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

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A B S T R A C T

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

1. Introduction

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

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

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

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

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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

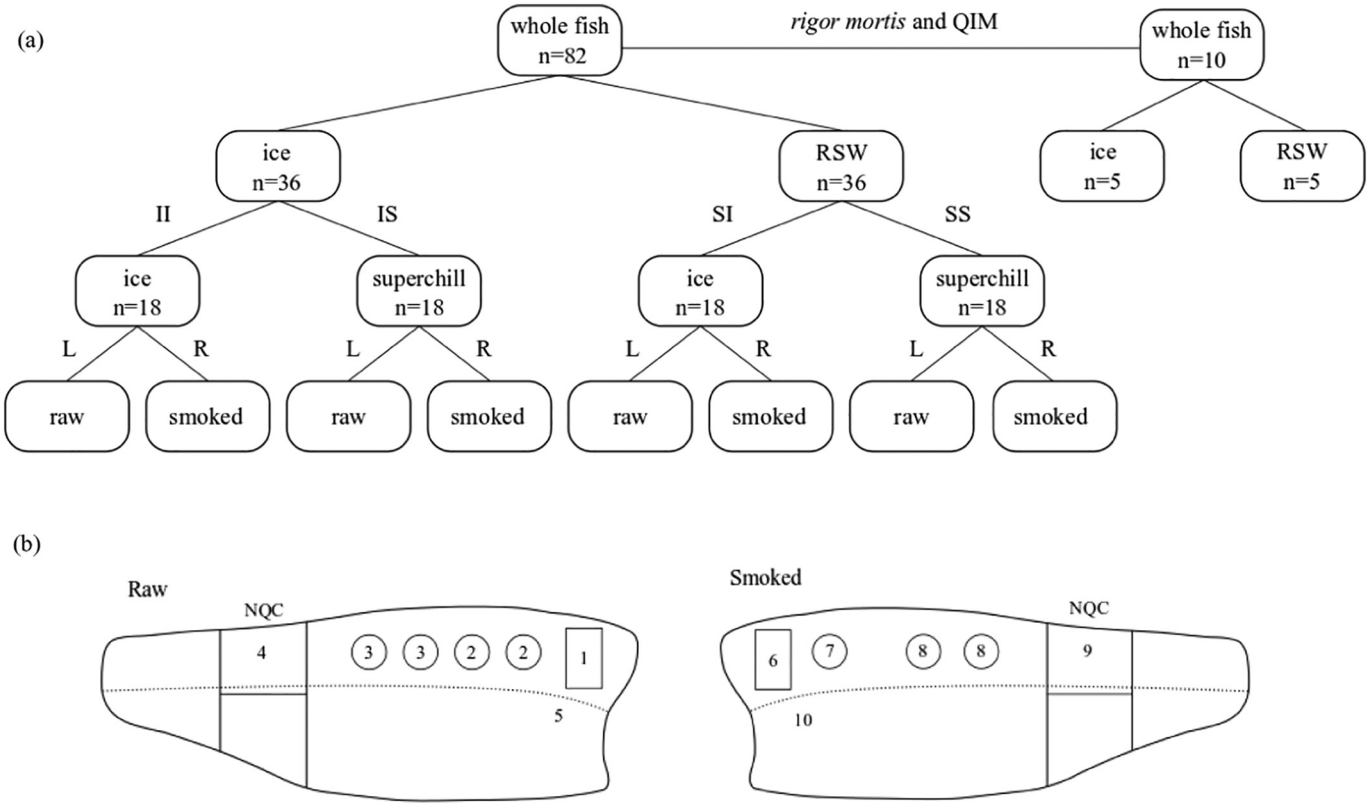

Fig. 1. (a) Experimental overview. 10 fish were used for *rigor mortis* and quality index measurements (QIM). II and IS represents whole fish on ice and stored in ice or superchilled after filleting respectively; SI and SS represents whole fish in RSW and stored in ice or superchilled after filleting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

