WHC in Atlantic salmon during raw fillet storage has not been extensively studied. This is possibly due to the high variations and different methods that are available to measure WHC. Predicting WHC can be possible during thermal processing through the correlation of other related quality parameters. Multivariate analysis has shown that WHC is highly correlated to heating temperature, pH, heating time and salt, in decreasing order (Varmbo et al., 2000). Heat treatment causes more destructive changes to the muscle structure, thereby affecting WHC, as shown by Ofstad et al. (1995). When the salmon muscle is heat-treated, a rapid water loss (i.e. WHC decreases) is observed at temperatures >30°C. With the reduction in myofibrillar space, a transverse shrinkage occurred between 45 and 50°C. This eventually leads to protein denaturation and more water loss. Between 60 and 70°C, WHC slightly increased, probably due to protein aggregation that holds water (Varmbo et al., 2000).

The numerical modelling of WHC as a function of temperature has been formulated by van der Sman (2007) as follows:

$$C_{eq}(T) = C_{eq,0} - \frac{a_1}{1+a_2 e^{(-a_3(T-T_\sigma))}} \quad (6)$$

where $C_{eq,0}$ is the initial WHC of the sample, T is the temperature in °C, T_σ is the centre of the sigmoid curve, and a_1, a_2 and a_3 are fitting parameters. This model has yet to be used on salmon. The model was used by Blikra et al. (2019) with farmed cod after cooking from 0 to 100°C. It was found that WHC follows a negative sigmoidal curve when the sample is heated, inducing a pressure gradient inside the muscle that

led to an expulsion of water. The decrease in WHC from 25 to 40°C to a minimum value signifies the loss of free water during heating. Heating further from 40 to 90°C gave no differences in WHC.

DL and WHC can have a high degree of uncertainly as affected by several post-mortem and processing conditions. For this reason, it can be challenging to create a one-size-fits-all model with minor errors. Vibrational spectroscopy methods like NIR have been used for early prediction of WHC in fresh pork (Forrest et al., 2000). A study to predict WHC using Raman spectroscopy in pork was a promising technique compared to NIR or fluorescence spectroscopy (Andersen et al., 2020; Andersen et al., 2018). The broad range of this application makes it a suitable method to characterize the macro-components of food, including carbohydrates, protein, fat and water (Li-Chan, 1996). Using multivariate analysis, Pedersen et al. (2003) reported a good correlation between DL and WHC and the Raman spectra. The spectral regions between 951-876 and 3128-3071 cm^{-1} can provide information about WHC, where 940 cm^{-1} is assigned to the peptide α-helix conformation. The latter region is attributed to the N-H stretching band of the amide group in the protein structure, which provides details on proteolysis and protein denaturation. However, as spectroscopy methods measure the potential DL formation, the real DL obtained may differ from the predictions (Andersen et al., 2018). The possibility of using this non-invasive technique in predicting water holding properties calls for more studies to be done and its applicability in industrial settings.

6. Conclusion

Water holding properties (WHC and DL) are essential attributes that can influence the entire value chain, from whole fish to filleting to further processing and storage. A common challenge for the fish industry in maintaining food quality is to obtain a low DL and good WHC, in other words, a high amount of immobilized water in the muscle. Various methods are available to measure WHC, and the demand for a rapid and low-cost approach introduces non-invasive techniques. Nevertheless, it must be noted that results obtained from various measurements are relative and should be compared with studies including many of

the same technical details and calculation methods. Several methods, including pre- and post-slaughter conditions, and processing and preservation technology combinations, can extend the product's shelf life and improve water holding properties. In addition, innovative technologies might be introduced and determining their potential needs more research to optimize the parameters in maximizing water holding properties without compromising quality.

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Conflict of interest

The authors declare no conflict of interest.

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Figure 1: A summary of various methods used in measuring water holding capacity (WHC) in Atlantic salmon. “Conventional” represents methods involving force where WHC is calculated from the water loss. “Non-invasive” represent methods that are rapid and non-invasive. LF-NMR, MRI, and NIR represent low field-nuclear magnetic resonance, magnetic resonance imaging, and near-infrared spectroscopy, respectively.

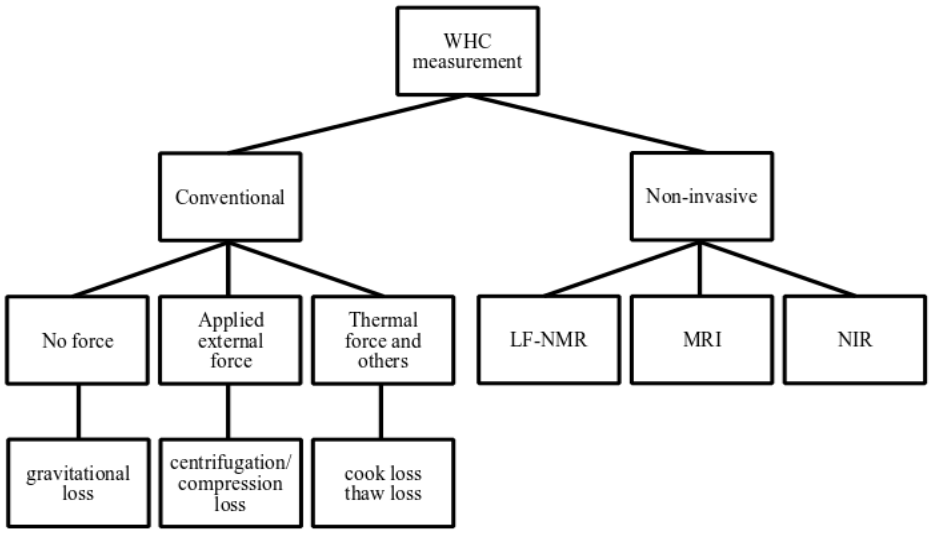

Figure 2: An overview of pre- and post-mortem factors reported affecting drip loss (DL) and water holding capacity (WHC) of Atlantic salmon.

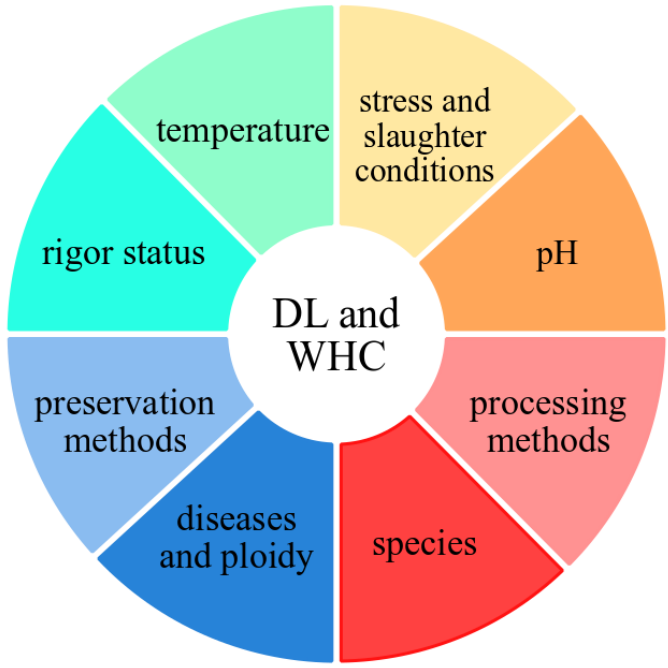

Table 1: Selected literatures on centrifugation parameters and calculation methods for measuring water holding capacity (WHC) of raw Atlantic salmon.

Salmon storage	Fillets storage conditions	Centrifugation Parameters	Calculation method[1]	Reference
Raw	iced storage, 3d	210g, 5min	Equation 1	Aursand et al. (2010)
	superchilling in seawater slurry (-1.9°C) or iced, 11d	230g, 5min	Equation 1	Erikson et al. (2011)
	iced storage, 11d	210g, 5min	Equation 1	Hultmann and Rustad (2002)
	superchilled storage (-1.4 or -3.6°C), 34d	210g, 5min	Equation 1	Duun and Rustad (2008)
	superchilled storage (-1.7°C), 28d	270g, 5min	Equation 1	Kaale et al. (2014)
	1d	1500g, 5min, 10°C	Equation 1	Løje et al. (2017)
	iced storage, 4d	500g, 10min, 10°C	Equation 1	Rørå et al. (2003)
	iced storage, 22d	530g, 15min, 4°C	Equation 2	Chan et al. (2020a)
	superchilled in N_2 (-1°C) or iced storage, 23d	530g, 15min, 4°C	Equation 2	Chan et al. (2020b)
	vacuum skin vs modified atmospheric packaging (60% CO_2:40% N_2), 4°C, 20d	530g, 15min, 4°C	Equation 2	Chan et al. (2021b)
	vacuum skin vs traditional vacuum packaging, 4°C, 20d	530g, 15min, 4°C	Equation 2	Chan et al. (2021a)

	vacuum storage, 60MPa/10°C, 30d	530g, 15min, 4°C	Equation 2	Fidalgo et al. (2020)
	iced storage, 19d	530g, 15min, 4°C	Equation 2	Lerfall et al. (2015)
	iced storage, 14d	530g, 15min, 4°C	Equation 2	Lerfall and Rotabakk (2016)
	iced storage, 18d	530g, 15min, 4°C	Equation 2	Rotabakk et al. (2017)

[1] Equation 1 and 2 are different calculations of WHC based on the centrifugation method. Equation 1 calculates WHC from the liquid lost after centrifugation relative to the initial sample weight, while Equation 2 includes the water content of the initial sample (Skipnes et al., 2007).

Table 2: Research overview on obtained drip loss (DL) and water holding capacity (WHC) from standard salting procedures (dry, injection, brine) combined with cold-smoking of Atlantic salmon.

Salting and smoking method	Process parameters	Storage conditions	Days post-mortem	DL after smoking	WHC condition	WHC calculation	WHC after storage	Conclusion	Reference
Dry salting and cold-smoking	99.8% NaCl, 68% humidity, smoked at 23°C	vacuum packaged and put in ice	4 and 5	-12%	500g, 10min, 10°C	Equation 1	95%	Compared to injection salting, dry salting had a lower yield.	Birkeland et al. (2004)
	pre- vs post rigor, 5 or 12h salting, 2.5 vs 4% final NaCl content, smoked at 15 vs 25°C	vacuum packaged stored for 6 weeks, 4°C	pre: 0 post: 5	-7%	-	-	-	A 2.5% NaCl target gave 1% better yield than 4% NaCl.	Bjørnevik et al. (2018)
	fresh vs frozen fish, refined salt, smoked at 65% (20°C) or 50% (30°C) humidity	vacuum packaged, 2°C	7	-7 to -9%	-	-	-	Freezing influences DL smoked at 20°C, but not 30°C. Drying at 20°C gives a greater DL than 30°C.	Cardinal et al. (2001)

	ice vs RSW stored fish, ice vs superchilled fillets, salted at 99.8% NaCl, 75% humidity, smoked at 22°C	vacuum packaged until 31 d post-mortem, 4°C	9 and 10	-7 to -8%	530g, 15min, 4°C	Equation 2	84 to 87%	Whole fish stored in RSW then ice storage after filleting had the least DL when kept raw (1.5%), but was insignificant to other groups after smoking.	Chan et al. (2020b)
	ice vs RSW stored fish, salted at 99.8% NaCl, 75% humidity, smoked at 22°C	vacuum packaged until 29 d post-mortem, 4°C	7 and 8	-7%	530g, 15min, 4°C	Equation 2	85 to 88%	No difference in DL was observed after smoking.	Chan et al. (2020a)
	sea caged vs land-based diploid and triploid salmon, starved vs not starved, salted at pure refined NaCl, 65% humidity smoked at 20°C	vacuum packaged, stored at frozen storage at -80°C	-	-	4000g, 10min, rt	Equation 1	99.8 to 100%	WHC increased after smoking due to the added salt. There were no differences between starvation nor stress.	Gomez-Guillen et al. (2000)
	salted at 99.8% NaCl, 68-73% humidity varying smoking temperatures from 20°C to 30°C	vacuum packaged until day 16, 0-4°C	5	-	500g, 10min, 10°C	Equation 1	96 to 97%	No difference was seen on WHC at varying smoking temperatures.	Hultmann et al. (2004)

	muscle temperature upon filleting at 2, 9 and 14°C, salted in 99.8% NaCl, smoked at 22°C	vacuum packaged and stored for 28 d, 5°C	6 and 7	-10%	-	Equation 2	-	DL was not affected by the muscle temperature upon filleting.	Lerfall and Rotabakk (2016)
	small vs large salmon size, salted (60g salt/kg fillet), 75% humidity, smoked at 26°C	vacuum packaged and stored for 20 d, 2°C	-	-	1500g, 5min, 10°C	Equation 1, Equation 5	81 to 94%, 2.0-2.6%	WHC decreasing during storage and is related to lipid loss.	Løje (2007)
	varying diets, salted (70:30 salt/sugar), smoked at 22°C	stored for 5d or 15d, 4°C or 14°C	4	-12 to -13%	500g, 10min, 10°C	Equation 1	96-99%	WHC was not influenced by dietary oil.	Rørå et al. (2003)
Injection salting and cold-smoking	test on various parameter settings, 20 and 26% brine (w/w), 70% humidity, smoked at 23°C	vacuum packaged, 4°C	1 and 2	+5%	-	-	-	Constant injections and increasing brine injection pressure gave better yields.	Birkeland et al. (2003)
	25% (w/w) brine, 68% humidity, smoked at 23°C	vacuum packaged and put on ice	4 and 5	+4%	500g, 10min, 10°C	Equation 1	96%	WHC were similar for both dry salted and injection salted salmon.	Birkeland et al. (2004)

	pre- vs post rigor processing. 25% (w/w) brine, 45% humidity , smoked at 27°C	vacuum packaged and stored for 14 d, 2-4°C	pre: 0 and 1 post: 4 and 5	-2 to -3%	-	-	-	No difference in processing yield was observed from pre-or post rigor injection salted fillets.	Birkeland and Akse (2010)
	pre- vs post rigor 2.5 vs 4% final NaCl content, 160 or 300g/l brine, smoked at 15 vs 25°C	vacuum packaged and stored for 6 weeks, 4°C	pre: 0 post: 5	+6 to +7%	-	-	-	Injection salting gave a 14-15% better yield than dry salting.	Bjørnevik et al. (2018)
Brine salting and cold-smoking	saturated brine (50% w/v), smoked at 65% (20°C) or 50% (30°C) humidity	vacuum packaged, 2°C	7	-5 to -6 %	-	-	-	There was a better yield using brine salting than dry salting.	Cardinal et al. (2001)
	saturated brine (360g/l), 65% humidity, smoked at 20 vs 30°C	vacuum packaged, 2°C	6	-5 to -6%	-	-	-	There was a better yield for smoking at 30°C than 20°C.	Sigurgisladottir et al. (2000)

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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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ABSTRACT

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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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

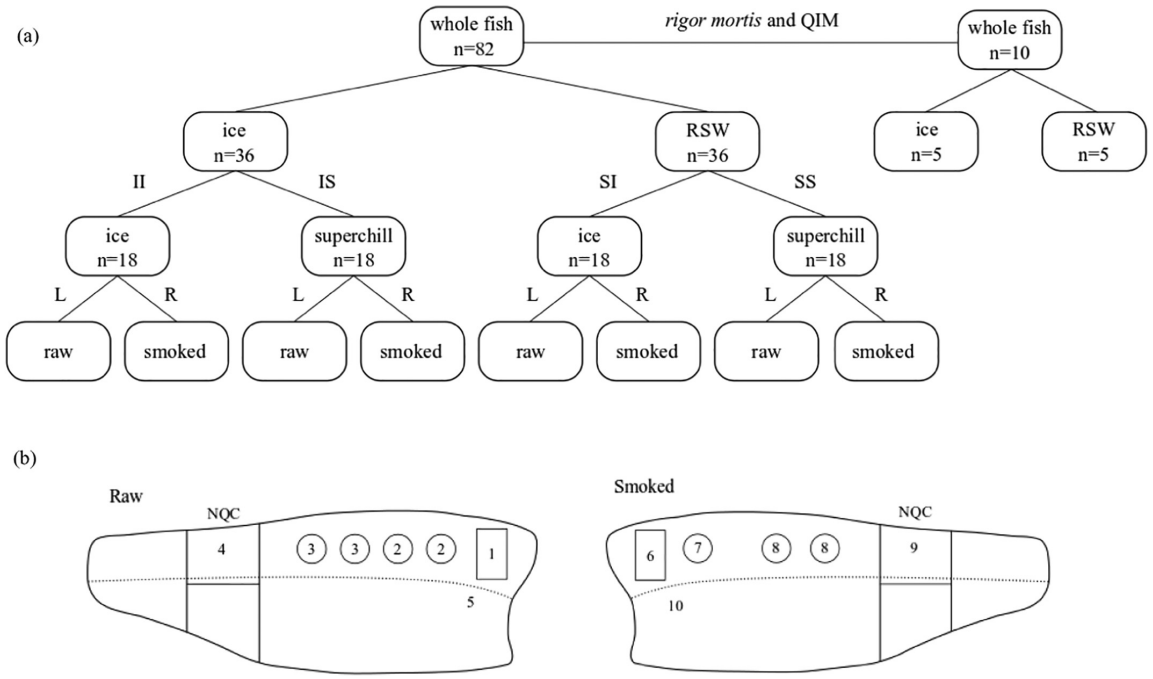

Fig. 1. (a) Experimental overview. 10 fish were used for *rigor mortis* and quality index measurements (QIM). II and IS represents whole fish on ice and stored in ice or superchilled after filleting respectively; SI and SS represents whole fish in RSW and stored in ice or superchilled after filleting 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

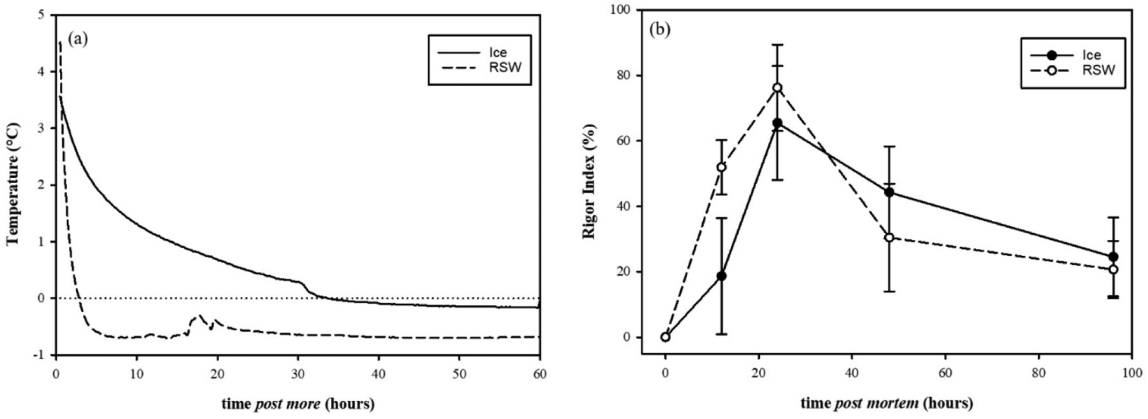

Fig. 2. (a) Temperature change and (b) rigor index of whole fish in ice and RSW (GLM, $p = .784$) over time.

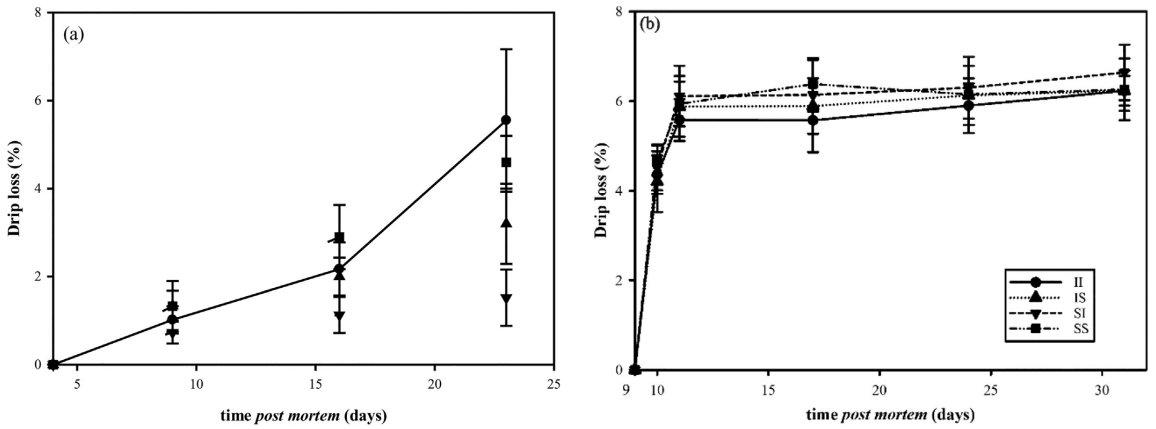

Fig. 3. (a) Drip loss of raw fillets after filleting on day 4 (GLM; storage days: $p < .001$; whole fish: $p = .039$; fillets: $p < .001$); and (b) smoked fillets processed at day 9 as a function of time after processing (GLM; storage days: $p < .001$; whole fish: $p = .002$; fillets: $p = .740$).

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.

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A comparative study of Atlantic salmon chilled in refrigerated seawater versus on ice: from whole fish to cold-smoked fillets

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Water and salt uptake, and water holding capacity (WHC) of whole gutted Atlantic salmon superchilled at sub-zero temperatures in refrigerated seawater (RSW) were compared to traditional ice storage. Following the entire value chain, the whole salmon was further processed, and fillets were either chilled on ice or dry salted and cold-smoked. Changes in quality parameters including colour, texture, enzyme activity and microbial counts were also analyzed for 3 weeks. Our results showed that when fish were removed from the RSW tank after 4 days and further chilled for 3 days, an overall weight gain of 0.7%, salt uptake of 0.3% and higher WHC were observed. In contrast, ice-stored fish had a total weight loss of 1% and steady salt uptake of 0.1%. After filleting, raw fillets from whole fish initially immersed in RSW had better gaping occurrence, softer texture, lower cathepsin B + L activity but higher microbiological growth. Otherwise, there were no differences in drip loss nor colour (L*a*b*) on both raw and smoked fillets from RSW and iced fish. Storage duration significantly affected quality parameters including drip loss, colour, texture, enzyme activity and microbial counts in raw fillets and drip loss, WHC, redness and yellowness in smoked fillets.

