



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

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## ARTICLE INFO

### Keywords:

Atlantic salmon  
Superchilling  
Slaughter vessel  
RSW

## ABSTRACT

The purpose of this study was to investigate the shelf life and quality of Atlantic salmon (*Salmo salar*) slaughtered onboard vessels and shipped to Denmark in  $-0.8\text{ }^{\circ}\text{C}$  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  $\text{H}_2\text{S}$  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  $200\text{--}300\text{ kg/m}^3$  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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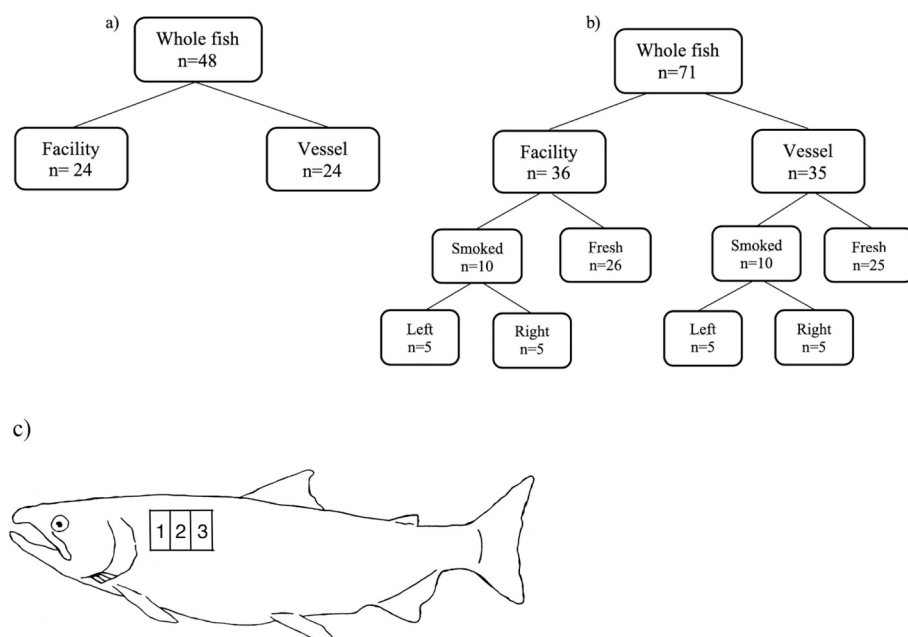
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<https://doi.org/10.1016/j.aquaculture.2021.736681>

Received 16 October 2020; Received in revised form 22 March 2021; Accepted 24 March 2021

Available online 26 March 2021

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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 (Pineiro 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. Twenty-six and twenty-five 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 Birke-land 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<sub>2</sub>S 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 Laboratoire, 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  $72 \pm 6$  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<sup>-1</sup>.

### 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 ( $L^* = 0 = \text{black}$ ,  $L^* = 100 = \text{white}$ ). The a\* value changes from -a (greenness) to +a (redness) while b\* value changes from -b (blueness) to +b (yellowness). Chroma 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<sup>-1</sup>, 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