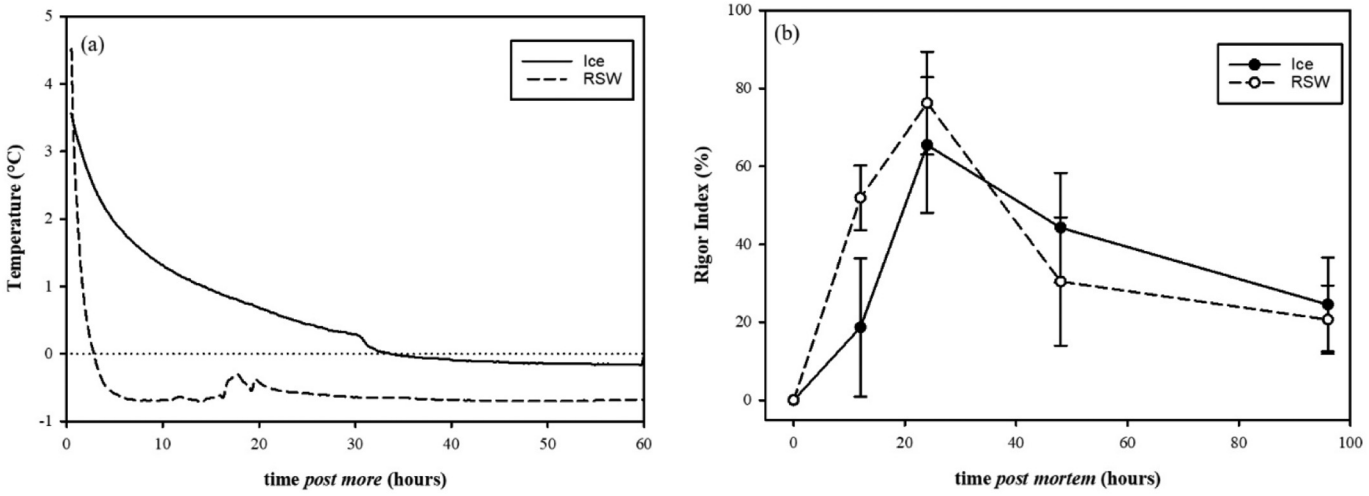

Fig. 2. (a) Temperature change and (b) rigor index of whole fish in ice and RSW (GLM, $p = .784$) over time.

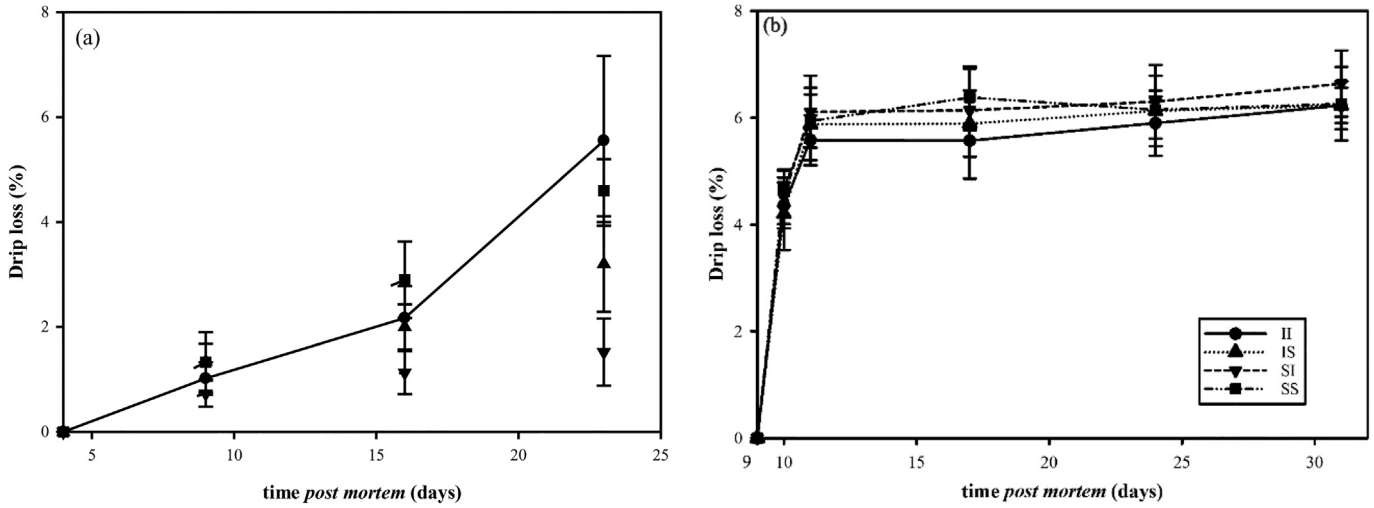

Fig. 3. (a) Drip loss of raw fillets after filleting on day 4 (GLM; storage days: $p < .001$; whole fish: $p = .039$; fillets: $p < .001$); and (b) smoked fillets processed at day 9 as a function of time after processing (GLM; storage days: $p < .001$; whole fish: $p = .002$; fillets: $p = .740$).

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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