Water accounts for 60–70% of total weight in Atlantic salmon (*Salmo salar*). The ability to retain water, known as water holding capacity (WHC), is regarded as one of the most important parameters in preserving fish quality. Most free water that can be easily released lies between the actin and myosin filaments of myofibrils in living or pre-rigor muscles. This water, lost as drip during postmortem storage, is also known as drip loss. Both WHC and drip loss can affect surface appearance and texture, thereby the sensory quality of food[1]. A low WHC is related to postmortem changes in the muscle such as myofibril shrinkage, and a high drip loss is usually related to greater protein denaturation[1,2]. These are undesirable as they lead to greater water and nutrients loss, and directly result in lower salmon quality and sale value. Therefore, maintaining a high WHC and low drip loss is a common aim for fish producers.

Superchilling is a food preservation method where the temperature of fish is kept between traditional chilling and freezing[3]. This slows down autolytic biochemical processes in the muscle and inhibits microbial spoilage, hence prolonging shelf life[4]. One way to achieve superchilling is by storing fish in refrigerated seawater (RSW), and the practice of storing fish under chilled conditions in RSW tanks has been widely used in well-boat industries to store bulk catches of live fish. These tanks are holding systems where the chilling medium of either RSW or brine of the same salinity as seawater (3.5%) is continuously pumped through mechanical chillers.

An unprecedented fish slaughter method has recently been introduced in the aquaculture industry, whereby fish slaughter is directly performed onboard fishing vessels at sea after the fish is pumped, gutted and bled then immediately superchilled at temperatures below 0 °C in RSW tanks during transportation[5]. This novel method condenses the traditional three-stage handling process, where fish are pumped into well-boats and transported to land before processing, into only one. Implementing fish slaughter directly on the vessel eliminates the need for storage and transportation of ice, thereby reducing costs. Therefore, applying the synergistic concept of superchilling and storage in RSW tanks potentially provides an environmentally friendly alternative to traditional chilling on ice, while maintaining a constant low internal fish temperature of below 0 °C at the beginning

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of the value chain. Since seawater has a higher heat transfer coefficient than ice, it removes heat at a faster rate and maximizes the contact between seawater and fish. Immersing fish in seawater thereby gives a comparative advantage over ice storage, as ice storage can be subjected to delays due to the manual labor involved. In addition, RSW preserves meat freshness by allowing gutted fish to bleed adequately and avoids blood retention in the flesh[6].

Although the storage life of whole fish in RSW is longer than on ice[7], this could be limited by water and salt uptake[8]. Usually, a 0.5–1% salt uptake is observed in fish stored in RSW[9]. This uptake may limit the performance of RSW systems and fish might acquire a salty taste and become distasteful, affecting further processing and marketing. The amount of salt absorbed depends on various factors such as size, species, lipid content, physiological state, storage time, temperature and salt content of RSW[10]. Nevertheless, only limited recent studies have been conducted on water and salt uptake of whole salmon in RSW[7,11,12]. A 1985 study on dress chinook salmon by Bronstein et al.[7] suggests that salmon can only be kept in RSW for less than 4 days and that chilled water systems at 0 °C appears to be more advantageous in terms of microbial quality, but their weight changes were more observable and sensory quality lower than iced fish. These findings are also similar to a 1994 study on pink salmon by Himelbloom et al.[11].

Despite existing commercial practices of storing live fish in chilled RSW tanks, there are only few studies which examine the new concept of superchilling gutted fish from direct cage slaughter, and how superchilling in RSW affects the water holding properties and other quality parameters of Atlantic salmon throughout the whole value chain. Since this is a new field, there is potential for this method to be widely adapted but there is currently a knowledge gap with little scientific research focusing on the quality of fish using this slaughter method. Therefore, the main objective of this study is to evaluate superchilling in RSW by comparing it to traditional storage on ice of Atlantic salmon. This was done by monitoring water and salt uptake, water holding properties and other quality parameters (colour, texture, microbiology, enzyme activity) from whole salmon to fillets, and further to processed cold-smoked fillets. A small scale RSW tank was constructed in the laboratory to mimic this condition.

Materials and methods

Experimental design. On 13th of May 2019, 77 Atlantic salmon (*S. salar*) were obtained from a fish slaughtering facility from the west coast of Norway. Fish were pumped, electrically stunned, automatically slaughtered, gutted and thoroughly bled (temperature: 3.9 ± 1.1 °C, weight: 4.5 ± 0.9 kg, condition factor: 1.4 ± 0.2). An 800-L polyethylene fish chilling tank containing RSW (− 0.60 to − 0.88 °C) was also obtained from the same facility.

A full factorial experiment was designed (Fig. 1a). Fish were individually tagged and weighed before placing them in either expanded polystyrene (EPS) boxes containing wet ice (n = 30) or in the tank containing RSW (n = 30) prior to a 2 h transport to Nofima AS, Stavanger. 17 fish were used as control, where they were wrapped in plastic then chilled in boxes containing ice, but without any contact with ice. Within this control group, the left fillets of 10 fish were used to determine the initial WHC after slaughter. These fish were then stored before sampling the right side for WHC on days 2 (n = 5) and 4 (n = 5). The remaining 7 fish were filleted on day 7, where left fillets were kept as raw and right fillets were dry salted and cold-smoked.

For each treatment group (ice, RSW, control), two TrackSense Pro temperature loggers (Ellab A/S, Denmark) were inserted in 2 random fish in the mid-abdomen towards the lower back. pH was also measured upon arrival at the laboratory using a Mettler Toledo SevenGo pro pH meter (Mettler Toledo Inc, USA). The tank and EPS boxes were stored in a 0 °C cooling room. Temperature in the tank was constantly monitored and maintained below 0 °C by periodically adding pre-prepared frozen seawater obtained from the International Research Institute of Stavanger (sand filtered, salinity ~ 3.5%). Weights of fish from all groups were recorded on days 2 and 4 in addition to sampling 6 fish from iced and RSW group for WHC, colour and texture analysis. The RSW tank was drained completely on day 4, and fish were gently dried and stored in EPS boxes without ice at 0 °C.

Processing. On day 7, fish were weighed before being mechanically filleted using a Carnitec fillet machine (Carnitec AS, Støvring, Denmark). The left fillets were stored at 0 °C and weekly sampling was done for quality analysis on 6 fillets from the ice and RSW group (Fig. 1b, t = 7, 15, 22 days postmortem), respectively. On the same day, right fillets were randomized on grids and dry salted with refined salt (GC Rieber, Norway) for 18 h, 0 °C. These fillets were rinsed briefly with cold tap water and gently dried with tissue paper before recording the weight, then cold-smoked using the wood chips protocol of Birkeland and Skåra[13]. The fillets were dried in the smoking chamber for 1 h before smoking and drying 5 consecutive times at alternating intervals of 45 min and 15 min at 22 °C, 75% humidity. They were then cooled, and colour measurements were done on all smoked fillets before vacuum packaging at 99% vacuum and stored at 4 °C. Quality analysis was also done weekly on 10 fillets from the ice and RSW group (t = 16, 23, 29 days postmortem). As for the control group, quality analysis was done on the last sampling day for both raw and smoked fillets (n = 7). Mini temperature loggers (Ellab A/S, Denmark) were placed in boxes containing the fillets from different treatments throughout storage.

Quality analysis. *Weight change and water holding capacity.* For drip loss calculation, whole fish from all groups were weighed on days 0, 2, 4 and 7. Raw fillets from the ice and RSW group were periodically weighed on t = 7, 15, 22 days while smoked fillets on t = 7, 8, 9, 16, 23, 29 days. Fillets from the control group were weighed on the last sampling day. Drip loss (%) was calculated using the formula $((m_0 - m_t)/m_0) \times 100$, where m_0 is the initial weight (g) and m_t the weight (g) on the sampling day t.

Water holding capacity (WHC) and water content (WC) were calculated involving an applied force described by Skipnes et al.[14]. Samples were measured in triplicates (diameter 31 mm, height 6 mm) from the dorsal back above the lateral line on the white muscle tissue during sampling days of whole and processed fish (Fig. 1b).

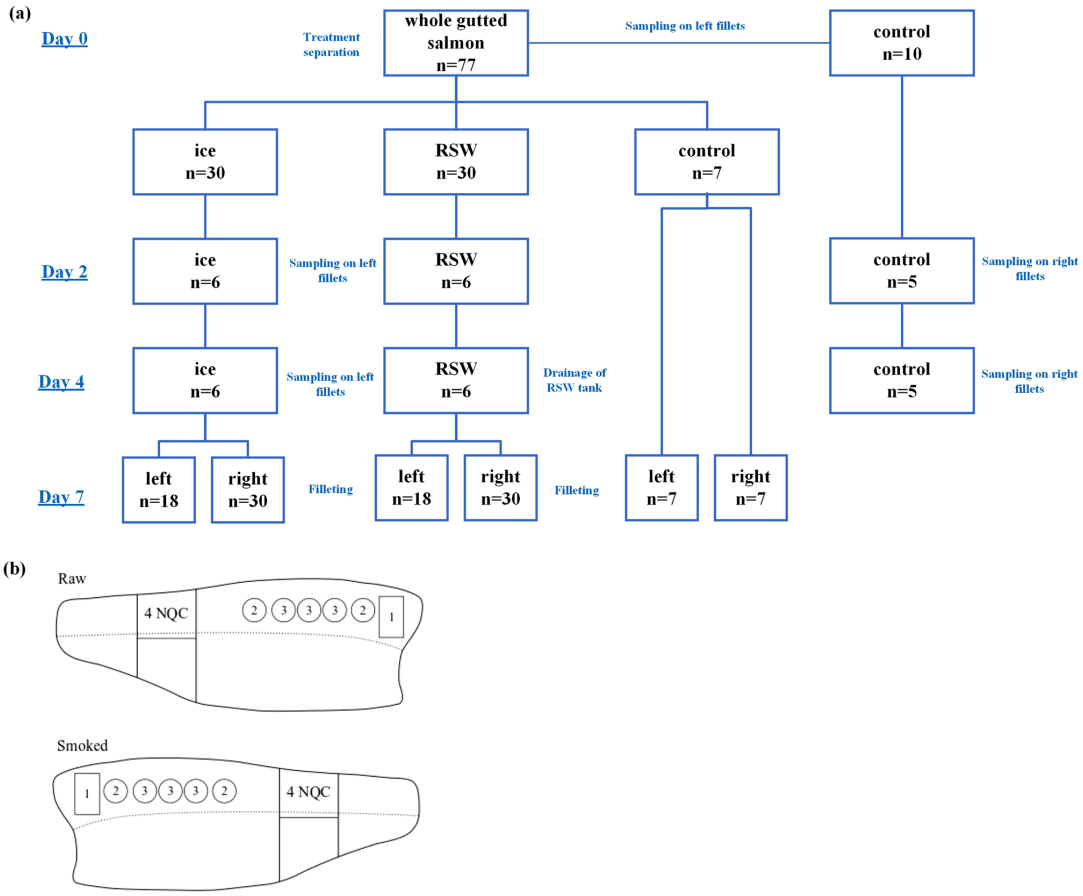

Figure 1. (**a**) Experimental overview, including sample size during storage days. "Control" represents fish wrapped in plastic surrounded by ice, where 10 fish were used to measure WHC on the left fillets from whole fish at t = 0 after slaughter. Thereafter, these fish were kept in a cold room and sampling of WHC was done on the right fillets on days 2 and 4 postmortem. "Ice" represents whole fish stored on ice, "RSW" represents fish stored in refrigerated seawater. "Left" and "right" represents left and right fillets respectively. On day 7 after filleting, left fillets were kept as raw while right fillets were dry salted and cold-smoked. The ice and RSW fillets were sampled periodically for 3 weeks, while control fillets were sampled on the last sampling week. (**b**) Schematic illustration showing weekly sampling locations (n = 6 for raw fillets, n = 10 for smoked fillets). Control fillets were sampled on the last sampling day (n = 7 for raw fillets, n = 7 for smoked fillets). (1) microbiology analysis, (2) frozen samples for enzyme and salt analysis, (3) Water holding capacity and water content, (4) Norwegian Quality Cut (NQC) for texture analysis.

Enzyme analysis. Approximately 1 g of frozen sample collected from raw fillets was added in a ratio of 1:5 into the solvent of 0.25 M sucrose solution containing 1 mM EDTA (Sigma-Aldrich, Norway) and 100 mM NaCl (Sigma-Aldrich, Norway) in phosphate buffer (3.38 mM Na_2HPO_4 (Merck, Germany), 15 mM NaH_2PO_4 (Merck, Germany), pH 7.5). The mixture was homogenized using an Ultra Turrax T25 (Janke and Kunkel IKA, Labortechnik, Staufen, Germany) at 13,500 rpm, 40 s, 4 °C. The homogenates were centrifuged, and supernatants collected for cathepsin B + L analysis using the method of Kirschke et al.[15]. Cathepsin B + L activity was measured in replicates fluorometrically at excitation and emission wavelengths 360 nm and 460 nm respectively, via the release of 7-amino-4-methylcoumarin from substrate Z-Phe-Arg-Nmec.

Flesh quality analysis. The extent of fillet gaping was visually recorded according to Andersen[16] on both raw and smoked fillets on a scale from 0 to 5, where 0 and 5 means no and severe gaping, respectively. For days 2 and 4, right fillets from whole fish used for sampling were manually filleted for colour and texture measurements. Colour analysis was implemented using computer vision with a digital colour measurement system (DigiEye full system, VeriVide Ltd, UK) connected to a SLR (Nikon D80, 35 mm lens, Nikon Corp., Japan). The fillets were placed in a lightbox (daylight, 6400 K) and the images taken were analyzed using the Digipix software (www.

verivide.com, version 2.8, VeriVide Ltd., UK) to measure L*a*b* values, where L* represents lightness (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)[17]. Colour analysis was also carried out on gills after filleting on day 7.

Texture analysis was measured by a puncture test using a Texture Analyzer TA-XT plus (Stable Micro Systems, UK) equipped with a 12.7 mm flat end cylindrical plunger to poke three consecutive punctures of each muscle sample above the mid-line of the Norwegian Quality Cut (NQC, Fig. 1b). The force (N) was recorded in a texture profile curve operated by the Texture Exponent light software (www.stablemicrosystems.com/, Stable Micro Systems) with a 5 kg load cell at a rate of 2 mm s^{-1} until the probe reached 80% of the fillet height.

Salt content. Salt content (% NaCl) was determined with a titration method using SI Analytics Titroline 7000 connected to the software TitriSoft 3.15 and an AgCl 62 electrode (www.xylemanalytics.com/en/, Xylem Analytics, Norway). The solvent and titration agent used were distilled water and 0.1 mol l^{-1} $AgNO_3$ (VWR International, Norway) respectively. 100 ml of warm deionized water was added to approximately 2 g of sample. The mixture was then homogenized using an Ultra Turrax T25 (Janke & Kunkel IKA, Labortechnik, Staufen, Germany) at 13,500 rpm for 40 s before adding 1 ml HNO_3 (VWR International, Norway). Automatic titration was performed, and titration stops at the equivalence point where AgCl is formed. Salt content is calculated using the formula (Eq-B)*T*M*F1/W, where Eq is the volume (ml) of $AgNO_3$ consumed at equivalence point; B = 0, blank value; T = 0.1 mol l^{-1}, the concentration of titrant; M = 58.44 g mol^{-1}, molecular weight of NaCl; F1 = 0.1, conversion factor for % and W = sample weight (g).

Microbiological analysis. Muscle pieces (~ 10 g, without skin) were aseptically excised from the anterior part of the epaxial muscle and transferred to a sterile stomacher bag diluted with 1:10 sterile buffered peptone water (Merck, Germany). The mixture was blended using a Smasher (AES Laboratorie, bioMérieux Industry, USA) for 120 s. Homogenates were further diluted to appropriate concentrations. For raw fillets, total psychotropic counts (TPC) were quantified using long and hammer (L&H) agar, while total mesophilic counts (TMC) and H_2S producing bacteria (HSPB) were quantified using iron agar supplemented with 0.04% L-cysteine (Sigma-Aldrich, Norway) according to the NMKL method No. 184[18]. 49.2 µl of each homogenate was transferred to L&H agar using an Eddy Jet 2 W Spiral Plater (IUL micro, Spain) while 1 ml was transferred to iron agar. L&H agar plates were incubated at 15 °C for 5 days, while iron agar at 25 °C for 72 ± 6 h. HSPB was quantified by the black colonies produced. For smoked samples, microbial analysis was done on the last sampling day using L&H and MRS (de man, Rogosa, Sharpe, Oxoid, UK) agar with Amphotericin B to quantify for TPC and lactic acid bacteria (LAB), respectively. MRS plates were incubated in anaerobic conditions at 25 °C for 5 days according to the NMKL method No. 140[19]. The end of shelf life was defined as the point where microbial counts exceeded 10^6 cfu g^{-1}.

Statistics. Minitab Version 19 (www.minitab.com, Minitab Inc., USA) were used for all statistical analysis, while Microsoft Visio 2016 (Microsoft Corporation, USA), Inkscape 1.0 (www.inkscape.org) and SigmaPlot 14.9 (www.systatsoftware.com/products/sigmaplot/, Systat Software, USA) were used for figures and plotting of results. Normality tests were assessed based on the normality probability plots. Association among treatment, storage days and response variables were analyzed using general linear model (GLM). Fillet height was added as an additional covariate for texture analysis in GLM. A model including the interaction effect was first tested and the interaction term included when it was significant. Otherwise, a non-interaction model was used. A non-parametric Kruskal–Wallis test was used for comparison of salt content of smoked sampling on the last sampling day, while a one-way ANOVA was used for comparing microbial counts of smoked salmon and gill colour after filleting on day 7. The significance level was set at $p < 0.05$. All results are presented as mean ± standard deviation.

Results and discussion

Temperature, water holding properties and salt content. The pH of fish measured 2 h after slaughter was 6.3 ± 0.2. Since a typical pH value for rested fish is around 7.5[6,20], this indicated that fish used in the present study were likely stressed prior to slaughter and went rapidly into rigor mortis. Roth et al.[6] and Lerfall et al.[21] reported that stress from slaughter and pumping can cause a significant decrease in muscle pH. In the experiment, fish were transported from a well-boat to resting cages, crowded then vacuum pumped to the slaughter site. This likely caused the pH decline, suggesting high lactic acid accumulation from glycogen reserves. Internal temperatures of whole fish after 20 h until filleting on day 7 were rather stable for all groups (Fig. 2a). The observed fluctuation and rise in temperatures at 48 h and 96 h were due to the need for weighing and sampling. Removal of fish from RSW tank also caused the temperature increase at 96 h for RSW fish.

Temperatures of raw and smoked fillets for all groups were kept stable during storage at 0 °C and 4 °C respectively (data not shown). It is established that temperature during superchilling must be kept as stable as possible to prevent repeated ice recrystallisation and prolong the product's shelf life[12]. In the present study, it was inevitable to disrupt the cold chain even only for a short period during sampling days. However, temperature is usually maintained rather constant during storage in a commercial scale so this should not greatly affect shelf life.

There was a significant effect of storage days and treatments on drip loss in whole fish (Fig. 2b, $p < 0.001$, $p < 0.001$, respectively). The drip loss of iced fish stabilized at around 1% while those of control fish gradually increased during the 4 days of storage. Similarly, Erikson et al.[12] reported that ice-stored salmon lost weight during the first 2 days before stabilising at a drip loss of 2%. The present study illustrates that RSW fish gained 1% seawater by day 4. After seawater was drained on day 4, RSW fish lost 0.3% in drip with reference to its initial weight during the additional 3 days of storage. Taking into account that the weight gained on day 4 was greater than weight lost on day 7 with respect to its initial weight, the overall weight gain after 7 days of storage

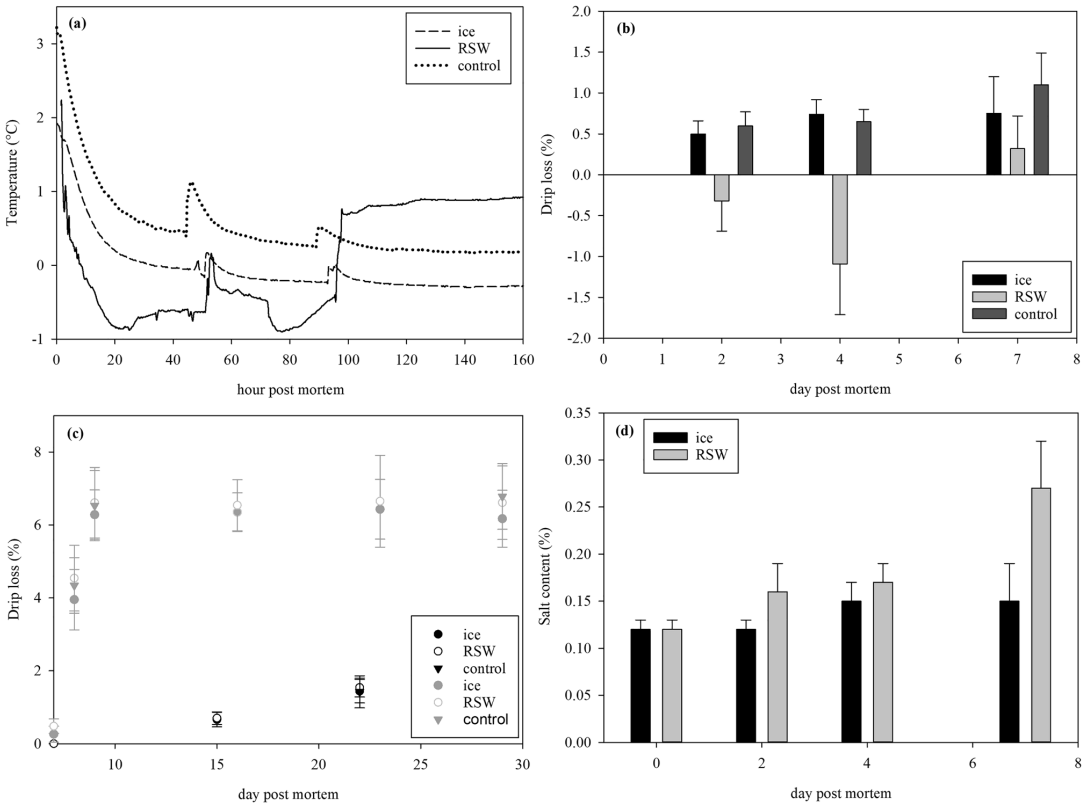

Figure 2. (**a**) Temperature change and (**b**) drip loss of whole fish on ice, RSW and control until day 7 (GLM; storage days: $p < 0.001$; treatment: $p < 0.001$); (**c**) drip loss of raw fillets (black line; GLM; storage days: $p < 0.001$; treatment: $p = 0.747$) and smoked fillets (gray line; GLM; storage days: $p < 0.001$; treatment: $p = 0.737$) from whole fish on ice, RSW and control; (**d**) salt content of whole fish on ice and RSW (GLM; storage days: $p < 0.001$; treatment: $p = 0.05$; days*treatment: $p < 0.001$).

for RSW fish was 0.7%. Previous studies have reported a higher weight gain of salmon kept in RSW for 4 days. Erikson et al.[12] reported a 2.5% gain in gutted Atlantic salmon kept in − 2 °C, while there was a 3% gain when pink salmon was stored in − 0.5 °C[11]. Bronstein et al.[7] measured a 2% gain at 0 °C. Based on these studies, the chilling temperature seemed to have minimal effect on weight gained in fish. Gutting fish before immersion in RSW results in water and salt uptake through the abdominal cavity, causing weight gain[12]. The comparatively lower gain observed in this experiment could be due to the larger salmon size used which renders a slower diffusion of water into the muscle. Differences in fat content of various salmon species reported may have also affected water and salt uptake.