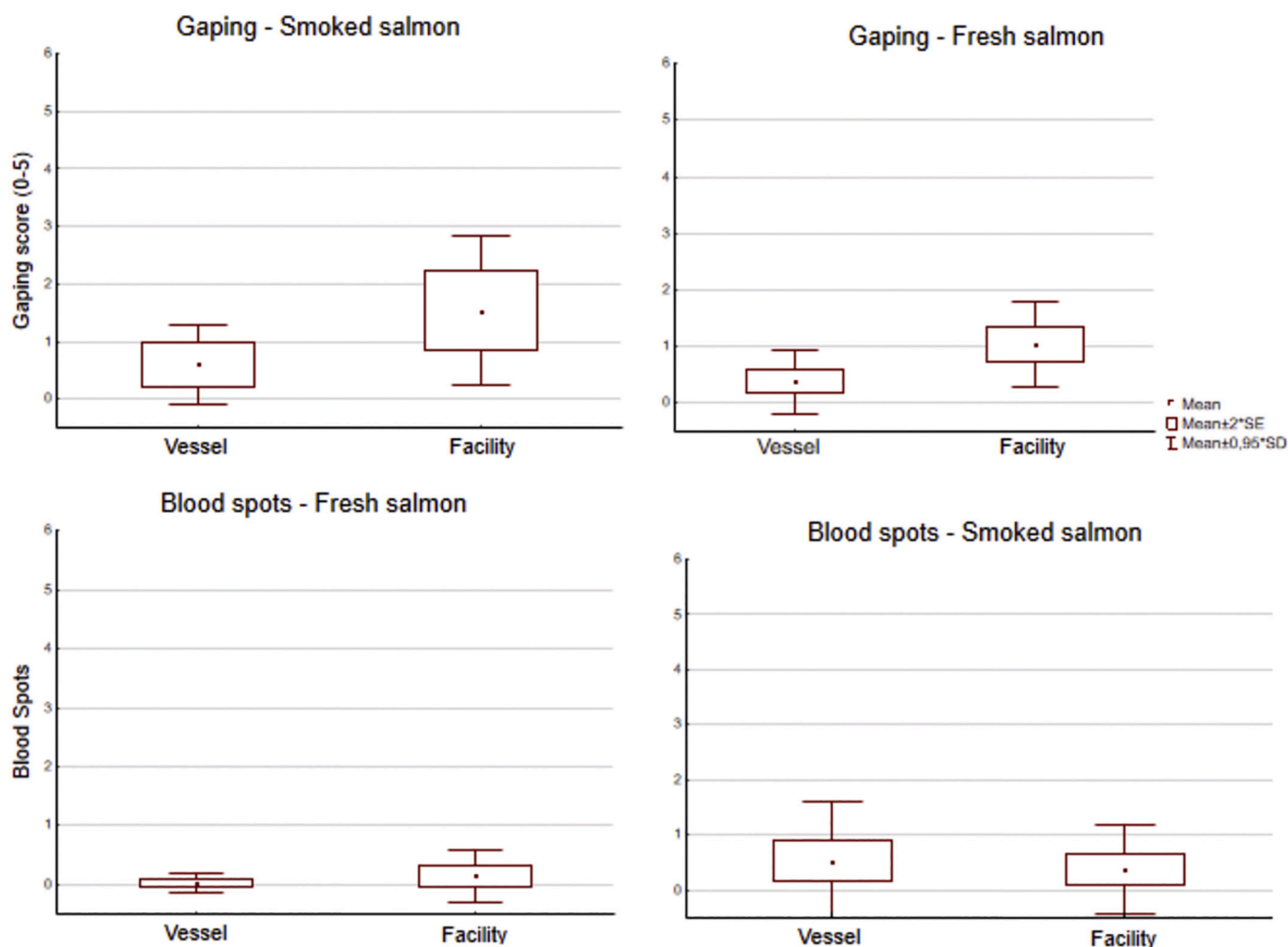


Fig. 2. Weighted result of gaping and blood spot counts from both fresh (n = 24) and smoked salmon (n = 10).

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  $0.4 \pm 0.60$ ) as compared to fish slaughtered at facility (on average  $1.0 \pm 0.79$ ). There was no significant difference between the groups in blood spots ( $p > 0.10$ ; 2 Sample Kolmogorov-Smirnov test), where fresh fish slaughtered

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                  | $21.5 \pm 9.4$  | $60.6 \pm$                   | $58.5 \pm 9.6$  | $24.3 \pm 4.9$  | 24 |
| Facility                | $20.4 \pm 17.9$ | $3.2$<br>$60.1 \pm$<br>$5.1$ | $58.4 \pm 17.5$ | $20.4 \pm 6.6$  | 24 |
| ANCOVA <sup>a</sup>     | $p > 0.35$      | $p > 0.89$                   | $p > 0.98$      | $p > 0.40$      |    |

<sup>a</sup> ANCOVA, analysis of covariance with fillet groups as factors and fillet height as covariant.

at facility (on average:  $0.1 \pm 0.45$ ) had a slightly higher blood spot count compared to fish slaughtered onboard vessel (on average:  $0.0 \pm 0.14$ ). 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:  $0.4 \pm 0.84$ ) than slaughtered at vessel (on average  $0.5 \pm 1.14$ ) (See Fig. 2).

#### 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.

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

<sup>a</sup> 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.

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

<sup>a</sup> Nested ANOVA analyses of dependent variables on slaughtering method with time nested into the design. Provided are the p and F values.

### 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  $2.1 \pm 0.10 \log \text{cfu g}^{-1}$  (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  $8.0 \pm 0.36 \log \text{cfu g}^{-1}$  as compared to the vessel with  $6.4 \pm 0.36 \log \text{cfu g}^{-1}$  ( $p < 0.00$ , post hoc test).