There was an effect of storage day ($p < 0.001$) but not of treatment ($p = 0.747$) on drip loss in raw fillets (Fig. 2c). Drip loss increased steadily to 1.4–1.5% for all three groups. The product yield after smoking for all fillets was 94% and there was no effect of treatment observed ($p = 0.737$). Drip loss of smoked fillets increased to 6.5% after smoking for all groups and plateaued throughout storage. There was also no difference in salt contents at the end of storage ($p = 0.733$; RSW: 3.2 ± 0.4%, ice: 3.1 ± 0.7%, control: 3.0 ± 0.2%). The large increase in drip loss before and after dry salting and cold-smoking was mainly due to the diffusion and evaporation of water from the muscle to the surface. Since the drip loss of fillets from all treatments were similar, the observed increase in drip loss was only affected by storage duration ($p < 0.001$). Therefore, the choice of storage regime on whole fish seems unlikely to affect the drip loss of cold-smoked fillets. Comparison of salt uptake indicated that iced fish had a consistent salt content at 0.1%, while RSW held fish almost doubled from 0.1 to 0.2% during 4 days of storage, and further gained to 0.3% after RSW removal (Fig. 2d, storage days: $p < 0.001$, treatment: $p = 0.05$). The effect of storage duration was also significantly influenced by the treatment method (interaction: $p < 0.001$). The results consonates with other studies where chinook salmon had a salt content of 1.1% after 7 days in RSW and 0.1% on ice[7]. Himelbloom et al.[11] further showed that salt content of pink salmon stored in chilled seawater (CSW) increased to 0.5% during 10 days of storage, while iced salmon maintained at 0.1%. In RSW systems, the addition of salt in water causes the structure of pure water to be disrupted as salt dissociates into Na^+ and Cl^- ions, increasing the ion–dipole interaction between salt and water. The salt gained on day 7 observed in the

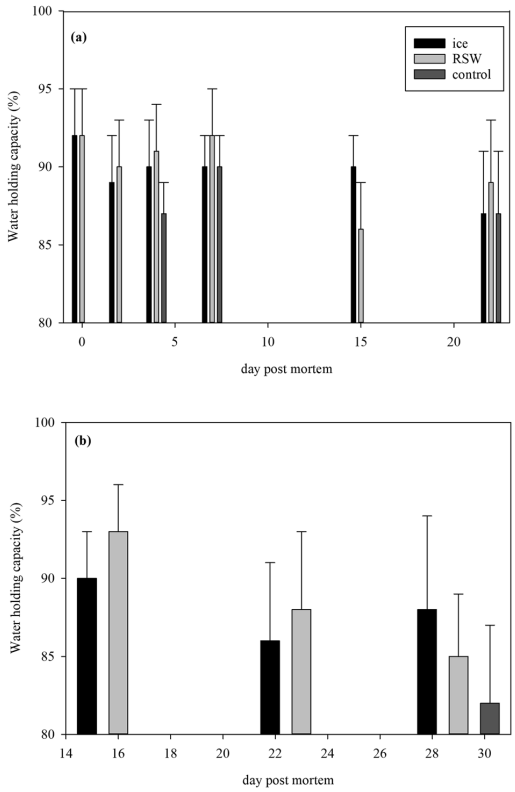

Figure 3. (**a**) WHC (%) of whole fish on ice, RSW and control until day 7 (GLM; storage days: $p = 0.002$; treatment: $p < 0.001$), and after filleting until day 22 (GLM; storage days: $p = 0.728$; treatment: $p = 0.032$); (**b**) WHC (%) of smoked fish from ice, RSW and control until day 29 (GLM; storage days: $p < 0.001$; treatment: $p = 0.002$).

present study after the removal of RSW possibly signifies that the retained Na^+ and Cl^- ions from seawater that was absorbed continued to diffuse into the fish muscle and binded with the muscle proteins. As such, the drip lost after removal of fish from RSW probably contains mainly water, likely from the free water located outside the myofibrillar network that can be easily lost from the tissue. Nevertheless, the application of salt uptake in Atlantic salmon has been considered relatively unimportant due to its large size and subcutaneous fat layer which hinders salt migration[22]. Hence salt uptake during RSW storage is not considered a problem as it is also dependent on other factors like species, lipid and salt content, temperature, physiological state and storage duration[10].

RSW fish had a notably higher WHC than iced and control fish ($p < 0.001$) until day 7 prior to filleting, with also a significant effect of storage days ($p = 0.002$, Fig. 3a). After filleting, there was no effect of storage duration on raw fillets ($p = 0.728$) and no clear pattern was observed among treatments, although statistical analysis revealed a difference ($p = 0.032$). In contrast, there was a general decrease in WHC through storage days for smoked fillets ($p < 0.001$). The control fish had the lowest WHC on day 29, while RSW fish had the highest WHC on days 16 and 23 (Fig. 3b).

Storage of fish in RSW is a brining method where fish is immersed in a 3.5% salt solution. Since the surrounding brine has a higher concentration than the intracellular fluid in the muscle, this results in salt entering the muscle by diffusion, thereby increasing yield. Degree of muscle swelling depends on the salt concentration where maximum swelling and WHC occurs at a 5–6%[23,24]. Previous studies reported that brining allows proteins to retain more moisture, as lower salt concentrations causes a lower degree of protein denaturation and induces swelling of muscle fibers, leading to a higher WHC[25,26]. This occurs due to the repulsive electrostatic forces of Cl^- anions weakly attached to the myofibrillar and sarcoplasmic proteins, causing protein to coagulate and entrap free water[25]. At higher salt concentrations, protein denaturation increases and myofibrillar proteins lose water, causing muscle dehydration and eventually yield loss[23]. Storing fish in RSW could therefore be beneficial in moisture retention and potentially give the product a better cooking yield and tenderness. Further studies such as sensory analysis could be done to examine and relate this with industries' and consumers' preferences.

	Raw fillets						Smoked fillets					
Group	Day	L*	a*	b*	80% compression force (N)	n	Day	L*	a*	b*	80% compression force (N)	n
Ice	2	63.3 ± 2.9	40.6 ± 1.6	30.9 ± 1.6	31.2 ± 5.3	5	9	54.4 ± 1.6	32.9 ± 1.9	34.9 ± 1.3	–	30
	4	56.8 ± 3.6	37.3 ± 1.6	32.9 ± 1.6	28.0 ± 4.8	6	16	53.8 ± 1.8	30.7 ± 1.3	35.7 ± 1.3	26.6 ± 4.6	10
	7	60.1 ± 2.7	34.5 ± 2.6	29.0 ± 2.0	24.1 ± 6.3	6	23	54.0 ± 0.7	29.6 ± 1.8	35.0 ± 1.0	24.6 ± 5.6	10
	15	65.3 ± 1.9	33.1 ± 2.1	29.7 ± 1.9	17.9 ± 4.1	6	29	53.3 ± 2.2	29.4 ± 1.6	33.3 ± 0.8	27.4 ± 6.6	10
	22	65.2 ± 1.6	29.6 ± 1.8	26.9 ± 1.5	17.2 ± 4.5	6						
RSW	2	66.4 ± 4.7	37.4 ± 2.1	28.9 ± 1.6	29.0 ± 6.6	6	9	54.1 ± 2.5	33.1 ± 2.1	34.6 ± 1.5	–	30
	4	58.7 ± 2.7	35.2 ± 2.5	31.7 ± 1.6	24.8 ± 7.7	6	16	54.6 ± 2.5	30.0 ± 1.8	34.5 ± 1.4	28.0 ± 4.4	10
	7	60.5 ± 1.5	37.5 ± 1.5	28.2 ± 1.3	19.8 ± 5.2	6	23	53.5 ± 2.2	30.0 ± 2.6	34.3 ± 1.4	24.0 ± 6.2	9
	15	65.9 ± 2.7	31.4 ± 3.5	28.4 ± 2.0	17.0 ± 4.1	6	29	53.0 ± 1.9	30.3 ± 0.9	33.7 ± 1.4	24.9 ± 5.9	10
	22	64.2 ± 1.4	31.6 ± 0.9	27.7 ± 0.7	19.0 ± 3.8	6						
Control	22	65.8 ± 1.7	29.7 ± 1.9	26.5 ± 1.2	18.7 ± 5.2	7	9	54.7 ± 1.9	33.3 ± 1.8	35.4 ± 1.7	–	7
							29	54.6 ± 1.8	27.8 ± 1.51	33.9 ± 1.0	24.3 ± 5.6	7
GLM[a]	P_D	0.001*	< 0.001*	< 0.001*	< 0.001*		P_D	0.057	< 0.001*	0.001*	0.120	
	P_G	0.478	0.823	0.106	0.006*		P_G	0.334	0.689	0.254	0.635	
	P_H	–	–	–	0.001*		P_H	–	–	–	< 0.001*	

Table 1. L*, a*, b* and 80% compression force of raw and smoked fillets throughout storage. [a]General Linear Model (GLM) analyses of variance with fillet groups as factors and storage days as covariance. Fillet height was added as an extra covariate for texture analysis. P_D, P_G and P_H are the significant levels for the effects of the storage days, groups and fillet height, respectively. *Significant when $p < 0.05$.

Surface appearance and texture. Lightness (L*) of fillets from whole fish decreased while yellowness (b*) increased until day 4. This is likely due to the flesh of fish becoming less translucent (more opaque), affecting the light absorption and reflection after slaughter of fresh fish[20,27]. The opposite was observed after filleting from days 7 to 15, where L* increased throughout fillet storage and became paler before leveling out ($p = 0.001$, Table 1). A similar phenomenon was observed by Erikson and Misimi[20], where the L* of ice chilled salmon fillets was darker during the first 20 h and then rose sharply afterwards. This could be influenced by the duration of rigor, as muscle contraction may cause differences in lightness[20]. Redness of raw fillets observed in this study decreased throughout storage ($p < 0.001$). Loss of fillet transparency and redness has been correlated with protein denaturation[28] and higher liquid loss[27], in line with observations seen after filleting. Previous studies also revealed that fillets resulted in a lighter, less reddish and yellowish colour in continuous ice storage for 7 days[20] which tallies to our results for iced fish after filleting.

There was no effect of treatment on the colour of raw fillets (L*: $p = 0.478$; a* $p = 0.823$; b*: $p = 0.106$). The RSW fish were slightly lighter and less yellowish in colour than iced. A common problem associated with RSW stored fish is the bleaching of fillets which may hinder its market value[10]. Bleaching was not seen in the fillets, as adjacent to the findings of Erikson et al.[12] who found that continuous storage of fish in RSW did not lead to lighter fillets. By visual observation, gill colour from the RSW fish seemed to be grayer and less reddish in colour which could be a more obvious evidence of bleaching, as also reported in RSW-stored cod for 4 days[29] and RSW-stored ocean pearch for several days[30]. This was further verified by the present study when the lightness observed on day 7 for the RSW fish was significatly higher ($p = 0.001$; ice: 36.2 ± 3.2; RSW: 41.1 ± 3.4; control: 39.4 ± 3.2), while redness lower ($p = 0.001$; ice: 18.1 ± 3.2; RSW: 13.2 ± 2.5; control: 16.0 ± 4.4) than both the iced and control fish. Nevertheless, quality of fillets from RSW stored fish are still considered highly acceptable with its shelf life surpassing traditionally iced fish[31].

The smoked fillets from all treatments had a decrease in redness and yellowness through storage (a*, $p < 0.001$; b*, $p = 0.001$), while no differences were observed among treatments (L*, $p = 0.334$; a*, $p = 0.689$; b*, $p = 0.254$). As accurately seen in this study, a general trend in cold-smoked salmon is that they are darker and less red, but more yellowish than the unprocessed fillets[32]. Dry salting is a process that affects colour and texture, causes cell shrinkage and decreases the thermal stability of actin and myosin which eventually leads to protein denaturation within the muscle. Limited reports are currently available on colour changes in both raw and smoked fillet as affected by RSW storage. However, results from the present study as well as visual observations indicated that the colour quality of raw and smoked fillets from RSW fish are comparable to those on ice.

The puncture test showed that texture of raw fillets decreased throughout storage ($p < 0.001$), an established fact of muscle tenderization due to the gradual disintegration of connective tissues[5,33]. Afterwards, the divergence becomes minimal and gradually stabilizes through storage. The compression force was significantly affected by the fillet height ($p = 0.001$). Storing and removal of fish in RSW also produced softer fillets as they had a significantly softer texture in comparison to iced fillets ($p = 0.006$). This was attested by Erikson et al.[12] and Chan et al.[5] who asserted that changing the chilling medium from RSW to storage with or without ice leads to significantly lower hardness. One would therefore expect that the fillets of RSW fish will cause a higher gaping incidence. Gaping is a damaging textural problem causing flesh softening from the collapse of muscle and collagen fibrils[16,34]. In the present study, the extent of gaping for the ice-stored and control raw fillets increased through time ($p < 0.001$), coinciding with the decrease in compression force. Interestingly, there was almost no gaping occurrence for

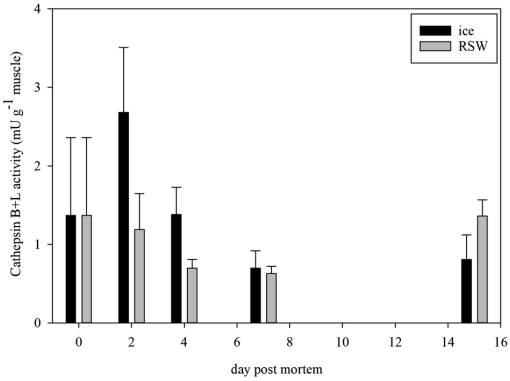

Figure 4. Cathepsin B + L activity of ice and RSW fish (n = 5; GLM; storage days: $p = 0.035$; treatment: $p < 0.001$; days * treatment: $p = 0.002$).

raw fillets from RSW fish on days 7 (ice: 0.5 ± 0.5; RSW: 0.0 ± 0.2) and 15 (ice: 1.0 ± 0.3; RSW: 0.0 ± 0.0; control: 3.0 ± 0.7). This suggests that softness might be due to other factors apart from fibral disintegration. It should also be noted that texture and gaping in fish can be influenced by a variety of factors such as harvest season, body size, collagen composition and water content[34].

There was no difference in textural quality among the smoked products neither in storage day ($p = 0.120$) nor treatment per se ($p = 0.635$), while the compression force was significantly affected by the fillet height ($p < 0.001$). In terms of gaping, the control fish had the highest gaping score on the last sampling day (ice: 1.0 ± 0.7; RSW: 1.0 ± 1.0; control: 2.0 ± 1.1). Therefore, the effect of treatment method on both texture and gaping seemed to apply only to the unprocessed fillets in this experiment. Hansen et al.[35] stated that textural deterioration occurs in cold-smoked salmon due to autolytic deterioration of tissue which develops rancid and oxidised off-flavours. However, the decline in textural properties for all smoked salmon groups was not apparent in our study, and is in agreement with earlier studies which found that shear force of cold-smoked salmon was stable during cold storage[36,37].

Enzyme and microbial activity. The lysosomal cathepsins B + L are proteases believed to degrade muscle collagen which causes tissue softening and has been used to explain the degree of proteolysis[38,39]. In the present study, significant differences in muscle cathepsin B + L activity were observed between the iced and RSW fish. Samples from whole iced fish had a consistently higher enzyme activity from days 2 to 7 (Fig. 4, $p < 0.001$) and decreased through storage ($p = 0.035$), while those from RSW fish increased in enzyme activity on day 15. There was also an interaction effect between storage duration and treatment ($p = 0.002$).

Temperature is a main determinant in both enzyme and microbiological activity. The enzyme activity observed in RSW fish during the first 7 days is possibly explained by a lower refrigerated temperature when fish were kept in RSW, suppressing the activity. When RSW fish were kept at the same temperature conditions as iced fish afterwards, the enzyme activity increased on day 15. Although whole fish from RSW resulted in a softer texture than iced fish before they were filleted, the enzyme activity apparently does not reflect this. A plausible explanation for the softer texture of RSW fish could be due to its water and salt uptake during immersion in seawater. Contrary to this, the observed softening in iced fish was likely due to increased enzyme activity during chilled storage especially during the first 48 h. Gaarder et al.[40] stated that cathepsin B + L activity increases to a threshold until 24 h postmortem and remains stable afterwards, while Duun[2] presented that the activity was stable during ice storage, indicating that these enzymes were still active and led to softening during storage. Based on our knowledge, the enzymatic activity including other enzymatic reactions involved in postmortem softening of tissue like collagenases and calpains from fish stored in RSW has not been thoroughly explored. This could be an interesting aspect for further studies which can include fish histology to identify the development of intra- and extracellular cell structures during RSW storage.

Microbial counts (TPC, TMC, HSPB) significantly increased throughout storage for both raw fillets from iced and RSW fish (Fig. 5a–c; TPC: $p < 0.001$, TMC: $p < 0.001$. HSPB: $p < 0.001$). Psychotropic and mesophilic bacterial species that can be present in salmon include *Shewanella* spp., LAB, *Photobacterium* spp., *Pseudomonas* spp. and *Brochothrix thermosphacta*[41]. A bacterial prevalence of $> 10^6$ cfu g^{-1} depicts the end of shelf life[35,42] so under this criteria both treatments were deemed spoiled after 15 days. RSW fish had a significantly higher counts on TPC ($p < 0.001$), TMC ($p = 0.046$) and HSPB ($p < 0.001$). This occurrence was presumably due to a variety of reasons such as challenges of recirculating seawater in the simulated tank. Despite ensuring proper hygiene when handling the fish from the tank, it was still an enclosed system with no recirculation of new seawater. In addition, contamination is a big risk and cross-contamination might have occurred as other experiments were ongoing concurrently when the tank was stored in the cold room. Insufficient cleaning of the RSW tank might have also contributed to the increased microbial growth. Prior to the experiment, the tank was cleaned with foam instead

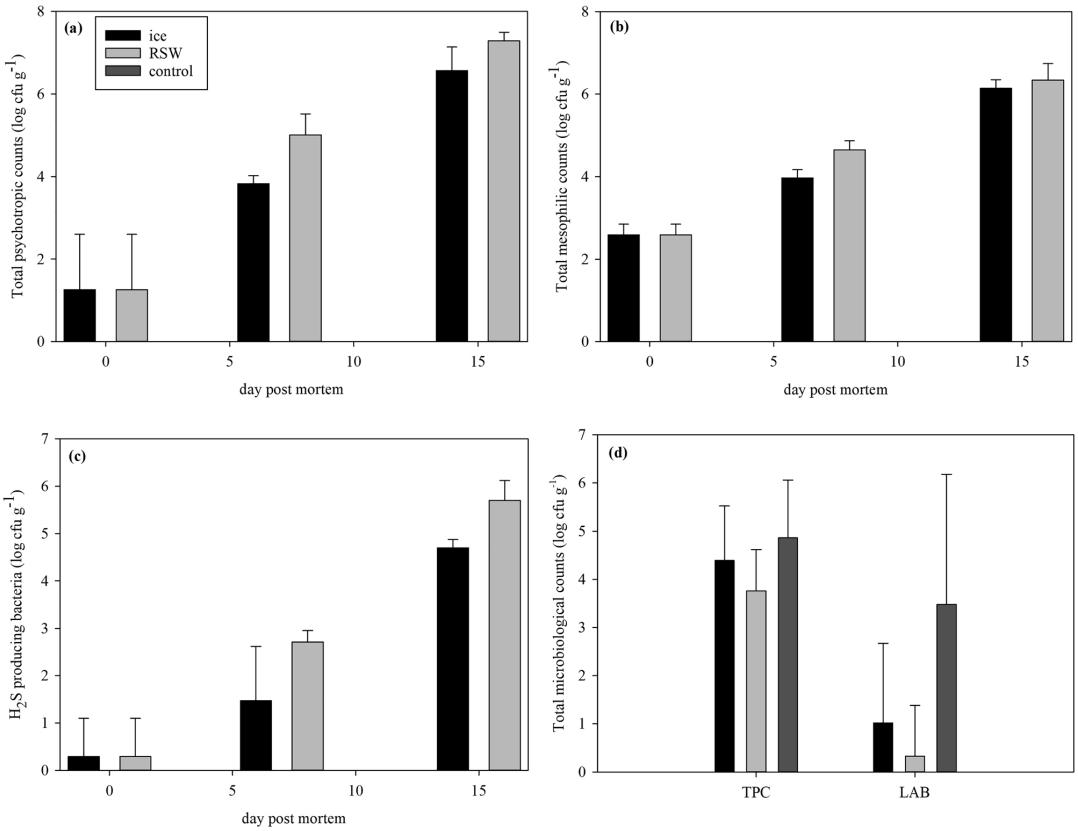

Figure 5. (**a**) Total psychotropic counts of raw fillets from ice and RSW through storage (n = 6; GLM; storage days: $p < 0.001$; treatment: $p < 0.001$); (**b**) Total mesophilic counts of raw fillets from ice and RSW through storage (n = 6; GLM; storage days: $p < 0.001$; treatment: $p = 0.046$); (**c**) H_2S producing bacteria counts of raw fillets from ice and RSW through storage (n = 6; GLM; storage days: $p < 0.001$; treatment: $p < 0.001$); (**d**) Total psychotropic counts (n = 10; One-way ANOVA, $p = 0.123$) and lactic acid bacteria counts ($p = 0.005$) of smoked fillets from ice, RSW and control treatment on last sampling day.

of using a strong detergent like lye, so thorough cleaning could have been challenging for this makeshift tank despite it being kept constant at sub-zero temperatures during the storage period.