There was a significant difference increase in the amount of H<sub>2</sub>S 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 ( $7.0 \pm 0.47 \log \text{cfu g}^{-1}$ ) than those slaughtered on vessel ( $6.0 \pm 0.37 \log \text{cfu g}^{-1}$ ,  $p < 0.00$ ; 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  $2.4 \pm 0.29$  (vessel) and  $2.9 \pm 0.33$  (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  $7.6 \pm 0.37$  and  $8.1 \pm 0.17 \log \text{cfu g}^{-1}$  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 ( $6.8 \pm 1.24$ ), than those slaughtered at vessel ( $5.8 \pm 1.02$ ), ( $p < 0.01$ ; ANOVA). The juiciness for the fish slaughtered at vessel had a slightly higher score ( $6.1 \pm 0.94$ ), compared to fish slaughtered on facility ( $5.8 \pm 0.72$ ), although a significant difference was not detected ( $p > 0.30$ ; ANOVA).

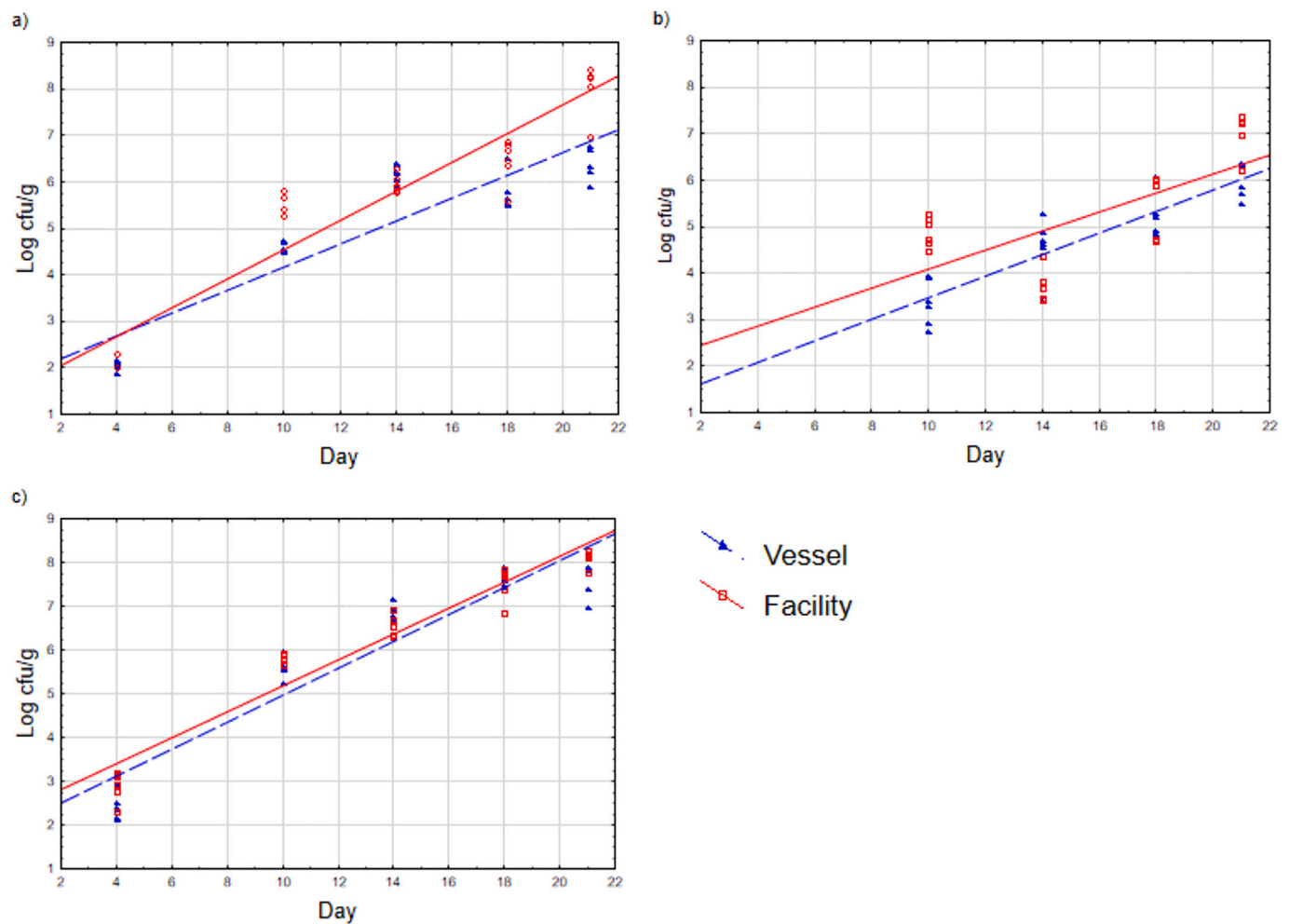


Fig. 3. (a) Total mesophilic counts (log cfu/g) (b) Hydrogen sulphide producing bacterial counts (log cfu/g) and (c) Total psychotropic count (log cfu/g) from day 4 to day 21 post mortem of fresh fish.