Contradictory results have been reported when fish were stored in RSW and on ice possibly due to the difference between laboratory and industrial based experimental scale[43]. The initial microbiological activity observed right after slaughter further suggested that the fish might have blood remnants remaining in the flesh after being bled in the bleeding tanks at the land-based slaughtering facility. The addition of seawater and seawater ice into the tank was also manually done as compared to mechanical procedures in commercial settings. Hence the rate of spoilage for the RSW fish was more pronounced in this study which affirms how easy contamination can occur. However, these problems should not arise in commercial settings due to more stringent rules with regards to filtering, ozonating and chlorinating of process water. Industries also use CO_2-based RSW tanks which improves energy efficiency, suppresses bacterial growth, and enhances sensory attributes of fish[8]. Good hygiene has been posed as a possible challenge posed by closed RSW tanks as spoilage of fish may affect the entire catch[44]. Therefore, a well-designed RSW system is important and proper considerations must be implemented including good insulation of tanks, evenly distributed and controlled temperatures, constant supply of clean seawater and adequate cleaning and disinfection of the factory after every harvest[10]. This ensures good recirculation of water, lessens microbial spoilage, maintains good quality and offers the flexibility for fishing vessels to travel to further distances.

TPC for RSW treated fish after cold-smoking tended to be lower than both chilled and control fish ($p = 0.123$), while a significantly higher LAB was produced for the control fish (Fig. 5d, $p = 0.005$). The shelf life of vacuum-packed whole fillet cold-smoked salmon at 5 °C was previously reported to be around 32–49 days[35], while the acceptable shelf life for commercial industries is 21–60 days[45]. LAB is prevalent in smoked salmon, producing organic acids and ethanol as fermentation products, hence off-flavours and off-odours associated with

spoilage[35,46]. Hansen et al.[35] further concluded that spoilage of vacuum packed cold-smoked salmon is due to microbiological activity combined with the autolytic deterioration of tissue, causing textural damage through storage. The actual shelf life of salmon was difficult to conclude in our study with only 3 sampling points conducted. Future considerations may include more sampling points and characterization of other prevalent spoilage microorganisms such as *Pseudomonas* spp. or *Photobacterium* spp. which may be a better microbiological indicator of shelf life[41]. Nevertheless, salting and smoking of salmon is a well-established method to prolong shelf life of fish and it is recommended to further process salmon fillets at early stages of the value chain.

Conclusion

This study presented several quality parameters examined on salmon stored in RSW throughout the whole supply chain. In comparison to traditional chilling methods, whole fish stored in RSW had an overall increase in water and salt uptake, with better WHC before filleting. After filleting, better gaping scores, softer texture, lower cathepsin B + L activity and higher microbiological growth were observed. Although the raw fillets from RSW fish had a softer texture, this was likely unaffected by the enzymatic process of cathepsins B + L causing postmortem degradation. The microbiological analysis on raw fillets suggested that RSW fish had a shorter shelf life, but this is not representative of commercial practices due to the experimental scale. Drip loss and colour of both raw and smoked fillets from the 2 treatments were comparable, and storage duration was the main determinant affecting these parameters. These results indicate that RSW-stored fish is a viable method in minimizing the need for ice storage and land transportation, thereby introducing economical benefits and contributing to a positive impact on the environment. The idea of shifting fish slaughter from land to sea further introduces several advantages including reduced transportation costs, reduced fish diseases and mortality, increased slaughtering capacity and improved fish welfare. Therefore, the cutting-edge concept of slaughter vessels can provide great potential to increase its competitive advantage in the salmon industry. Industries seeking to understand more about the quality changes during storage of fish in RSW tanks, how this differs from the traditional chilling method on ice and how this affects fillet quality after primary and secondary processing to cold-smoked fillets can consider the results of this study during the formularization and streamlining of their processes.

As temperature is a critical aspect in superchilling, it is crucial to maintain a constant sub-zero temperature during storage combined with proper recirculation and good hygiene practices of RSW systems to lower the risk of contaminating the whole catch. Since this is a relatively new concept, more comprehensive research like shelf life and consumer acceptance studies can be performed to explore more potentials and solutions for such vessels.

Data availability

The datasets generated during/or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

S.S.C. wrote the manuscript text; S.S.C., B.R., F.J., T.L., A.N.J. and J.L. conceived and designed the study; S.S.C. and B.R. performed the experiments; All authors analyzed the data and reviewed the manuscript.

Competing interests

The authors declare no competing interests.

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Paper IV

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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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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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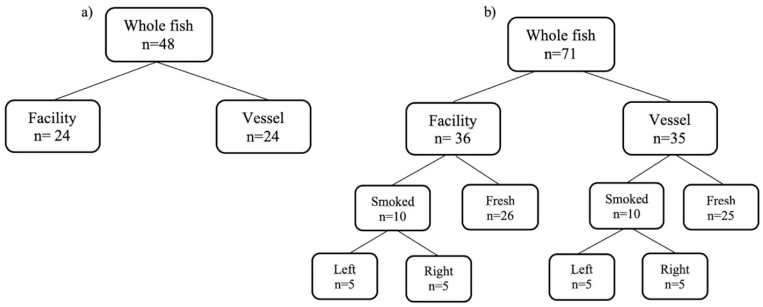

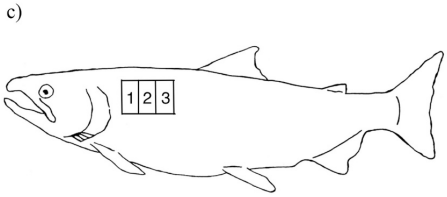

Fig. 1. (a) Experimental overview of the experiment conducted in December 2018, where texture, colour, gaping and blood spot counts were done (b) Experimental overview of the experiment conducted in October 2019. 10 fish from each group were filleted, dry salted and cold-smoked. The remaining 26 fish 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

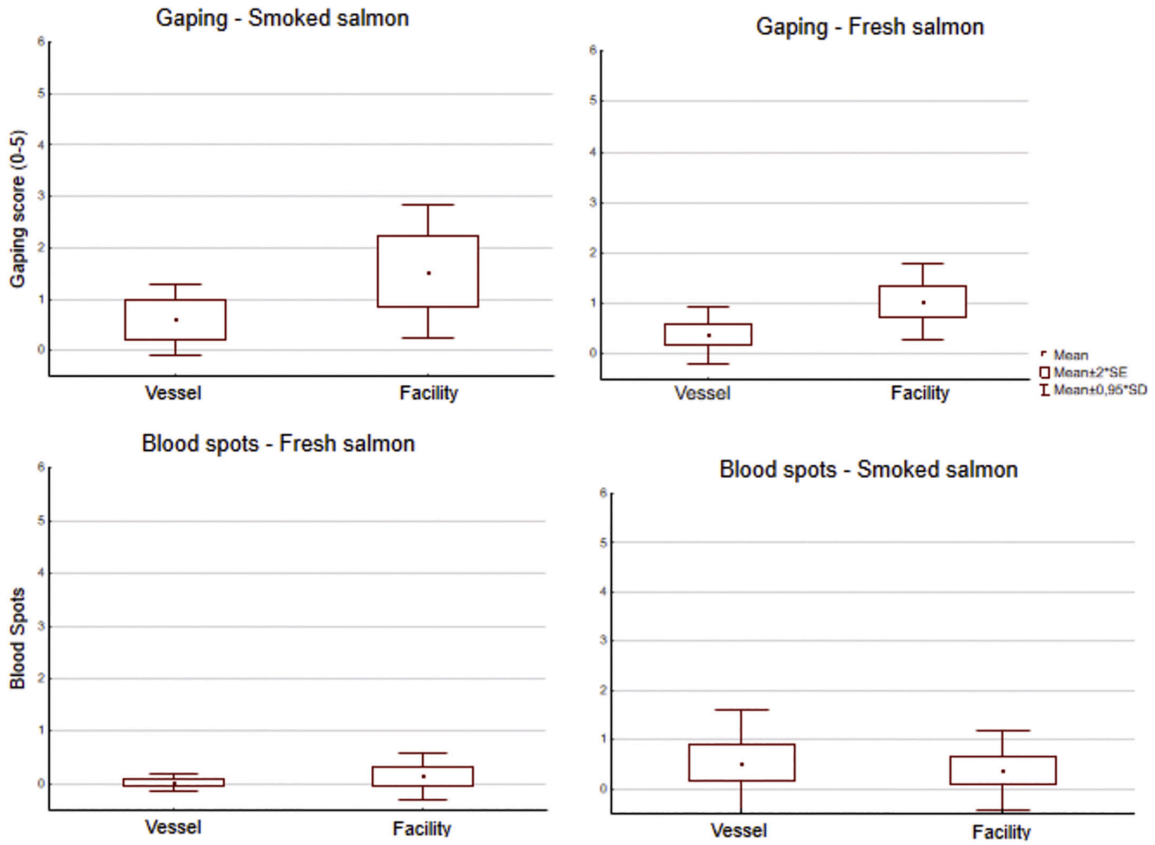

Fig. 2. Weighted result of gaping and blood spot counts from both fresh ($n = 24$) and smoked salmon ($n = 10$).

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.

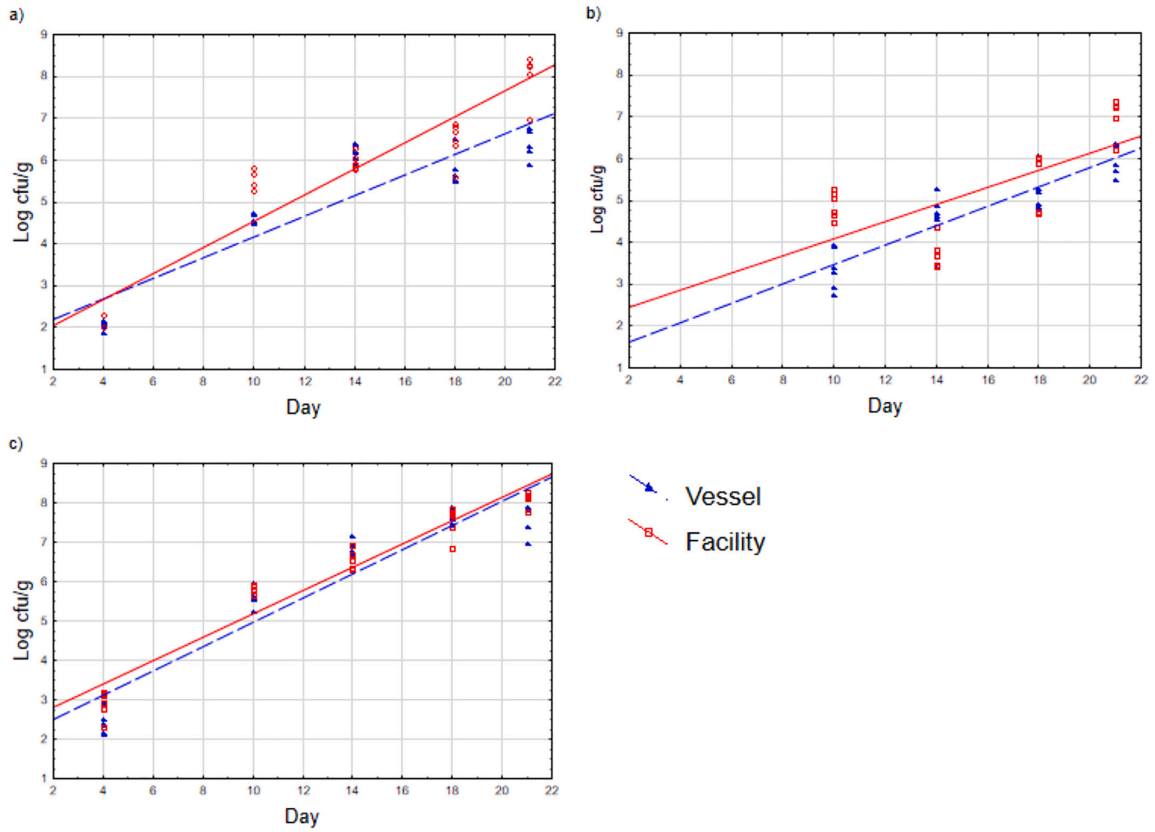

Fig. 3. (a) Total mesophilic counts (log cfu/g) (b) Hydrogen sulphide producing bacterial counts (log cfu/g) and (c) Total psychotropic count (log cfu/g) from day 4 to day 21 post mortem of fresh fish.

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

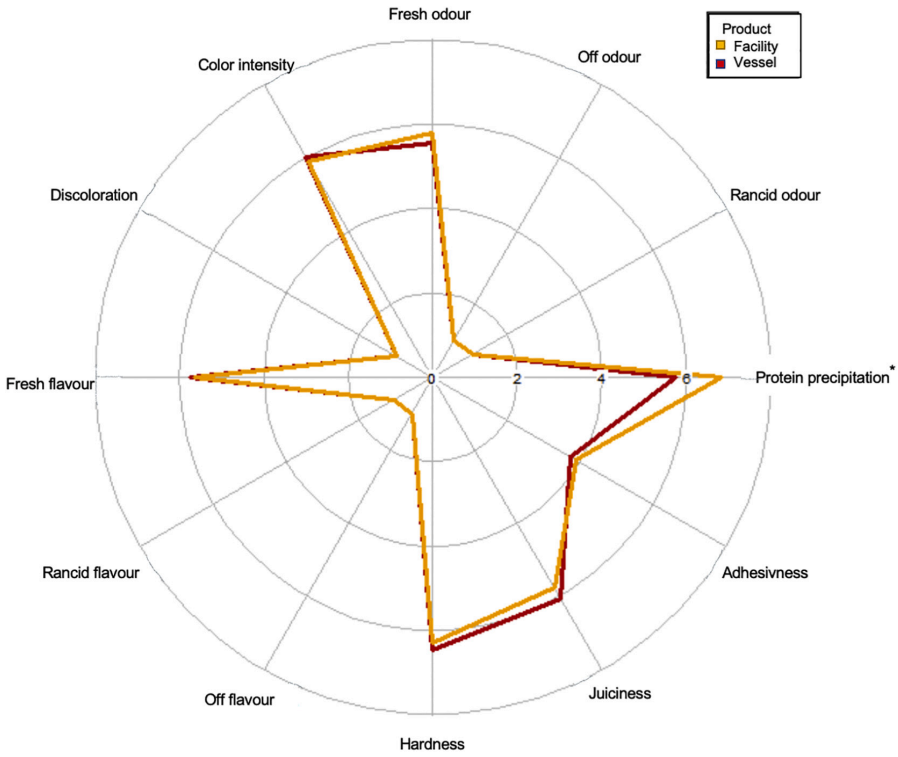

Fig. 4. Spider Plot showing the score for each attribute for both groups ($n = 5$ from each group), Day 18 post mortem (October experiment). *significant levels with $p < 0.05$.

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 $\pm$ 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.

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Paper V

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Skin and vacuum packaging of portioned Atlantic salmon originating from refrigerated seawater or traditional ice storage

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ABSTRACT

The effect of gutted whole fish storage method after slaughter (refrigerated seawater (RSW) −1 °C, ice 0 °C) and common fish packaging methods (vacuum skin, traditional vacuum) in refrigerated storage at 4 °C were investigated. Quality parameters such as pH, drip loss, water holding capacity, colour, texture and microbial growth were analyzed. There was an overall weight gain of 0.9% and salt uptake up to 0.23% for fish stored in RSW for 4 days, followed by ice storage for 3 days. After packaging, pH and hydrogen sulphide producing bacteria in RSW stored fish were lower than iced fish. Drip loss and microbial growth increased through packaged storage, while water holding capacity decreased in all samples. Skin packaged fillets presented a higher drip loss and were lighter, less reddish and less yellowish than traditionally vacuum packaged fillets. Lightness increased, and firmness decreased throughout the experimental period, while redness and yellowness decreased after packaging. Predictive microbiology gave good agreements with the experimental values. It is concluded that storage in RSW extends the lag time of microbial growth and presents good quality traits as those from ice, and the main difference between the packaging methods was drip loss and colour.

1. Introduction

The supply chain for food to reach from farms to the retail shelves requires a significant amount of time, especially when food is transported through long distances. Fish and fishery products are among the most perishable foods due to their high water content value, polyunsaturated fatty acids, pH, protein and non-protein nitrogenous compounds (Fidalgo, Castro, Trigo, Aubourg, Delgadillo & Saraiva, 2019; Gokoglu, 2020). Therefore, they must be stored appropriately to avoid undesirable biochemical and enzymatic reactions such as enzymatic autolysis, lipid oxidation, and microbial growth. A combination of food packaging and low temperature storage are critical ways of extending the microbiological shelf-life of food and fishery products. Vacuum packaging is one of the preferred packaging types for fresh fish, whereby air is removed before sealing the fish in a low O_2 permeable film (Nagarajarao, 2016). This provides an anaerobic environment with good barrier properties towards air and water, inhibiting the growth of aerobic spoilage microorganisms like, e.g. *Pseudomonas* spp. and limiting oxidative rancidity. However, vacuum packaging involves applying pressure on the fish and cause the formation of wrinkles on the package. The accumulation of purge may also induce bacterial growth and render it aesthetically unappealing to consumers (Łopacka et al., 2016).

An extended type of vacuum packaging is vacuum skin packaging, where food is placed on a tray before tightly sealing it with a thin, flexible film. The wrinkle-free application from the upper film's shrinkage by heating reduces the accumulation of oil and water exudates (Lagerstedt et al., 2011; Vázquez, Carreira, Franco, Fente, Cepeda & Barros-Velázquez, 2004). Besides, the perfectly contoured seal enhances the product's shape and appearance, providing ease in packing durability and better consumer perceptibility. Most studies on vacuum skin packaging were carried out on meat products like beef (Kameník, Saláková, Pavlík, Bořilová, Hulanková & Steinhauserová, 2014; Lagerstedt et al., 2011; Li, Lindahl, Zamaratskaia & Lundström, 2012; Łopacka et al., 2016; Strydom & Hope-Jones, 2014; Vázquez et al., 2004), pork (Kameník et al., 2014) and lamb (Bellés, Alonso, Roncalés & Beltrán, 2017). In contrast, only a few focused on the effect of this technique on fish species like Atlantic salmon, a commercially important product. A study on Atlantic pomfret fillets by Pérez-Alonso, Aubourg, Rodríguez, and Barros-Velázquez (2004) revealed that the vacuum skin packaged fillets kept at 4 °C lead to better biochemical and sensory quality and significant shelf-life extension than traditional vacuum and air packaged fillets. Duran-Montgé et al. (2015) further suggested that skin packaging

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combined with superchilling can be a viable preservation technique to prolong the shelf-life of sea bream fillets.

Slaughter vessels are one of the recent vessel innovations in the Norwegian aquaculture industry. This concept conducts the fish slaughtering process directly in the slaughter vessel by the cage and superchills the gutted whole fish in refrigerated seawater (RSW) at −1 °C. Utilizing RSW allows the core temperature of the fish to cool at a faster rate than the traditional storage method on ice due to its higher convection rate. Studies comparing the quality of RSW stored fish showed higher water and salt uptake and better water holding capacity during whole fish storage. Better gaping and blood spot counts were also observed, but differences in drip loss were minimal after filleting and cold-smoking compared to ice-stored fish (Chan, Roth, Jessen, Løvdal, Jakobsen & Lerfall, 2020). Since this is a new method, extensive studies are needed to observe the quality through the whole value chain, specifically when the fresh product reaches the retail shelves. Therefore, this paper aims to study the combined effect of storage method and packaging technique, with the increasingly popular vacuum skin packaging, on various quality parameters such as pH, drip loss, water holding capacity, texture, colour, and microbial growth. The references chosen as control samples were traditional vacuum packaging and storage on ice, which are currently practiced commercially.

2. Materials and methods

2.1. Experimental design

A two factorial (2 × 2) design, with chilling method (RSW vs ice) and packaging technology (traditional vacuum vs vacuum skin) as experimental factors, was carried out. Before the experiment, a makeshift RSW tank was made using an 800-L polyethylene tank filled with seawater (sand filtered, ~3.5% salinity) and kept in a 0 °C storage room, as Chan et al. (2020) described. In parallel, seawater ice was made by freezing filtered seawater at −30 °C.

A graphical summary of the experimental timeline is shown in Fig. 1a. Farmed Atlantic salmon (*Salmo salar*) was obtained from a local fish slaughtering factory (n = 84, starved 9 days, average weight: 3.6 ± 0.23 kg, condition factor: 1.1 ± 0.09). Fish were electrically stunned, bled, and gutted before recording their individual weights and storing them in either the RSW tank (n = 39) or boxes containing ice (n = 39). The rest fish (n = 6) were used to obtain the initial values for pH, water holding capacity (WHC), colour, texture and microbial growth. Two temperature loggers (TrackSense Pro, Ellab A/S, Denmark) were inserted in the abdomen of random fish from each group. The tank and boxes were kept in a 0 °C storage room for 4 days, and the temperature of seawater in the tank was kept at −1 °C by periodically adding the premade seawater ice. On day 4, the RSW was drained out, and 5 fish from each group were used for sampling. All fish from both groups were weighed before storing in boxes containing ice and stored for another 3 days before processing on day 7.

2.2. Processing

On day 7, each fish was weighed before manually filleting. They were first classified into 2 main groups based on the storage method (R: RSW, I: ice). Then, the right and left fillets were categorized based on the packaging method in either vacuum skin (RS, IS) or traditional vacuum (RV, IV). This results in 4 treatment groups (RS, RV, IS, IV), as illustrated in Fig. 1b. Each fillet was then portioned into three pieces for packaging (Fig. 2, portions A, B and C, n = 102/group). A Multivac T2000 Tray sealer (Multivac, Norway) was used to seal the vacuum skin packages using the lidding film Skintite HB 125 alu/pet (thickness 125 μm, oxygen permeability 2 cm^3/m^2/24 h/atm (23 °C, 50% RH), water vapour transmission rate 4 g/m^2/24 h (38 °C, 90%RH), Plus Pack, Norway) and packaging tray C2187–1 F black crystallized polyethylene terephthalate trays (CPET, 187×137×40 mm, Faerch, Denmark). For traditional vacuum packaging, the portions were packed in 99.9% vacuum in plastic bags designed for vacuum packaging (Vakpak, PA/PE film, Lietpak UAB, Lithuania). All the packaged portions were stored at 4 °C for further analysis.

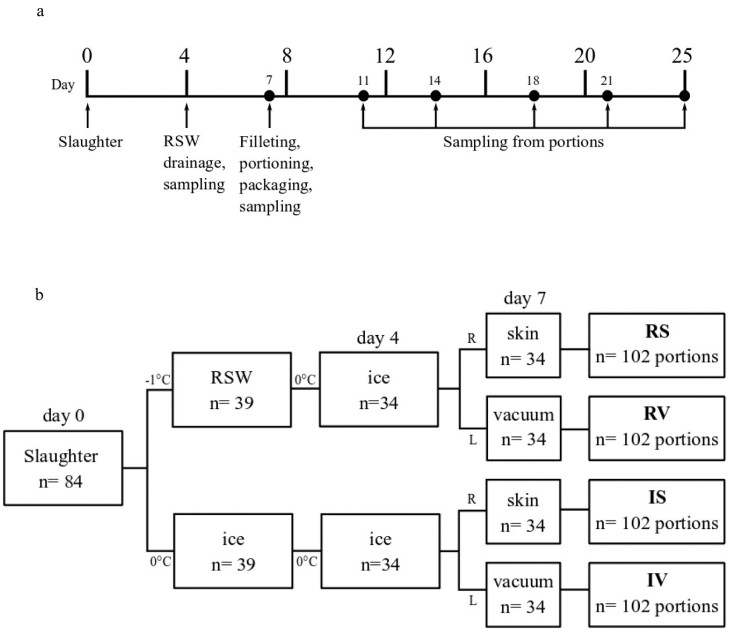

Fig. 1. A graphical illustration of the (a) experimental timeline and (b) experimental setup. RSW and ice represents gutted whole fish stored in refrigerated seawater and on ice, respectively. R ad L represents right and left fillets, respectively. RS and RV represents portions where whole fish was initially stored in RSW then packaged in vacuum skin and traditional vacuum, respectively. IS and IV represents portions where whole fish was intially stored on ice then packaged in vacuum skin and traditional vacuum, respectively.

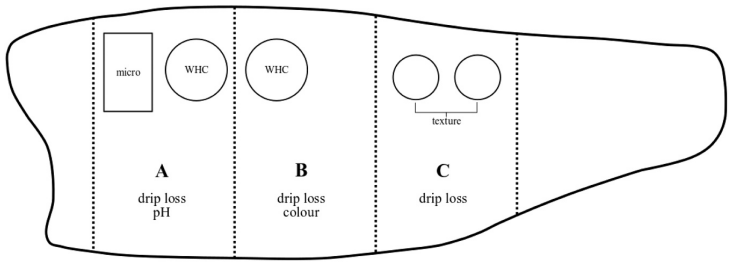

Fig. 2. A graphical illustration on fillet portioning into A, B and C and sampling positions on the skin and vacuum packaged portions. "micro" and WHC represents samples used for microbiological and water holding capacity analyses, respectively.

2.3. Quality analysis

Initial proximate composition was measured after slaughter on day 0. Samples were also collected and frozen at −80 °C for salt, protein and fat content analyses. pH was measured using a Mettler Toledo SevenGo pro pH meter (Mettler Toledo Inc., USA). Protein and fat contents were measured using the Bligh and Dyer (1959) and the Kjeldahl method (AOAC 928.08). A factor of 6.25 was used to convert nitrogen to total protein content. The salt content was also analyzed on days 4 and 7, where approximately 1.5 g of the sample was homogenized in an Ultra Turrax T25 (Janke and Kunkel IKA, Labortechnik, Staufen, Germany) for 13,500 rpm. Afterwards, the automatic titration method was performed using an SI Analytics Titroline machine (Xylem Analytics, Norway) with 0.1 M $AgNO_3$ (VWR International, Norway), as described by Chan et al. (2020).

After portioning and packaging on day 7, quality analysis was done periodically on days 11, 14, 18, 21 and 25 post mortem (Fig. 1a). Portions A, B and C from the same fish were randomly chosen from the 4 groups. pH was measured on portion-A at each sampling day.

2.3.1. Drip loss and water holding capacity

Drip loss was measured immediately after opening the packages on each portion. The portions were gently wiped with paper before weighing, and drip loss was calculated as the percentage difference (%) of the weighed sample (g) with respect to its initial weight (g). Water holding capacity (WHC) was analyzed in parallel on portions A and B using the low-speed centrifugation method of Skipnes et al. (2007). The muscle sample was punched with a metal cylinder (diameter 31 mm, height 6 mm) and transversally sliced into 2 pieces. The top piece was weighed and centrifuged in metal cups (Part No. 4750, Hettich Lab Technology, Germany) at 1800 rpm (15 min, 4 °C), and the bottom piece was used for moisture content analysis by heating the sample (105 °C, 72 h) to ensure complete water evaporation.

2.3.2. Instrumental texture and colour

Colourimetric assessments were analyzed on portion B with a DigiEye complete system (VeriVide Ltd, UK) connected to a Nikon D80 SLR camera (Nikon Corp, Japan). The L*a*b* values were measured using the DigiPix software (VeriVide Ltd, UK), where L*, a* and b* represent lightness, redness and yellowness, respectively (CIE, 1994). Chroma was calculated using the equation $\sqrt{a^2 + b^2}$. The hue angle (H°) was calculated using the equation $\tan^{-1}(\frac{b}{a})$, where $H^\circ = 0$ and $H^\circ = 60$ represent red and yellow hue, respectively.

Instrumental texture analysis was done with a Texture Analyzer TA-XT® plus (Stable Micro Systems Ltd, UK) equipped with a 5 kg load cell. A flat end cylindrical probe (12.7 mm P/0.5) was used to create 2 punctures on portion C from each group at each sampling day. The force-time graph was recorded using the Texture Exponent Light software, where the 80% compression force was determined as firmness.

2.3.3. Microbiological analysis

Total psychrotrophic count (TPC), total mesophilic count (TMC) and hydrogen sulphide producing bacteria (HSPB) were quantified using the NMKL method No. 184 (NMKL, 2006). Approximately 10 g of sample (without skin) was cut from portion-A and homogenized in filtered stomacher bags containing 90 mL of buffered peptone water (Merck, Germany) for 120 s. Serial dilutions on homogenates were carried out, and aliquots (49.2 μL) of each dilution was transferred to Long and Hammer (L&H) agar plates using the Eddy Jet 2 W Spiral Plater (IUL micro, Spain) and incubated for 5–7d at 15 °C. 1 mL of each aliquot was transferred to Iron agar (IA, Lyngby, Oxoid, Norway) supplemented with 0.4% L-cysteine (Sigma-Aldrich, Norway) and incubated for 72 h at 25 °C. TMC and HSPB were quantified by counting the total and black colonies in IA, respectively. The microbial populations are expressed in log cfu/g. If no colonies were detected on a plate, the value of 0.5 was assigned as the number of colonies for calculation.

In addition, predictive modelling was done using the Baranyi and Roberts (1994) model with the online DMFit in ComBase Predictor software (https://www.combase.cc/index.php/en/) to examine the growth kinetics of the obtained microbial population. The 4 parameters that characterize these models are lag time (λ), specific growth rate (μ_{max}), initial population size (N_0) and maximum population density (N_{max}). The r^2 value was included to examine the goodness of fit.

2.4. Statistics

Statistical analysis was done in Minitab® v.19 (Minitab, USA). A general linear model (GLM) was carried out where the whole fish storage method was set as a categorical factor and storage days as a continuous independent variable. After packaging, the packaging method was added as an additional categorical factor. Fillet height was added as an additional covariate for texture analysis. All results are presented as mean ± standard deviation unless otherwise stated. The α-value was set to 0.05.

3. Results

3.1. Raw material composition and pH

The initial compositions of the fish after slaughter were 66.1 ± 3.1% (water), 22.0 ± 0.6% (protein), 9.2 ± 0.9% (fat) and 0.14 ± 0.08% (salt). Temperatures during RSW and ice storage of whole fish were kept constant at −0.9 ± 0.2 °C and −0.1 ± 0.1 °C until day 4. From day 4–7, all fish from both groups were stored in ice, where the internal temperature was −0.7 ± 0.1 °C (RSW) and −0.1 ± 0.0 °C (ice), respectively (data not shown).

The initial pH was measured to be 6.2 ± 0.0, then 6.1 ± 0.0 (RSW) and 6.2 ± 0.1 (ice) on day 4. Before packaging on day 7, the pH increased slightly to 6.3 ± 0.1 (RSW) and 6.3 ± 0.1 (ice). After that, the pH for the 4 packaging groups decreased through storage time ($p < 0.001$, Fig. 3a), where the vacuum and skin packaged fish

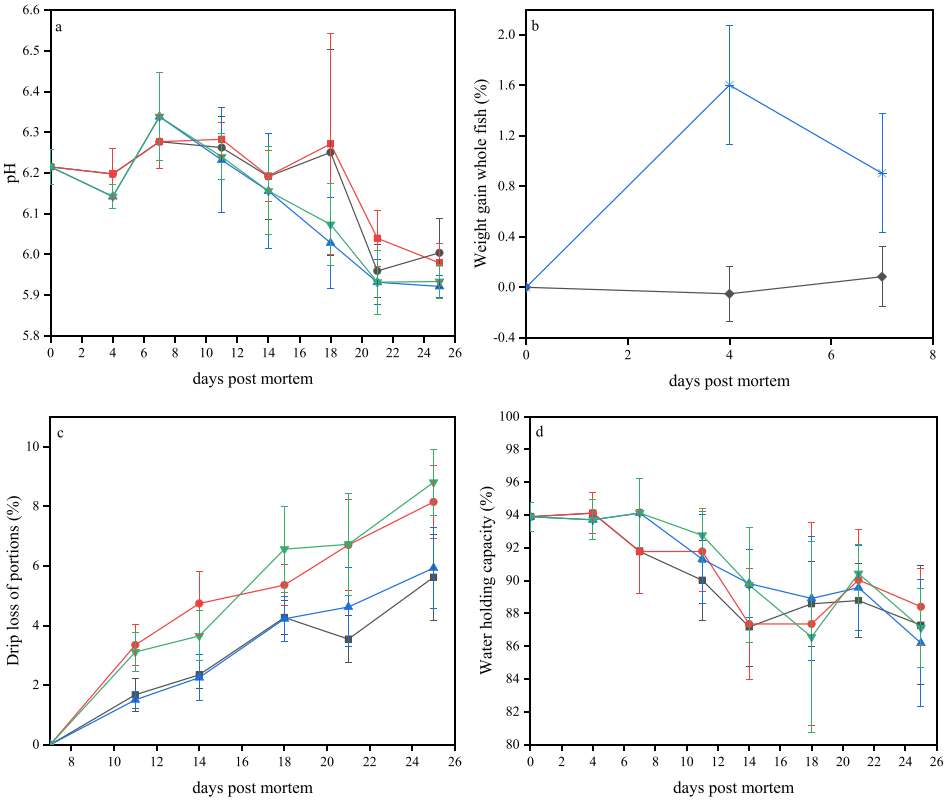

Fig. 3. Graphs showing IV (●), IS (■), RV (▲) and RS (▼) during storage. (a) pH of whole fish (GLM; days: $p = 0.015$, whole fish: $p = 0.915$) and portions after packaging (GLM; days: $p < 0.001$, whole fish: $p < 0.001$, portions: $p = 0.449$). (b) Weight gain of whole fish from RSW (✳) and ice (◆) (GLM; days: $p < 0.001$, whole fish: $p < 0.001$). (c) Drip loss of portions after packaging (GLM; days: $p < 0.001$, whole fish: $p = 0.115$, portions: $p < 0.001$). (d) Water holding capacity of whole fish (GLM; days: $p = 0.627$, whole fish: $p = 0.333$) and portions after packaging (GLM; days: $p < 0.001$, whole fish: $p = 0.617$, portions $p = 0.087$.

originally stored in RSW (RV and RS) had a slightly lower pH ($p < 0.001$) than those in ice (IV and IS). The final pH obtained on the last sampling day were 6.0 ± 0.1 (IV), 6.0 ± 0.1 (IS), 5.9 ± 0.0 (RV), 5.9 ± 0.0 (RS).

3.2. Drip loss, water holding capacity and salt content

There was an effect of the storage method on the weight gain of whole fish ($p < 0.001$). A weight gain of 1.6 ± 0.5% was observed when fish was stored for 4 days in RSW. After seawater was drained from the tank and fish were further stored on ice for 3 days, there was a weight loss of 0.7%. Therefore, the overall weight gain for RSW fish after 7 days of whole fish storage was 0.9%. In contrast, the ice stored fish had a relatively stable weight gain of 0.1 ± 0.2% and weight loss of 0.1 ± 0.2% on days 4 and 7, respectively (Fig. 3b). The drip loss of all portions after packaging increased throughout storage (Fig. 3c, $p < 0.001$). Regardless of how the whole fish was stored ($p = 0.115$), skin packaged fillets gave a higher drip loss than the vacuum packaged ones ($p < 0.001$), reaching the highest value of 8.8 ± 1.1% (RS) as compared to 5.9 ± 1.4% (RV) on day 25. The salt content for iced fish remained stable until day 7 (day 4: 0.15 ± 0.01%, day 7: 0.16 ± 0.02%). In contrast, RSW fish gave a higher salt content ($p = 0.027$) and gradually increased until day 7 ($p = 0.006$, day 4: 0.20 ± 0.07%, day 7: 0.23 ± 0.04%).

As seen in Fig. 3d, the storage regime of whole fish did not affect WHC from days 0–7 ($p = 0.333$). However, when only day 7 was compared, it was observed that the WHC of fish stored in RSW was higher than those stored in ice ($p = 0.042$). After packaging, WHC of the portions decreased through storage ($p < 0.001$), but there was no influence of whole fish storage ($p = 0.617$) nor packaging method ($p = 0.087$).

3.3. Colour and texture

Lightness increased ($p = 0.014$), while yellowness decreased ($p = 0.003$) throughout the whole fish storage from days 0–7, and there was no effect of storage treatment on the colour values (L*: $p = 0.372$, a*: $p = 0.738$, b*: $p = 0.664$, C: $p = 0.983$, H°: $p = 0.421$). After packaging, lightness increased ($p < 0.001$), while redness ($p = 0.001$), yellowness ($p < 0.001$), chroma ($p < 0.001$) and hue values ($p < 0.001$) generally decreased throughout storage (Table 1). The storage regime of whole fish affected the redness after packaging, whereby RSW stored fish gave a slightly more reddish colour ($p = 0.016$) and lower hue values ($p = 0.005$) than those stored in ice. In addition, skin packaging gave a lighter ($p = 0.001$), less reddish ($p < 0.001$), less yellowish fillets ($p < 0.001$) and lower chroma value ($p < 0.001$) than vacuum packaged fillets.

The initial firmness of the fillets after slaughter was 31.2 ± 6.0 N. Afterwards, the firmness significantly decreased until day 7 ($p < 0.001$), but no difference between the storage method ($p = 0.949$) nor fillet height ($p = 0.624$) were observed. The firmness of fillets after packaging also generally decreased through increasing storage duration

Table 1
Results of colour and texture analysis from whole fish to packaged fillet portions throughout storage.

Day	Group	Colour					Firmness[b] (N)	n
		L*	a*	b*	Chroma	Hue		
0	Raw	56.6 ± 1.8	37.9 ± 1.1	33.5 ± 1.7	50.6 ± 1.6	41.4 ± 1.3	31.2 ± 6.0	6
4	Ice	58.3 ± 1.9	35.2 ± 1.8	31.7 ± 1.0	47.4 ± 1.7	42.0 ± 1.3	13.6 ± 1.8	5
	RSW	59.3 ± 1.7	35.5 ± 2.7	32.6 ± 2.5	48.2 ± 3.6	42.5 ± 0.4	13.1 ± 1.8	5
7	Ice	58.2 ± 2.9	38.0 ± 1.9	31.5 ± 1.5	49.3 ± 2.4	39.6 ± 0.5	12.2 ± 1.4	6
	RSW	59.2 ± 2.4	37.1 ± 0.5	31.4 ± 1.0	48.6 ± 1.0	40.3 ± 0.6	12.6 ± 2.3	6
11	IV	62.0 ± 1.3	39.8 ± 1.7	36.1 ± 1.8	53.7 ± 2.4	42.2 ± 0.5	9.6 ± 1.4	6
	IS	63.2 ± 1.1	37.3 ± 1.5	32.4 ± 1.8	49.4 ± 2.3	41.0 ± 0.6	9.7 ± 1.4	6
	RV	61.3 ± 2.2	39.6 ± 1.3	35.7 ± 1.3	53.4 ± 1.8	42.0 ± 0.5	10.7 ± 3.3	5
	RS	62.5 ± 2.2	38.5 ± 1.3	33.2 ± 1.5	50.8 ± 1.8	40.7 ± 0.7	10.3 ± 1.1	6
14	IV	61.7 ± 1.6	36.7 ± 1.1	31.5 ± 1.1	48.4 ± 1.5	40.7 ± 0.7	10.4 ± 1.4	6
	IS	62.9 ± 1.6	36.6 ± 1.6	32.1 ± 1.5	48.7 ± 2.2	41.3 ± 0.4	9.0 ± 1.4	6
	RV	60.6 ± 1.3	37.7 ± 1.7	32.7 ± 1.2	49.9 ± 1.9	41.0 ± 0.9	10.8 ± 2.0	6
	RS	61.5 ± 1.2	37.4 ± 1.4	32.5 ± 0.7	49.6 ± 1.4	41.0 ± 0.7	9.6 ± 1.7	6
18	IV	61.5 ± 2.1	35.2 ± 1.2	29.3 ± 0.8	45.8 ± 1.3	39.8 ± 0.7	7.8 ± 1.1	6
	IS	62.5 ± 1.9	34.4 ± 0.9	28.7 ± 1.1	44.9 ± 1.4	39.8 ± 0.6	8.7 ± 2.2	6
	RV	61.2 ± 2.0	36.4 ± 1.5	30.2 ± 1.4	47.3 ± 2.0	39.7 ± 0.7	7.9 ± 1.0	6
	RS	62.5 ± 0.5	35.6 ± 1.5	29.1 ± 1.7	46.0 ± 2.1	39.7 ± 1.0	8.8 ± 1.2	6
21	IV	62.7 ± 2.2	38.2 ± 2.1	31.8 ± 2.0	49.7 ± 2.8	39.7 ± 0.7	8.7 ± 1.0	6
	IS	64.0 ± 2.0	36.1 ± 2.1	29.8 ± 2.0	46.8 ± 2.9	39.6 ± 0.6	8.9 ± 1.1	6
	RV	63.4 ± 1.8	38.2 ± 1.3	31.3 ± 0.8	49.4 ± 1.5	39.4 ± 0.5	9.3 ± 1.7	6
	RS	62.5 ± 1.6	35.6 ± 2.0	29.1 ± 2.0	47.3 ± 2.7	39.0 ± 0.6	9.0 ± 2.3	6
25	IV	63.9 ± 1.7	37.3 ± 1.9	30.4 ± 2.2	48.1 ± 2.8	39.2 ± 1.0	9.3 ± 1.8	6
	IS	65.1 ± 2.2	34.8 ± 1.9	29.1 ± 1.3	45.4 ± 2.2	39.9 ± 0.8	8.9 ± 1.6	6
	RV	64.1 ± 2.7	38.2 ± 1.9	30.8 ± 1.6	49.0 ± 2.5	38.9 ± 0.5	9.1 ± 2.1	6
	RS	65.1 ± 2.2	36.1 ± 1.6	28.8 ± 1.6	46.2 ± 2.2	38.6 ± 0.6	10.0 ± 2.1	6
GLM[a]	P_d	<0.001*	0.001*	<0.001*	<0.001*	<0.001*	<0.001*	
	P_f	0.397	0.016*	0.479	0.094	0.005*	0.002*	
	P_p	0.001*	<0.001*	<0.001*	<0.001*	0.111	0.859	
	P_h	–	–	–	–	–	<0.001*	

* Significant levels with less than 0.05.
[a] General Linear Model (GLM) analysis of variance after portioning from days 11–25 with whole fish storage (RSW, ice) and packaging type (vacuum, skin) as factors, and storage days as covariance. The fillet height was added as a covariance for texture analysis. pd, pf, pp and ph are the significant levels for the effects of the storage days, whole fish storage, packaging type and fillet height, respectively.
[b] Firmness is described by the 80% compression force.

($p < 0.001$). It was further observed that fish initially stored in RSW gave a firmer texture ($p = 0.002$) regardless of the packaging method ($p = 0.859$). In addition, fillet height affected the firmness of fillets ($p < 0.001$).