#### 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), where 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 (Lamboojij 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

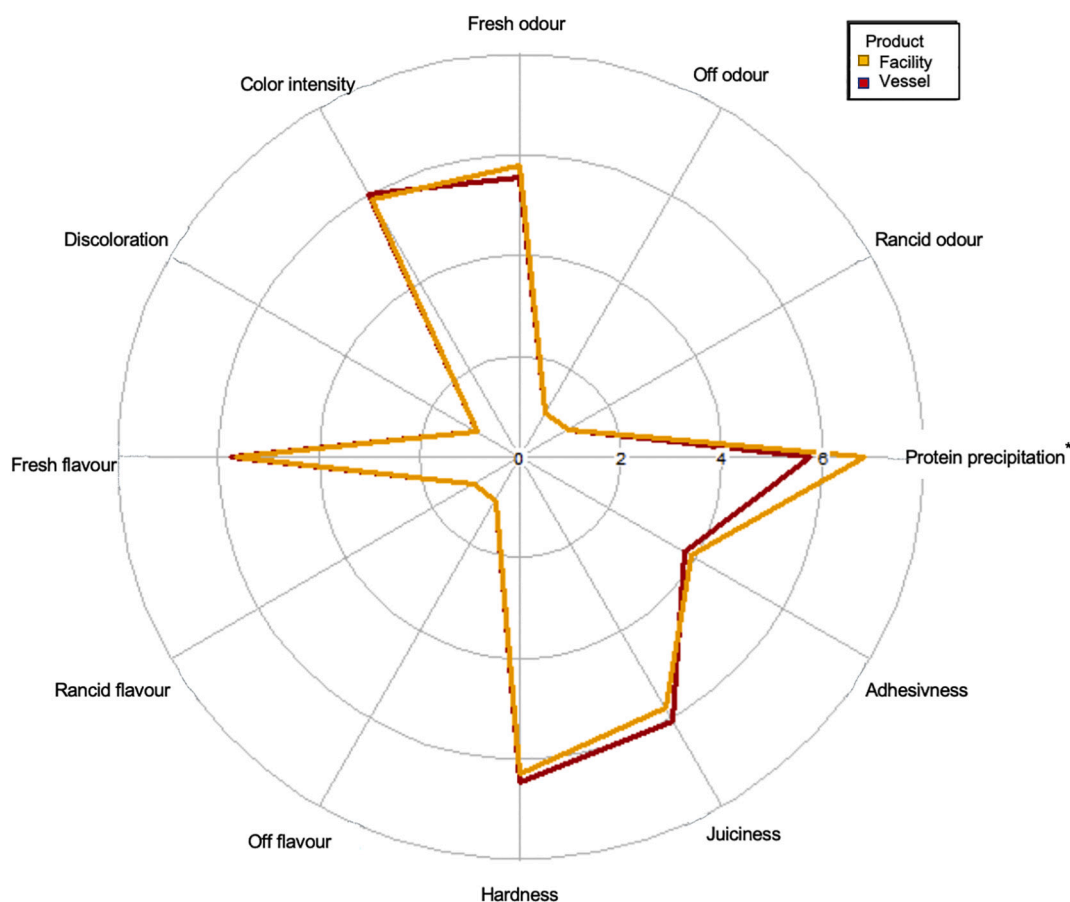


Fig. 4. Spider Plot showing the score for each attribute for both groups ( $n = 5$  from each group), Day 18 post mortem (October experiment). \*significant levels with  $p < 0.05$ .

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  $-2\text{ }^{\circ}\text{C}$  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  $5\text{--}6\text{ log cfu g}^{-1}$ . The HSPB observed may indicate a longer shelf life for fish slaughtered onboard vessel compared to fish slaughtered at the facility, although there is no consensus as to which bacterial species should be used to monitor the shelf life of the fish.

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

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

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

## 5. Conclusion

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

## Declaration of Competing Interest

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

## Acknowledgement

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

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