3.4. Microbiological analysis

The microbial growth was under the quantification limits on days 0 and 4. Thus, the values of TPC (Fig. 4a), TMC (Fig. 4b) and HSPB (Fig. 4c) were assigned to 1.7 ± 0.0, 0.7 ± 0.0 and 0.7 ± 0.0 log cfu/g, respectively. Microbial growth increased during whole fish storage until day 7 (TPC: $p = 0.006$; TMC: $p < 0.001$; HSPB: $p = 0.015$). After packaging, TPC ($p < 0.001$) and TMC ($p < 0.001$) increased from day 7–14 before plateauing. On day 14, the microbial counts for TPC were 6.5 ± 0.1 (IV), 6.6 ± 0.3 (IS), 6.9 ± 0.3 (RV) and 7.1 ± 0.3 (RS) log cfu/g; while those for TMC were 5.1 ± 0.2 (IV), 4.5 ± 0.7 (IS), 5.3 ± 0.2 (RV) and 5.2 ± 0.1 (RS) log cfu/g. HSPB increased until day 18 ($p < 0.001$) and the counts were 4.7 ± 0.2 (IV), 4.7 ± 0.9 (IS), 4.3 ± 0.5 (RV) and 3.7 ± 0.0 (RS) log cfu/g. Storing the fish in RSW gave significantly lower HSPB values after packaging (RS and RV) than iced (IS and IV) fillets ($p = 0.002$). Otherwise, no difference between the treatment groups after packaging was detected.

Table 2 presents the growth kinetics estimations obtained using the Baranyi and Roberts model. All r^2 values obtained were at least 0.91, indicating good agreements with the observed values. The lag phases (λ) for RSW stored fish were generally longer than those on ice, extending 1–2 days for HSPB. Moreover, the maximum population density of RSW fish was lower on TMC and HSPB, but the specific growth rate (μ_{max}) of RSW fish were slightly higher on TPC and TMC.

4. Discussion

This study demonstrated that superchilling whole fish in RSW presented a higher water and salt uptake and a lower HSPB growth than ice-stored fish. After portioning and packaging, the main differences lie in the packaging method. Skin packaged fillets resulted in a higher drip loss with a lighter, less reddish and less yellowish colour than those traditionally vacuum packaged.

The initial pH of 6.2 obtained indicated that fish were in a stressed condition, as a typical pH for rested fish is 7.5 (Erikson & Misimi, 2008; Roth, Grimsbø, Slinde, Foss, Stien & Nortvedt, 2012). In the present study, the decrease in pH observed through packaged storage was likely due to postmortem anaerobic glycolysis. The depletion of glycogen reserves gives rise to the formation and accumulation of lactic acid. This observation was similar to Chan et al. (2021) during cold storage for skin packaged Atlantic salmon fillets.

Previous studies reported that whole fish storage in RSW leads to weight gain (Bronstein, Price, Strange, Melvin, Dewees & Wyatt, 1985; Chan et al., 2020; Erikson et al., 2011; Himelbloom, Crapo, Brown, Babbitt & Reppond, 1994; Perigreen, Pillai, Surendran & Govindan, 1975). In a similar study, Chan et al. (2020) observed an overall weight gain of 0.7% on salmon stored for 4 days in RSW followed by 3 days on ice. The slight difference seen in the weight changes is likely attributed to the difference in the size of whole salmon. In the present study, the increase in drip loss through storage is an established fact of postmortem degradation that increases the amount of free water released as purge between the actin and myosin filaments (Offer & Trinick, 1983). This purge mainly contains water, proteins and lipids (Ofstad, Kidman, Myklebust, Olsen & Hermansson, 1995; Rotabakk et al., 2017).

So far, limited studies have been done on skin packaged fish species. The packaging technique with traditional vacuum and vacuum skin can

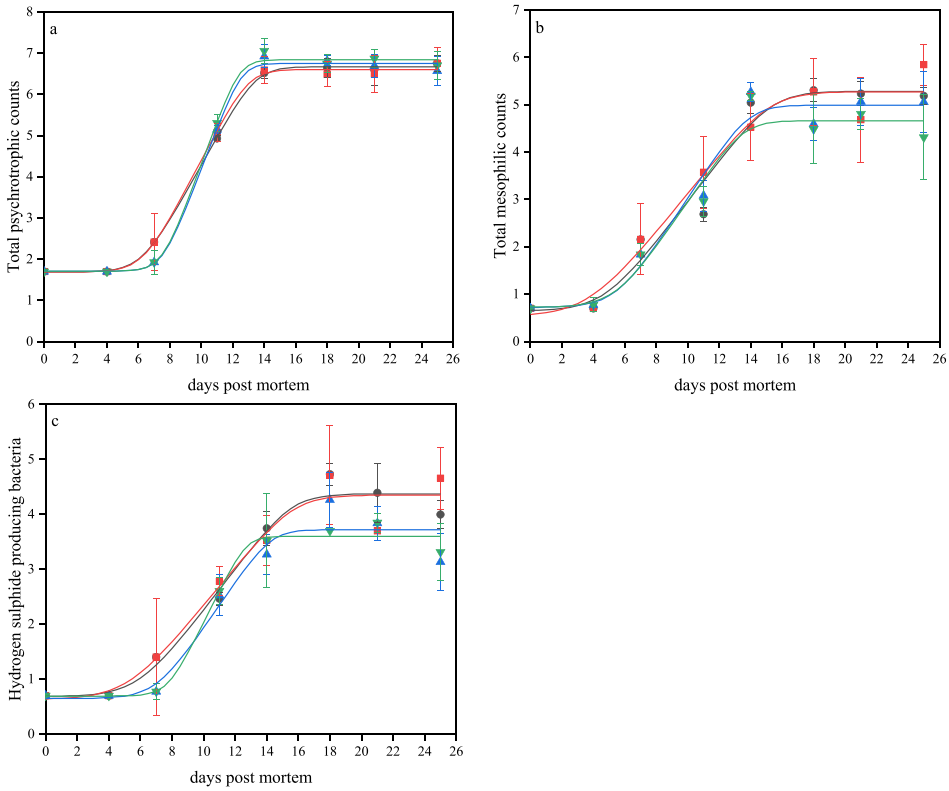

Fig. 4. Graphs showing microbial growth of IV (●), IS (■), RV (▲) and RS (▼) during storage. The solid lines represent the predicted values of IV (—), IS (—), RV (—) and RS (—) with DMFit in ComBase using the Baranyi and Roberts model. Since microbial growth was under the quantification limits on days 0 and 4, the value of 0.5 was assigned as the number of colonies, translating to the respective values in log cfu/g presented in the figures. (a) Total psychrotrophic counts of whole fish (GLM; days: $p = 0.006$, whole fish: $p = 0.247$) and portions after packaging (GLM; days: $p < 0.001$, whole fish: $p = 0.119$, portions: $p = 0.689$), (b) Total mesophilic counts of whole fish (GLM; days: $p < 0.001$, whole fish: $p = 0.681$) and portions after packaging (GLM; days: $p < 0.001$, whole fish: $p = 0.623$, portions: $p = 0.158$), and (c) Hydrogen sulphide producing bacterial counts of whole fish (GLM; days: $p = 0.015$, whole fish: $p = 0.286$) and portions after packaging (GLM; days: $p < 0.001$, whole fish: $p = 0.002$, portions $p = 0.998$). The numbers are expressed in log cfu/g.

Table 2
Parameters of growth kinetics obtained from predictive modelling with DMFit in ComBase using the Baranyi and Roberts model. r^2 depicts goodness of fit. λ, $\log(N_0)$, μ_{max} and $\log(N_{max})$ represent lag time in days, initial population size in log cfu/g, specific growth rate in day^{-1} and maximum population density in log cfu/g. Results are given in mean ± standard error.

Parameters	TPC				TMC				HSPB			
	IV	IS	RV	RS	IV	IS	RV	RS	IV	IS	RV	RS
r^2	1.00	1.00	1.00	1.00	0.94	0.95	0.95	0.93	0.97	0.94	0.91	0.99
λ (day)	6.2 ± 0.2	6.3 ± 0.3	7.5 ± 0.5	7.5 ± 0.5	4.7 ± 2.1	3.8 ± 2.1	5.5 ± 1.6	5.4 ± 2.0	5.9 ± 1.5	5.2 ± 2.4	7.0 ± 2.2	7.3 ± 0.6
$\log(N_0)$	1.7 ± 0.0	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	0.7 ± 0.4	0.6 ± 0.4	0.7 ± 0.3	0.7 ± 0.4	0.7 ± 0.2	0.6 ± 0.4	0.7 ± 0.3	0.7 ± 0.1
μ_{max} (day^{-1})	0.7 ± 0.0	0.7 ± 0.0	1.0 ± 0.1	1.0 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.1
$\log(N_{max})$	6.7 ± 0.0	6.6 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	5.3 ± 0.3	5.3 ± 0.3	5.0 ± 0.2	4.7 ± 0.3	4.4 ± 0.2	4.3 ± 0.3	3.7 ± 0.3	3.8 ± 0.1

render some physical pressure on the food sample that may cause more significant drip loss than other techniques like modified atmosphere packaging (Huff-Lonergan, 2009). This study observed that skin packaged fillets gave a higher drip loss than those traditional vacuum packaged, similar to the study on skin packaged beef retails cuts by Vázquez et al. (2004). In contrast, Strydom and Hope-Jones (2014) reported that skin packaging produces a lower amount of purge due to the lesser space available in the package for purge to be collected. Several factors influence drip loss. Stella et al. (2018) explained that this loss is related to pH, as meat acidification results in a pH closer to the isoelectric point, so muscle proteins are less polar to bind to water molecules. Ofstad et al. (1995) also indicated that a low pH induces drip loss in the muscle. However, this was not observed in this study as the packaging type was the primary determinant for drip loss, but not the storage technique for whole fish. The lack of research on skin packaging for fish species and contradicting results necessitates more studies to be done. As purge can accumulate in the crevices of the plastic pouches during traditional vacuum packaging, this may render the retail package unattractive to consumers. For skin packaged products, absorbent pads are usually included on the base of the trays, which helps absorb the exuded fluid and moisture, and presents a more aesthetically attractive package.

Water holding capacity is an important quality parameter that measures the ability of muscles to retain water. Chan et al. (2020) found that storing whole fish in RSW led to a higher WHC, in line with the result found on day 7 in the present study. This is explained by the "salting in" process at low salt concentrations where swelling of proteins occurs, and the open network in the tissues retain water. The WHC observed in the present study decreased through the storage period, and there was no difference between treatments after filleting and packaging. This confirms that drip loss and WHC are inversely related, as the muscle would hold the remaining water more tightly. pH is also known to influence WHC. A pH close to the isoelectric point would lower WHC as the net charge of myofibrillar proteins becomes closer to 0, and no charge is available to bind the immobilized and water (Huff-Lonergan & Lonergan, 2007). The observed salt content for the RSW stored fish indicated that salt was likely absorbed through the abdominal cavity even when they were removed from the RSW tanks on day 4. This result agreed well with Chan et al. (2020), where a similar RSW tank set up was performed.

Colour properties can change during chilled storage. The observed decrease through storage in redness, yellowness and hue on fillets after packaging was consistent with the results of Chan et al. (2021), who found similar trends in L*a*b* measurements during chilled storage of skin packaged salmon fillets. In addition, Chan et al. (2021) also observed a lighter, less reddish and less yellowish colour for skin packaged fillets than those in modified atmosphere. This increase in lightness may be explained by protein denaturation and the greater drip loss, causing differences in the reflective properties of the fillet surface (Lerfall & Rotabakk, 2016; Robb et al., 2000). Hue represents the observable visual colour. The decrease in hue values indicated that the fillets gradually leaned towards the reddish side (0°) of the spectra. However, this was not explained by an increase in redness, possibly due to the observed decrease in chroma, suggesting that the colour intensity weakened during storage. As observed in this study, the significant decrease in firmness after slaughter implies that muscle softening occurred within the flesh. Further softening after packaging was due to protein denaturation leading the muscle tenderization (Erikson et al., 2011; Hultmann & Rustad, 2002). In correlation to previous research (Chan et al., 2020; Chan et al., 2021), the fillet height can significantly affect the texture properties of the fillet.

To prevent microbiological contamination, the RSW tank was thoroughly washed with concentrated lye before adding seawater. If an aerobic plate count of 6–7 log cfu/g is considered the maximum level of microbial acceptance (Dalgaard, Mejlholm, Christiansen & Huss, 1997; Hansen et al., 1998), the portions were deemed spoiled after day 14. Pérez-Alonso et al. (2004) found that skin packaged Atlantic pomfret fillets had a significantly lower aerobic mesophilic count than those traditionally vacuum packaged. The study of Duran-Montgé et al. (2015) also revealed that superchilled storage (−1 °C) gave the lowest drip loss. In addition, such storage for skin packaged sea bream fillets had the lowest microbial growth compared to refrigerated storage. This shows the potential of superchilling in increasing the microbiological shelf-life, and it would be interesting to study this condition on packaged salmon fillets.

From the r^2 values obtained in this study, the Baranyi and Roberts model fit the experimental values well. Specific spoilage organisms such as HSPB responsible for off-odours can be prevalent in vacuum and air stored fish, resulting in an increased amount of total volatile base nitrogen (TVB-N) (Fogarty et al., 2019). The present study suggested that storing fish in RSW extends the lag phase of HSPB by 1–2 days with a lower maximum population count. Delgado and Sun (2012) reported that shelf-life extension is directly related to the length of the lag phase. Therefore, based on the HSPB counts, RSW storage presents the possibility of an extended shelf-life, which may be attributed to the lower temperature during whole fish RSW storage. Similar to Skare, Chan, Handeland, Løvdal, Lerfall, and Roth (2021), RSW stored fish resulted in lower HSPB counts. Eliasson, Arason, Margeirsson, Bergsson, and Palsson (2019) also revealed that superchilling whole gutted Atlantic cod in RSW onboard fishing trawlers at −1 °C slowed the growth of HSPB and production of TVB-N. They further observed that the effect of superchilling onboard did not show a noticeable effect of shelf-life extension, which could be explained by the short storage time of fish in RSW during transportation. The superchilled storage of fillets gave a more obvious extension of freshness and shelf-life. Nevertheless, slaughtering fish by the cage in slaughter vessels and superchilling them in RSW during transport can give a comparably good quality as storing on ice, which is currently practiced today. Further studies could observe the differences in these 2 storage methods on sensory quality aspects and shelf-life in the future.

5. Conclusion

We conclude that whole gutted fish superchilled in RSW have higher water and salt uptake, retain water better and provide overall good quality traits as ice-chilled fish. Further packaging of the fillet portions provides an extension in microbiological shelf-life. Predictive microbiology presented good agreements with the experimental values and indicated that the lag times of bacterial growth was extended in RSW fish compared to the iced fish. Traditional vacuum packaging better affects drip loss with a darker and more reddish and yellowish colour. Nonetheless, the application of superchilled RSW storage combined with vacuum skin packaging may be a possible strategy for consumer preferences due to its more attractive packaging. Further research is recommended to utilize the potential of this packaging method.

CRediT authorship contribution statement

Sherry Stephanie Chan: Conceptualization, Methodology, Formal analysis, Writing – original draft; Bjørn Tore Rotabakk: Methodology, Formal analysis, Writing – review & editing; Trond Løvdal: Methodology, Formal analysis, Writing – review & editing; Jørgen Lerfall: Methodology, Formal analysis, Writing – review & editing, Supervision; Bjørn Roth: Project administration, Conceptualization, Methodology, Writing – review & editing, Supervision.

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CRediT authorship contribution statement

Sherry Stephanie Chan: Conceptualization, Methodology, Formal analysis, Writing- original draft; **Bjørn Tore Rotabakk:** Methodology, Formal analysis, Writing- review and editing; **Trond Løvdal:** Methodology, Formal analysis, Writing- review and editing; **Jørgen Lerfall:** Methodology, Formal analysis, Writing- review and editing, Supervision; **Bjørn Roth:** Project administration, Conceptualization, Methodology, Writing- review and editing, Supervision.

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Modelling water and salt diffusion of cold-smoked Atlantic salmon initially immersed in refrigerated seawater versus on ice

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ABSTRACT

The effect of dry salting during the cold-smoking process was evaluated on Atlantic salmon initially stored in refrigerated seawater (RSW) or ice. A 2D mathematical model was developed from first principles, simulating the heat and mass transfer process during dry salting at increasing salting duration. This model was validated using experimental values and compared with the empirical model of Zugarramurdi and Lupín. The predicted values were used for water activity prediction and validated. It was found that salting duration influenced drip loss, redness and yellowness, texture, water activity, salt uptake and water loss. Smoked salmon from RSW fish were more reddish with a lower water activity than iced fish after vacuum storage. Drip loss and colour were significantly influenced by the processing step (salting, smoking and storage). Overall, the model presented reasonable predictions for temperature, salt and water content, water activity and was in close agreement with the empirical model.

1. Introduction

As the population becomes more mindful of their health, "The Keyhole" is a voluntary Nordic label on food packages that encourages consumers to choose healthier products. In Norway, to obtain this label for cold-smoked salmon, the regulation states that the final salt (sodium chloride, NaCl) content must be less than 3 g NaCl/100 g product (Ministry of Health and Care Services, 2015). Cold-smoked salmon is a lightly preserved fish product with a salt content of 1.7–5.1 % in the water phase. Its water content is between 65 and 70 % (Cardinal et al., 2004) and pH between 5.8 and 6.3 (Hansen et al., 1995).

The recent advancement in the salmon processing industry introduces a novel fish slaughter method that effectively circumvents several steps in the value chain. This method slaughters fish directly by the net pens onboard a slaughter vessel followed by superchilling (<0 °C) them in refrigerated seawater (RSW) tanks during transportation. The study of Chan et al. (2020a) showed that immersing fish in RSW leads to slightly higher water and salt uptake than the traditional method of storing on ice. Nevertheless, there were minimal differences in quality when both groups were cold-smoked, and the primary determinant was storage duration. This gives an insightful notion that RSW fish can also produce high-quality cold-smoked salmon like those on ice. Thus, in addition to the successive benefits the new slaughter method brings (for instance, reduced environmental impact, shortened lead time and increased fish welfare), there presents a possibility that this slaughter method could revolutionize the fish processing industry.

Water activity (a_w) is a dimensionless physical parameter that measures the availability of water and is related to microbial growth. Various standard salting procedures are used in the salmon processing industry that can change the product's functionality, such as organoleptic properties, dehydration, solubilizing proteins and changing osmotic pressure to prevent microbial growth. Dry salting is one of the oldest food preservation methods by depressing a_w. It enhances shelf life by spreading crystalline NaCl on the product's surface until it diffuses into the product and equilibrates. The primary process in salting is diffusion, causing counter current water and salt transport between the salt and muscle and resulting in a high salt concentration in the fillet surface during the smoking process (Lerfall et al., 2011). The conformation of muscle protein is affected, causing changes in water holding capacity (WHC) and potentially, protein denaturation. At low salt

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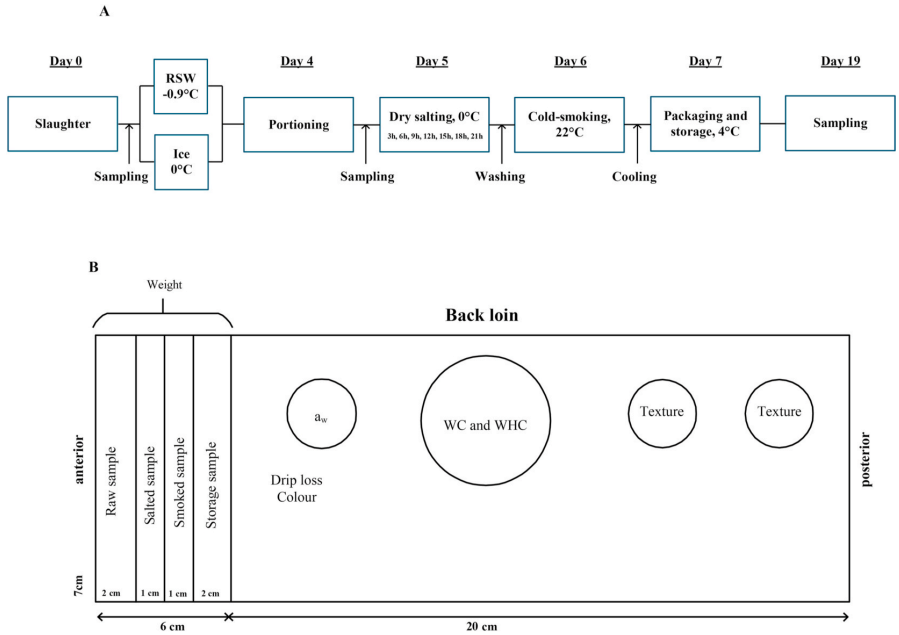

Fig. 1. (a) Graphical illustration of the experimental timeline. (b) Graphical illustration where sampling was done on the fillet portion after 12 days of storage, on day 19 post mortem. Small pieces were cut off (total of 6 cm) at every processing step and deep-frozen as backup analysis.

concentrations (<5–6%), a lower degree of protein denaturation occurs and causes swelling from the electrostatic repulsion of chloride ions weakly attached to the myofibrillar and sarcoplasmic proteins (Larsen and Elvevoll, 2008; Offer and Trinick, 1983). This expands the filament lattice and entraps free water, leading to an increase in WHC. Therefore, salting influences several quality parameters like WHC, taste, shelf life, texture, colour and fillet yield in the final smoked product (Birkeland and Bjerkeng, 2005; Birkeland et al., 2004; Løje, 2007).

The salting process can be simulated with a modelling approach. Quality measurements for food can often be time-consuming and dependent on laboratory-based premises. Some of the bottlenecks in laboratory analysis include cost, rate and labour intensity. Mathematical modelling is a powerful tool that can be used as an alternative for a wide variety of purposes in the food industry. For the past decades, it has received much attention in food science, technology and engineering, significantly reducing the experimentation process (Banga et al., 2008; Datta, 2008). The heat and mass transport phenomena in food can be described with coupled partial differential equations (PDEs) under various assumptions (including appropriate initial and boundary conditions) and solved numerical method to predict the state variables (e.g., temperature, concentration) as a function of space and time. The model predictions are validated with experimental data. The established model can aid in predictions, process designing and optimization in the industry concerning food quality and safety. Several researchers have used numerical methods based on mechanistic principles to study the prediction of heat and mass transfer in a convection oven heating process on fish and meat products like cod (Blikra et al., 2019), chicken (Rabeler and Feyissa, 2018), chicken patties (Chen et al., 1999) and pork (Feyissa et al., 2009, 2013). Modelling the mass transfer phenomena has also been done in salting of cod (Andrés et al., 2002; Blikra et al., 2020), brining on herring (Laub-Ekgreen et al., 2019) and brining (Wang et al., 1998, 2000) and dry salting on salmon (Martínez-López et al., 2019).

On the other hand, empirical models are built solely on mathematical equations developed from experimental data. The application of empirical models, like the Zugarramurdi and Lupín (1980) model (Z&L) for fish salting, is also used in predicting the development of average salt and water concentration over time. This uses an exponential approach to the equilibrium values and has been verified on several fish species like catfish (Corzo et al., 2015), sardines (Bellagha et al., 2007) and anchovies (Bellagha et al., 2007).

A better understanding of salting kinetics can help industries evaluate existing conditions and develop new products. Therefore, this study aims to compare the quality parameters of cold-smoked salmon produced from fish initially chilled in RSW and on ice subjected to different salting times. A mathematical model was developed to predict the temperature during salting, water and salt profiles, and water activity at increasing salting duration with a fixed cold-smoking procedure. In addition, to demonstrate the robustness of the model for RSW and ice stored fish, it was compared to the empirical model of Zugarramurdi and Lupín (1980).

2. Materials and methods

2.1. Experimental design

Before the experiment, an 800-L polyethylene tank was obtained and thoroughly washed with lye before filling in refrigerated seawater (RSW, 3.5 % salinity) kept at a temperature between −0.5 and −1 °C. After that, 54 farmed Atlantic salmon (*Salmo salar*) were obtained from a local slaughterhouse (November 2020) with an average weight of 3.6 kg (starved for 9 days, core temperature ~1.6 °C). The fish were electrically stunned, thoroughly bled, and gutted before being weighed and packed in either the RSW tank (n = 24) or expanded polystyrene (EPS) boxes containing ice (n = 24). Six fish were used to sample for raw material determination of pH, water content (WC), water holding capacity (WHC), salt, protein, and fat content. In each group, two TrackSense Pro temperature loggers (Ellab A/S, Denmark) were inserted into the mid-abdomen of two random fishes while one logger was placed in the surrounding environment. The tank and boxes were transported to the laboratory within 2 h and kept in a 0 °C storage room for 4 days. The

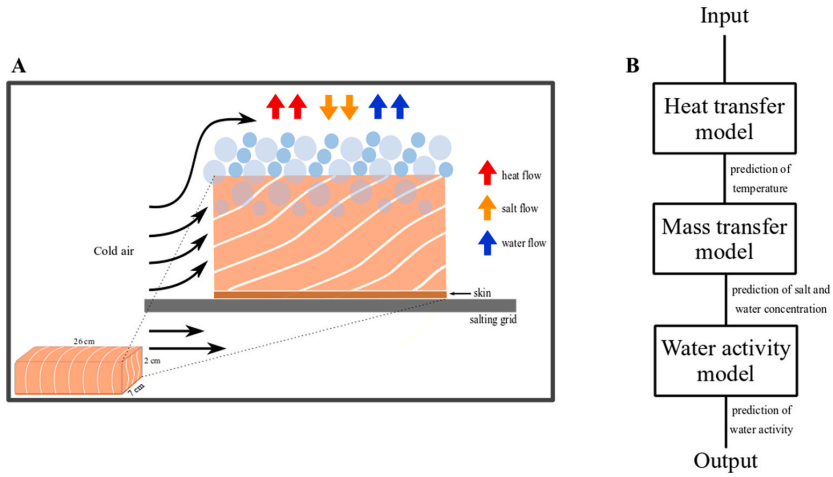

Fig. 2. (a) Transport process during dry salting in a cold room. (b) Input and output parameters used for prediction.

tank's temperature was monitored continuously and kept below 0 °C by adding clean seawater ice, similar to the study of Chan et al. (2020a). A graphical illustration of the experimental timeline is shown in Fig. 1a.

2.2. Processing and quality analysis

On day 4, the RSW tank was drained and the fish were gently wiped with paper. All fish from both groups were individually weighed and manually filleted, and five fish from each group were used for sampling. The remaining fillets were portioned on the back loins into rectangular parallelepiped-shaped samples (26 cm × 7 cm x 2 cm). Weights and colour measurements were taken prior to dry salting the samples' surface with excess refined salt (99.8 % NaCl, GC Rieber, Norway) in randomized grids at 0 °C for every 3 h interval until 21 h. Thermocouples type K (PR Electronics Inc., USA) was inserted at the center of one random salmon portion. The temperature was logged in an Eval Flex recorder every second (Eval Flex, Denmark). After salting, the portions were washed with cold tap water (6–8 °C), gently dried and weighed before cold-smoking using the alternating drying and smoking protocol of Birkeland and Skåra (2008). After smoking, the portions were cooled overnight at 1 °C, then vacuum-packaged (99.9 % vacuum) and stored for 12 days at 4 °C. Sampling was done after storage, as seen in Fig. 1b. Colour analyses were also taken after smoking and after storage. The weights of the portions were recorded during every processing step before cutting a small piece of sample on the anterior part and frozen at −80 °C for further analysis. The weight change is calculated as the % difference with respect to the initial weight before processing.

For quality analysis, WHC was measured based on the centrifugation method of Skipnes et al. (2007), which also calculates WC. Acidity was measured using a portable pH meter (Mettler Toledo SevenGo pro, Mettler Toledo Inc, USA). Water activity (a_w) analysis was done using the NMKL (2001) method no. 168 with an a_w meter (AquaLab Series 3, METER Group, USA). Fat and protein content were extracted using the Bligh and Dyer (1959) and the Kjeldahl method (AOAC 928.08). Salt analysis was done using the automatic titration method with 0.1 M $AgNO_3$ (VWR International, Norway) using SI Analytics Titroline 7000 (Xylem Analytics, Norway) (Chan et al., 2020a). Colour analysis was implemented using the DigiEye complete system (VeriVide Ltd, UK) connected to a Nikon D80 camera (Nikon Corp, Japan). The L*a*b* values were calculated using the DigiPix software (VeriVide Ltd, UK), where L* represents lightness, a* redness and b* yellowness (CIE, 1994). The ΔE value was further calculated by

$$\Delta E = \sqrt{\left(L_2^* - L_1^*\right)^2 + \left(a_2^* - a_1^*\right)^2 + \left(b_2^* - b_1^*\right)^2} \quad (1)$$

Texture analysis was performed in replicates with the puncture test using a Texture Analyzer TA-XT plus (SMS Ltd, UK) connected to a 12.5 mm flat-ended cylinder at a constant speed of 2 mm/s. The force-time graph was recorded using the Texture Exponent software, and the force to press the cylinder down to 80 % of the fillet height represents the firmness.

2.3. Statistical analysis

Statistical analysis was performed using Minitab® 19. The association of treatment (RSW, ice), salting time (3 h–21 h), processing step (raw, smoked, stored), and response variables were analyzed using the general linear model (GLM) at a 95 % confidence interval. Fillet height was added as a covariate for texture analysis. Normality assumptions were tested before the analyses. The root mean square error (RMSE) was used to evaluate the performance between the experimental data and model predictions. All results are presented as mean ± standard deviation and statistical significance set at $p \leq 0.05$.

3. Model formulation

Dry salting in cold storage is a cooling process involving heat transfer from the product to the surrounding air. The primary mechanism during salting of salmon is molecular diffusion through the aqueous phase of the muscle, which induces water from the muscle fibers to migrate out (Gómez-Salazar et al., 2015) (Fig. 2a). During dry salting and cold-smoking, cold air is transferred by convection to the product surface and conduction within the product. The temperature at the centre of the salmon portion in cold storage during salting was first predicted using the input parameters, including the estimated heat transfer coefficient, coupled with the moisture and salt transport. Afterwards, the obtained moisture and salt concentrations were used to predict the water activity (Fig. 2b).

The model focused mainly on water and salt transport. Certain assumptions were made for model simplification to formulate the coupled heat and mass transfer for a rectangular parallelepiped shape of fish meat, as follows. There was no internal heat generation. The shrinkage during salting was also neglected. Furthermore, the skin was present only on the bottom side, so it did not hinder water and salt transport. Since the length of the sample was much larger than the thickness of the

Table 1
Model input parameters.

Parameter	Symbol	Value	Unit	Source
Initial temperature	T_0	279.35	K	measured
Initial composition				
protein	y_p	0.22	kg/kg	measured
fat	y_f	0.09	kg/kg	measured
water	y_w	0.661	kg/kg	measured
ash	y_a	0.018	kg/kg	Aas et al. (2019)
Initial concentration				
salt				
RSW	C_{s0}	0.002	kg/kg	measured
ice		0.0015	kg/kg	measured
water				
RSW	C_{w0}	0.668	kg/kg	measured
ice		0.66	kg/kg	measured
Surrounding concentration				
salt	C_{s1}	0.15	kg/kg	estimated
water vapour in ambient air	C_{w1}	0.05	kg/kg	Rabeler and Feyissa (2018)
Density				
water	ρ_w	998	kg/m^3	Rao et al. (2014)
protein	ρ_p	1330	kg/m^3	Rao et al. (2014)
fat	ρ_f	926	kg/m^3	Rao et al. (2014)
ash	ρ_a	2424	kg/m^3	Rao et al. (2014)
fish	ρ_s	1071	kg/m^3	calculated
Moisture diffusion coefficient	D_w	3.98×10^{-10}	m^2/s	Martínez-López et al. (2019)
Salt diffusion coefficient	D_s	6.64×10^{-10}	m^2/s	Akköse and Aktaş (2016)
Heat transfer coefficient	h_a	15.0	W/(m^2 K)	estimated
Thermal conductivity of fish	k_s	0.47	W/(m K)	Rao et al. (2014)
Mass transfer coefficient between muscle and salt	$k_{m,s}$	3.54×10^{-7}	m/s	Martínez-López et al. (2019)
Mass transfer coefficient between muscle and water	$k_{m,w}$	1.73×10^{-8}	m/s	estimated
Specific heat capacity salmon	c_p	3436	J/(kg K)	calculated

sample, 2D geometry was used for the modelling.

3.1. Calculation of heat transfer coefficient

The heat transfer coefficient of convective heat transfer during the salting and cooling process was determined by comparing the experimental and predicted temperature profiles. Thermocouples type K were also inserted in the center of three Teflon cylinders (d = 2 cm, x = 20 cm) hanged at the bottom, middle and top positions of the salting grid concurrent to the salmon salting process in the cold room. The temperature was also logged in an Eval Flex every second until it stabilized, and the average temperature was calculated. The lumped system analysis was first tried to calculate the heat transfer coefficient using Eqs. (2a) and (2b) (Isleroglu and Kaymak-Ertekin, 2016).

$$\ln\left(\frac{T_\infty - T(t)}{T_\infty - T_0}\right) = -bt \tag{2a}$$

$$b = \frac{h_a A}{\rho C_p V} \tag{2b}$$

where h_a is the combined heat transfer coefficient (W/(m^2 K)), T_∞ is the surrounding temperature (K), T_0 is the initial temperature (K), A is the surface area (m^2), V is the volume (m^3), ρ is the density (2200 kg/m^3), c_p is the specific heat capacity (1172 J/(kg K)) of the material, and t is time (s). The obtained h_a was 13.2 W/(m^2 K) with an RMSE value of 1.50 W/(m^2 K). However, as the Biot number of the Teflon cylinder was slightly higher than 0.1, this obtained value may be inaccurate. Hence, a heat transfer model of the Teflon cylinder was predicted in COMSOL Multiphysics as described in Section 3.3.1 and 3.4 using the reverse estimation method, replacing the thermophysical properties with those of Teflon. The h_a value was adjusted until the predicted and measured temperature profiles showed close agreement. The final value obtained was 15.0 W/(m^2 K) and RMSE value 0.15 W/(m^2 K), which was a better fit than the Lumped capacity method. This value was therefore used as the input parameter for modelling water and salt transport.

3.2. Thermophysical properties

The model input parameters are presented in Table 1. The density of salmon and its heat capacity was estimated from its composition using Eqs. (3) and (4), respectively (Choi and Okos, 1986).

Density of salmon:

$$\rho = \frac{1}{\sum \frac{y_i}{\rho_i}} \tag{3}$$

Specific heat capacity of salmon:

$$c_p = \left(2y_p + 2y_f + 4.2y_w + 2.4y_a\right)\cdot 10^3 \tag{4}$$

where ρ, ρ_w, ρ_p, ρ_f and ρ_a are the densities (kg/m^3) of fish, water, protein, fat and ash respectively, y_i is the mass fraction of each component and c_p is the specific heat capacity of fish (J/(kg K)). Thermal conductivity of 0.47 W/(m K) was used (Rao et al., 2014).

3.3. Governing equations

3.3.1. Heat and mass transfer

The heat transfer within the salmon muscle is based on the heat diffusion equation, given by

$$\frac{\partial T}{\partial t} = \frac{k_s}{c_p \rho_s}\nabla^2 T \tag{5}$$

where c_p, ρ_s and k_s are the specific heat capacity (J/(kg K)), density (kg/m^3) and thermal conductivity (W/(m K)). ∇ is the Nabla operator, i.e. partial derivative in x, y and z-direction $\left(\nabla = \frac{\partial}{\partial x} + \frac{\partial}{\partial y} + \frac{\partial}{\partial z}\right)$. T is the temperature (K), and t is the time (s).

The governing equation for mass transfer of water (Eq. (6a)) and salt (Eq. (6b)) within the muscle is based on the conservation of mass (Fick's second law), given by

$$\frac{\partial C_w}{\partial t} = D_w \nabla^2 C_w \tag{6a}$$

$$\frac{\partial C_s}{\partial t} = D_s \nabla^2 C_s \tag{6b}$$

where C_w and C_s are the water and salt concentrations (kg/kg), respectively. D_w and D_s are the water and salt diffusion coefficients (m^2/s), respectively.

3.4. Initial and boundary conditions

We assume a uniform initial temperature, moisture, and salt distribution throughout the whole sample.

$$T(x, y, z, 0) = T_0 \tag{7}$$

$$C_w(x, y, z, 0) = C_{w0} \tag{8a}$$

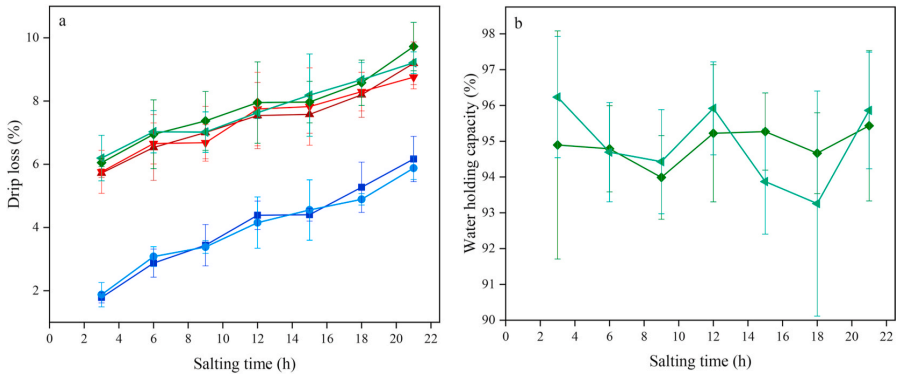

Fig. 3. (a) Drip loss for RSW fish after salting (■), ice fish after salting (●), RSW fish after smoking (▲), ice fish after smoking (▼), RSW fish after storage (◆), ice fish after storage (◀) (GLM; salting hour – after salting: $p < 0.001$, after smoking: $p < 0.001$, after storage: $p < 0.001$; treatment – after salting: $p = 0.596$, after smoking: $p = 0.951$, after storage: $p = 0.499$). (b) WHC for RSW fish after storage (◆), ice fish after storage (◀) (GLM; salting hour: $p = 0.895$, treatment: $p = 0.975$). All results are presented in mean ± standard deviation.

$$C_s(x, y, z, 0) = C_{s0} \tag{8b}$$

where is T_0, C_{w0} and C_{s0} are the initial temperature (K), water and salt concentration (kg/kg) respectively.

The boundaries that are exposed to the surrounding air and heat flux is given by:

$$-k_s \nabla T = h_a (T_\infty - T_s) \tag{9}$$

where k_s is the thermal conductivity of salmon (W/(m K)),h_a is the heat transfer coefficient (W/(m^2 K)), T_∞ is the surrounding temperature (K) and $T_s$is the surface temperature (K) of the salmon.

The boundary condition for the mass transfer is:

$$D_w \nabla C = k_{m,w} (C_{w1} - C_w) \tag{10a}$$

$$D_s \nabla C = k_{m,s} (C_{s1} - C_s) \tag{10b}$$

where $k_{m,w}$ and $k_{m,s}$are the mass transfer coefficient (m/s) between muscle and water, and muscle and salt, respectively. C_{w1} and C_{s1} are the concentration of water vapour in the surrounding air and salt (kg/kg), respectively. C_w and C_s are the concentration of water vapour and salt at the surface of the fillet (kg/kg), respectively.

3.5. Calculation of water activity

Water activity can be calculated as a salt molality function that couples salt and water transport, given by Eq. (11) (Martínez-López et al., 2019; Pazuki, 2005).

$$\ln\ a_w = -M_w \cdot \varphi \cdot \upsilon \cdot m = -\frac{M_w}{M_s} \varphi \cdot \upsilon \cdot \frac{C_s}{C_w} \tag{11}$$

where M_w and $M_s$are the molecular weights of water (0.018 kg/mol) and salt (NaCl, 0.0583 kg/mol), φ is the osmotic coefficient, υ is the number of solute ions (2), C_s and C_w are the water and salt concentrations on wet basis (kg/kg). The value of the osmotic coefficient as a function of molality at 0 °C was obtained from Pitzer et al. (1984). The value of molality was derived from the equation given by Fernández-Salguero et al. (1993).

3.6. Comparison with Zugarramurdi and Lupín model

As the Z&L model has been used previously for fish salting processes, this exponential approach to the equilibrium values of salt and water concentrations was included to compare with the numerical model:

$$\text{Salt uptake: } X_s = X_s^0 e^{-k_s t} + X_s^1 \left(1 - e^{-k_s t}\right) \tag{12}$$

$$\text{Water exudation: } X_w = X_w^0 e^{-k_w t} + X_w^1 \left(1 - e^{-k_w t}\right) \tag{13}$$

where X_s^0 and X_s^1 are initial and final salt contents (kg/kg), X_w^0 and X_w^1 are initial and final water contents (kg/kg). k_s and k_w are two theoretical coefficients (h^{-1}) calculated based on the experimental values obtained from 3 h salting time, while the equilibrium values used were obtained from 21 h salting time.

3.7. Model solution and validation

The mathematical model with coupled PDE of heat and mass transfer was solved in COMSOL Multiphysics v5.5 using the finite element method (FEM). A 2D rectangular geometry representing the salmon portion thickness with dimensions 7 cm × 2 cm was created and meshed. The salt bulk concentration was estimated to be 15 % using trial and error to minimize the RMSE value between predicted and obtained value. For the determination of salt transport parameters, the data sets of 0 h and 21 h from the RSW fish were used, and the rest for validation. For the determination of water transport parameters, the data sets from 0 h to 18 h from RSW fish were used, and the rest for validation.

4. Results and discussion

4.1. Initial composition, drip loss and water holding capacity

The pH and initial chemical composition of the sampled fish on day 0 after slaughter were 6.2 ± 0.0, 22.0 ± 0.6 % (protein), 9.2 ± 0.9 % (fat), 66.1 ± 3.1 % (water) and 0.1 ± 0.1 % (salt). The temperature during RSW and ice storage was kept stable until day 4 at around −0.9 °C and 0 °C, respectively (data not shown). On day 4, the RSW fish gained 1.6 ± 0.5 % while iced fish remained stable at 0.1 ± 0.2 % in weight ($p < 0.001$), but there were no differences in salt content ($p = 0.173$; RSW: 0.2 ± 0.1 %, ice: 0.2 ± 0.0 %). The results were consistent with Chan et al. (2020a), where salt uptake was similar for both treatments while RSW fish gained 1 % weight after 4 days.

Weight gain during RSW storage is a common phenomenon, as observed in several studies on various eviscerated salmon species (Bronstein et al., 1985; Erikson et al., 2011). When slaughtered fish is kept in seawater, the flesh equilibrates with the surrounding solution and increases in weight (MacLeod et al., 1960). As Erikson et al. (2011) explained, the weight gain observed in the present study probably came

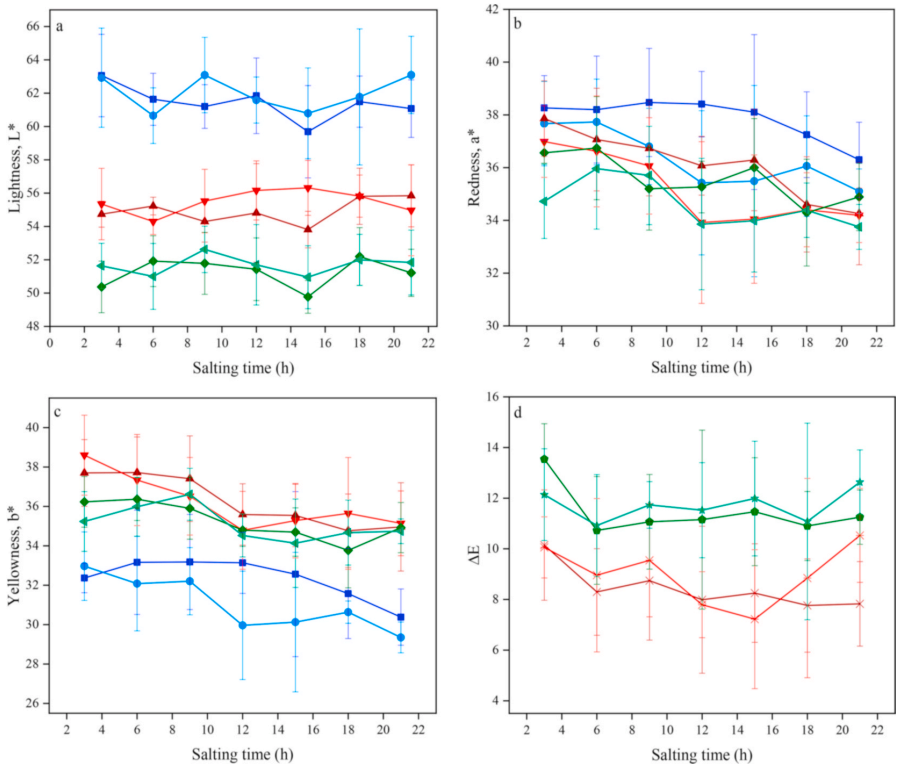

Fig. 4. Colour parameters of RSW raw fish (■), ice raw fish (●), RSW fish after smoking (▲), ice fish after smoking (▼), RSW fish after storage (◆), ice fish after storage (◀) (a) lightness, L* (GLM; salting hour: $p = 0.947$, treatment: $p = 0.029$, processing step: $p < 0.001$). (b) Redness, a* (GLM; salting hour: $p < 0.001$, treatment: $p < 0.001$, processing step: $p < 0.001$). (c) yellowness, b* (GLM; salting hour: $p < 0.001$, treatment: $p = 0.052$, processing step: $p < 0.001$). (d) ΔE of RSW fish after smoking (×), ice fish after smoking (✳), RSW fish after storage (●), ice fish after storage (★) (GLM; salting hour: $p = 0.219$; treatment: $p = 0.204$; processing step: $p < 0.001$). All results are presented in mean ± standard deviation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

mainly from the abdominal cavity since the salmon were bled and gutted. However, the % weight gain may vary according to species size and be unapparent, especially during the initial storage days when the differences are minor. Furthermore, Tomlinson et al. (1965) reported that the stress response before slaughter might influence the weight changes. As the initial pH observed in this study was lower than a typical pH of 7.5 for rested fish (Erikson and Misimi, 2008; Roth et al., 2012), this suggested that the fish used were possibly in a stressed condition during slaughter. Tomlinson et al. (1965) further found that the uptake of sodium ions significantly penetrated trout muscle only after adenosine triphosphate of the muscle was used. There was also a difference between the stress status, where unexercised fish initially lost weight while stressed fish gained weight after immersion. Hence, several factors could explain the rate of weight changes during RSW storage.

Heavy salting causes a significant weight reduction due to the consequential osmotic pressure of the salt on the moisture of muscle cells (Lauritzsen et al., 2004). The drip loss for both treatments increased linearly through increasing salting time at every processing step after salting ($p < 0.001$), after smoking ($p < 0.001$) and after 2 weeks of storage ($p < 0.001$, Fig. 3a). This result was expected since salting is a diffusion process, and water is dragged out of the muscle until equilibrium is attained. The highest drip loss was observed after storage, where drip loss ranged from 6.1 % to 9.7 % for RSW fish and 6.2 %–9.2 % for iced fish salted for 3 h and 21 h, respectively. When both treatments were compared, no effect in drip loss during the different processing steps was found. In line with previous studies (Chan et al., 2020a, 2020b), there were no differences in drip loss on smoked salmon from RSW treated or iced fish.

The most significant loss occurred after the smoking process, with the highest loss for fillets salted for 21 h (RSW: 9.2 ± 0.7 %, ice: 8.8 ± 0.4 %). The process yield decreased with increasing salting time from 94 % to 91 % in both groups, which was close to the previously reported values of 86%–92 % (Birkeland et al., 2004; Lerfall and Rotabakk, 2016; Sigurgisladottir et al., 2000). This decrease in yield can be explained by the increasing salt content, which decreases the hydrophilic surface and enhances protein-protein interaction, causing water loss (Bjørnevik et al., 2018). As cold-smoking involves both drying and smoking operations, the drying process also causes heat or pressure to expel water from the product's interior and mechanical energy to remove water from the surface (Sebastian et al., 2005). In addition, the smoking process allows the product to absorb volatile components, which provides antioxidant and antimicrobial effects and the required taste (Sebastian et al., 2005). In this study, vacuum storage of the smoked salmon for a further 2 weeks only gave a 1 % loss in drip. Like the findings of Løje (2007), little liquid was lost during cold vacuum storage of smoked salmon for almost 3 weeks. The liquid loss during vacuum storage can be related to the fatty acid profile, where a high liquid loss is linked to high amounts of monounsaturated and n-6 fatty acids (Lerfall et al., 2016).

The initial WHC after slaughter was 93.9 ± 0.9 %. On day 4, the WHC for RSW and iced fish were 93.7 ± 2.1 % and 94.1 ± 2.5 %, respectively.

Table 2
Firmness (80 % compression force) of smoked fillets after storage through different salting time. [a]General Linear Model (GLM) analysis of variance was done with treatment as a factor and salting time and fillet height as covariates. p_S, p_T and p_H are the significant levels for the effects of salting time, treatment, and fillet height, respectively. All results are presented in mean ± standard deviation.

Firmness(N)		Salting time (h)						
		3	6	9	12	15	18	21
RSW		17.3 ± 2.3	19.3 ± 2.4	16.2 ± 3.2	19.8 ± 3.9	19.3 ± 1.3	21.1 ± 5.7	20.6 ± 3.6
ice		17.8 ± 3.4	18.1 ± 2.6	17.9 ± 4.1	20.5 ± 3.3	20.6 ± 5.4	19.9 ± 2.6	18.0 ± 3.5
GLM[a]	p_S				0.021*			
	p_T				0.986			
	p_H				<0.001*			

The WHC observed after vacuum storage for different salting times appears not to give a general trend. For iced fish, WHC for 3 h salted fillets were at 96.2 ± 1.7 % as compared to 94.9 ± 3.2 for RSW fish (Fig. 3b). This was reversed after 15 h (iced: 93.9 ± 1.5 %, RSW: 95.3 ± 1.1 %) and 18 h (iced: 93.3 ± 3.1, RSW: 94.7 ± 1.1 %) of salting. As observed in other studies (Chan et al., 2020b; Løje, 2007), chilled storage of smoked salmon decreases WHC due to changes in water distribution. A higher salt content would also lead to a higher WHC (Løje, 2007). However, these observations were not seen in the present study when comparing the WHC of smoked and raw fillets. This might be explained by the variations in lipid content which can also influence other factors like salt content.

4.2. Effects on colour and texture

The colour of cold-smoked salmon plays an important role in the purchasing decisions of consumers. In this study, statistical analysis showed that the processing step (raw, smoked, stored) affected the colour parameters (Fig. 4a–c; L*: $p < 0.001$; a*: $p < 0.001$; b*: $p < 0.001$), and treatment affected L* ($p = 0.029$) and a* ($p < 0.001$). The dry salting and cold-smoking process significantly lowered lightness ($p < 0.001$) and redness ($p < 0.001$), and increased yellowness ($p < 0.001$) respective to the unprocessed fillets. This is a general trend for smoked salmon fillets, as confirmed in previous studies (Birkeland et al., 2004; Cardinal et al., 2001; Chan et al., 2020a, 2020b; Løje, 2007), and is due to the physical and chemical reactions that occur between the product and smoking compounds during smoking (Pittia and Antonello, 2016). Dry salting can affect colour and texture due to protein denaturation and precipitation in the muscle. Carotenoids, the pigments that give the red colouration in salmon, can be lost during processing due to their decomposition or extraction during the dry salting procedure (Lerfall et al., 2016).

Further vacuum storage for 2 weeks showed a greater reduction in colour (L*a*b*) than the freshly smoked counterparts. This is in agreement with Chan et al. (2020a) and could be explained by the liquid leakages accumulated during vacuum storage that can negatively affect the product appearance (Birkeland et al., 2004). As colour measurements are sensitive to fillet surface changes, possibly influenced by water content or surface structure, light scattering properties could be affected and make the fillet appear darker (Bjørnevik et al., 2018). There was no significant difference in salting duration and treatment on L* when individual processing steps were compared. As salting duration increases, there was a decreasing trend on a* (smoked: $p < 0.001$; stored: $p = 0.002$) and b* (smoked: $p < 0.001$, stored: $p = 0.001$), and smoked fillets initially stored in RSW appeared more reddish than those in ice (smoked: $p = 0.023$; stored: $p = 0.015$).

Total colour change (ΔE) helps determine the colour differences during storage (Fig. 4d). In this study, both smoking and storage altered the surface properties, and the ΔE was more significant after storage than after smoking ($p < 0.001$). Earlier studies reported a stepwise increase in ΔE from dry salting to cold-smoking (Birkeland and Bjerkeng, 2005). The smoking process influences colour through carbonyl-amino reactions of Maillard browning and protein and lipid oxidation (Hall, 2011). Besides, the observed colour differences in L*a*b* and ΔE may be explained by how the smoke components reacted with the chemical compounds like fatty acids in the muscle (Lerfall et al., 2016). Lerfall et al. (2016) and Lerfall and Rotabakk (2016) reported that the colour of refrigerated vacuum stored smoked salmon was restored and more similar to the raw fillets. This was not observed in this study, possibly due to the denaturation of the surface or other mechanisms that affect surface properties as a function of time.

Before salting on day 4, the firmness of unprocessed fillets was measured to be 13.1 ± 1.8 N (RSW) and 13.6 ± 1.8 N (ice). After processing and storage, firmness generally increased through salting time ($p = 0.021$), while there was no effect on treatment (Table 2). Textural properties for processed fillets are usually higher than the unprocessed counterparts, causing the muscle to be denser and more elastic (Chan et al., 2020b; Løje, 2007). As texture firmness is negatively related to the water content of smoked salmon (Birkeland et al., 2004), this likely explains the observed increase in firmness as water content decreases through salting. The slight dip observed at 9 h and 21 h might be explained by various factors such as variations in lipid and collagen content (Løje, 2007). As also verified by Chan et al. (2020a), fillet height significantly influences the firmness of the final product ($p < 0.001$).

4.3. Prediction of temperature

Fig. 5 presents the predicted temperature at the centre position of the salmon portion as a function of time in the cold room during salting. The sample temperature profile decreased rapidly to the surrounding temperature during the first 2 h and remained relatively stable afterwards. The model showed good agreement between the measured and predicted values (RMSE = 0.28 °C).

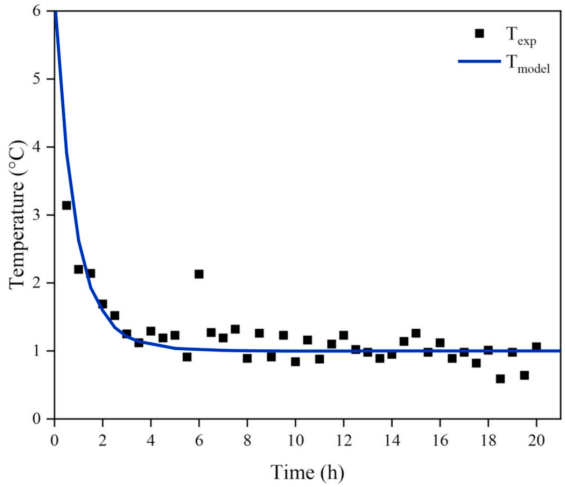

Fig. 5. Measured and predicted temperature profile at the middle position of the salmon portion during dry salting in a cold room (RMSE = 0.28 °C).

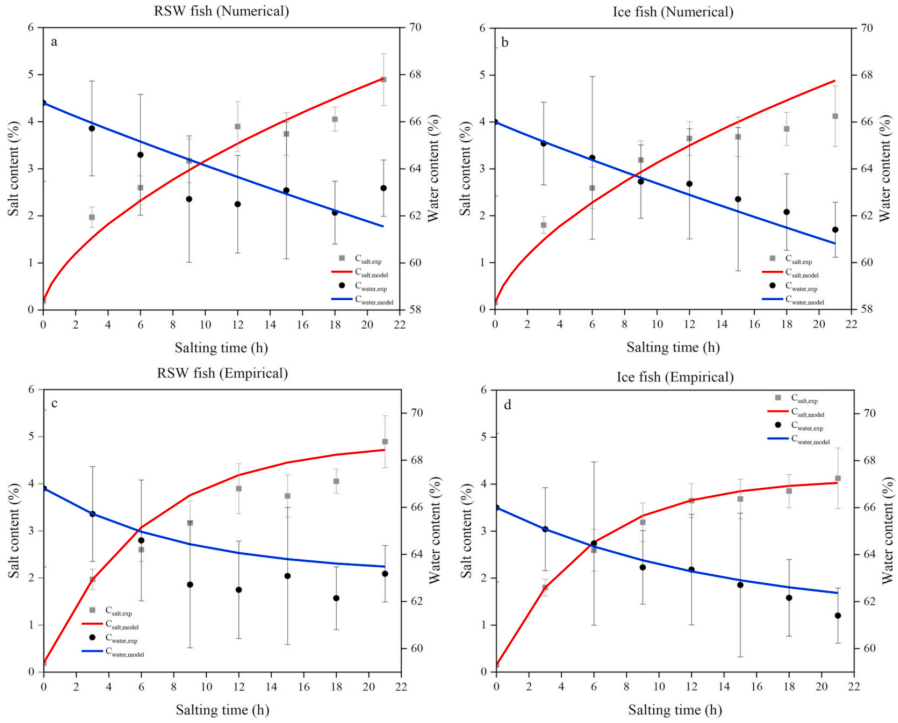

Fig. 6. Measured and predicted salt and water content for smoked salmon at different salting times from numerical modelling of (a) RSW (salt: RMSE = 0.30 %, water: RMSE = 0.95 %) and (b) ice fish (salt: RMSE = 0.41 %, water: RMSE = 0.41 %). Empirical modelling of (c) RSW (salt: RMSE = 0.43 %, water: RMSE = 1.02 %) and (d) ice fish (salt: RMSE = 0.11 %, water: RMSE: 0.40 %). Statistical analysis for measured salt content (GLM; salting time: $p < 0.001$, treatment: $p = 0.207$). Statistical analysis for measured water content (GLM; salting time: $p < 0.001$, treatment: $p = 0.511$). All results are presented in mean ± standard deviation.

4.4. Prediction of salt and water transport

Statistical analysis revealed that there was an effect of salting time on salt (NaCl, $p < 0.001$) and water content ($p < 0.001$), but no differences was observed between treatments. Similar to Chan et al. (2020b), minimal differences were detected after salmon initially stored in RSW and ice were dry salted and cold-smoked. In this study, the water content obtained before salting was 66.8 ± 3.3 % and 66.0 ± 3.2 % for the RSW and ice fish, respectively. An inverse relationship was observed between water loss and salt uptake at increasing salting time for both treatments. The measured salt content at 21 h reached up to 4.9 ± 0.6 % (RSW) and 4.1 ± 0.7 % (ice), while water content to 63.2 ± 1.2 % (RSW) and 61.4 ± 1.2 % (ice).

Salt uptake and water loss simultaneously affect each other because of concentration and osmotic pressure difference, so water diffuses out while salt solubilizes in the water phase and diffuses into the muscle until equilibrium is attained, and the net rate of mass transfer is zero (Barat et al., 2003; Bellagha et al., 2007). In this experiment, the samples were analyzed after almost 2 weeks of vacuum storage. This is to ensure equilibrium as salt diffuses into the product at different speeds. During the dry salting process, a saturated layer of salt is first formed on the product's surface before salt migrates into the product (Pittia and Antonello, 2016). The evident increase in salt content during the first few hours of salting, as seen in this study, was likely due to the large concentration gradient between dry salt and muscle tissue. Thereafter, layer formation with high salt content close to the muscle surface acts as a barrier against further salt uptake (Akköse and Aktaş, 2016). This phenomenon was also observed in several studies (Akköse and Aktaş, 2016; Bellagha et al., 2007; Wang et al., 2000). The bulk salt concentration was estimated to be 15 % (0.15 kg/kg) in the model. Since salt is used in excess during dry salting, this value can be influenced by the rate of salt diffusion and several factors such as surface-fillet thickness ratio and lipid content (Lerfall et al., 2016).

The calculated Z&L's specific constants k_s were 0.158 and 0.179 h^{-1}, and k_w were 0.118 and 0.074 h^{-1} for the RSW and iced fish. These values were similar to previous studies. For example, Bellagha et al. (2007) reported that k_s and k_w for dry salting of sardines were 0.139 and 0.191 h^{-1}. Corzo et al. (2015) also found that k_s and k_w salting of catfish sheets to be 1.125 and 1.489 d^{-1}.

The RMSE values obtained from the numerical model for RSW fish were 0.30 % (salt) and 0.95 % (water), while those of iced fish were 0.41 % (salt) and 0.41 % (water). In addition, the RMSE values from the Z&L model for RSW fish were 0.43 % (salt) and 1.02 % (water), while those of iced fish were 0.11 % (salt) and 0.40 % (water). Hence, the numerical and empirical model gave good agreements between the measured and predicted salt and water content for RSW and ice fish (Fig. 6).

Empirical models are developed by fitting the data with empirical correlations, relying on actual experiments. This can be beneficial and serve as a quick solution to observe the salting behaviour. However, such models cannot predict beyond the experimental range for a specific experiment, and biological variations like seasonal changes and raw material composition are omitted. In contrast, numerical models are based on the first principles and physical laws, which provides a better understanding of mechanisms. Food is a complex system with different physical properties such as shape, form, specific heat, thermal conductivity, density, and viscosity, changing with temperature. Therefore, numerical models can better manipulate process variables and adapt to

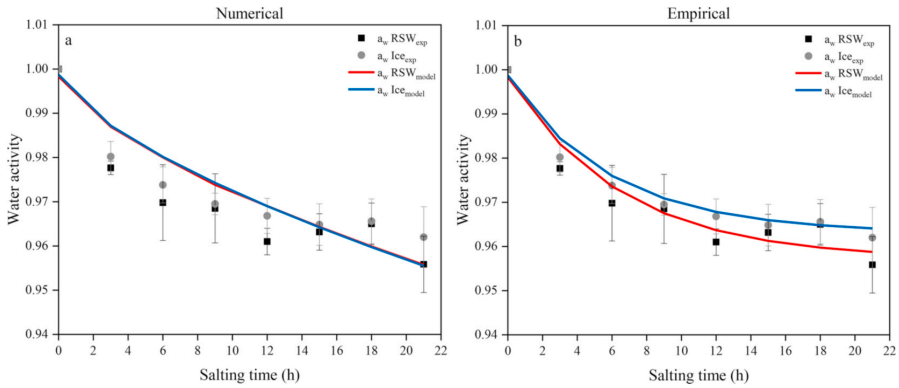

Fig. 7. Measured and predicted water activity for smoked salmon at different salting times from (a) numerical modelling of RSW (RMSE = 0.0063) and ice fish (RMSE = 0.0049); and (b) empirical modelling of RSW (RMSE = 0.0035) and ice fish (RMSE = 0.0021). Statistical analysis for measured water activity (GLM; salting time: $p < 0.001$, treatment: $p = 0.011$). All results are presented in mean ± standard deviation.

changes in the conditions, providing a more flexible solution to include different input parameters and data extrapolation. It could be especially useful to observe spatial distribution and variations in temperature and water and salt concentration over time during salting to better understand the mechanism and control the salt uptake.

4.5. Prediction of water activity

In this study, the measured a_w values decreased during the whole salting process ($p < 0.001$), and the RSW fish generally had a lower a_w than the ice fish ($p = 0.011$). As water activity measures the amount of free water in the product, this could be the factor that explains the difference in a_w between the two treatments. The salt gain and water loss at increasing salting time explain the decrease in a_w, in agreement with other studies (Bellagha et al., 2007; Corzo et al., 2015).

The predictions of a_w using the predictions from salt and water content were validated against the experimental values. In general, the numerical (Fig. 7a) and empirical models (Fig. 7b) can accurately predict the a_w changes during dry salting for the two treatments. The RMSE values for RSW and ice fish predictions were 0.0063 and 0.0049 using the numerical model. Similarly, the empirical model gave RMSE values of 0.0035 and 0.0021 for the RSW and ice fish. Therefore, with the knowledge of water and salt content, the estimation of a_w is possible without needing to conduct laboratory analysis.

5. Conclusion

This study examined the quality parameters of dry salted and cold-smoked salmon that were initially immersed in RSW or ice, subjected to different salting times. According to the results, drip loss and colour were affected by the processing steps during salting, smoking and vacuum storage. Drip loss and salt (NaCl) content increased, while redness, yellowness and water content decreased with increasing salting duration. WHC was not affected by the salting time. In general, the smoked salmon from the RSW fish had redder and lower water activity values than the iced fish.

The heat transfer coefficient of 15 W/(m^2 K) gave a better fit using the reverse estimation method. The mathematical model of heat and mass transfer of salt and water during dry salting of salmon gave a reasonable agreement between the measured and simulated temperature, salt and water content. The predicted values of salt and water content were also able to simulate the water activity accurately. Comparison between numerical and empirical model showed good agreements from the low RMSE values obtained. Shrinkage occurs during dry salting due to moisture loss, which tightens the solid structure of the product. Therefore, additional details may be included in the future to make the mathematical model more robust, such as measuring local concentrations, dimensional changes and fat distribution. Nevertheless, as more emphasis is given on the amount of salt added and salting time of the product, the model in this study can be a valuable tool for process optimization in the industry and understanding of the kinetics during dry salting of fish.

Author contributions

S·S.C. – conceptualization, methodology, investigation, writing original draft; A.H.F. – methodology, investigation, writing (review and editing); F.J. – supervision, writing (review and editing); B.R. – resources, supervision, writing (review and editing); A.N.J. - supervision, writing (review and editing); J.L. – conceptualization, investigation, supervision, writing (review and editing).

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.

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