

Effect of Season, Location, Filleting Regime and Storage on Water-Holding Properties of Farmed Atlantic Salmon (*Salmo salar* L.)

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SUMMARY

The effect of season, localization, filleting regime and storage on water-holding properties of Atlantic salmon (*Salmo salar* L.) was investigated. Salmon was sampled at two different slaughter facilities (in the north and south of Norway) in autumn and spring and divided in *pre-* and *post-rigor* groups, which were sampled before and after filleting. This gave a total of 16 groups that were analyzed for water-holding capacity (WHC), water content and pH. In addition, a storage trial was performed to assess the effect of all the design variables on drip loss and the composition of the drip loss during up to 18 days of storage. WHC was significantly affected by both *rigor* status and filleting, while water content was affected by localization and filleting. In addition, *post-rigor* filleting gave significantly decreased drip loss compared to *pre-rigor* filleting. However, storage time had the highest impact on the drip loss. Based on this, it is concluded that *pre-rigor* filleted salmon have excellent water-holding properties and a great potential for early processing (*pre-rigor* processing). It was however difficult to find a clear connection between the drip loss and the water-holding capacity of the muscle.

Key words: Atlantic salmon, water-holding capacity, drip loss, *rigor mortis*

INTRODUCTION

Atlantic salmon (*Salmo salar* L.) is highly valuable species for the Norwegian aquaculture. Norway produced 980 000 metric tonnes of Atlantic salmon with an export value of 61.4 billion Norwegian kroner (approx. €6.63 billion) in 2016. Drip loss and the ability of the salmon muscle to retain water during primary processing and ice storage are important factors that affect both consumer perception and the profitability for the producer (1-3). Most of the water in muscle tissue is kept within the structure of the muscle and muscle cells (4). Within the muscle cell, water is found inside the myofibrils, between the myofibrils themselves and between the myofibrils and the sarcolemma (cell membrane), between muscle cells and between groups of muscle cells (5). Water is moreover bound to proteins by hydrogen bonds, where the capacity of the specific protein depends on the net surface charge (affected by pH) and the polarity of the specific proteins.

Drip loss from muscle foods occurs due to changed capacity of the muscle structure to retain its natural water (2,4) and is closely related to the water-holding capacity (WHC) of the flesh. WHC of Atlantic salmon is known to be affected by several *pre-* and *post mortem* factors including stress prior to slaughtering (6,7), starvation (8), and state of *rigor mortis* (9,10). The WHC is also known to be affected by pH, because pH close to the isoelectric point is known to lower the WHC (4). The drip loss from salmon fillets consists mainly of water, proteins and lipids, and is affected by a drop in muscle pH due to anaerobic glycolysis (9) and ultrastructural changes during *rigor mortis* (10). A rapid drop of pH can, in all likelihood, also lead to denaturation of muscle and sarcoplasmic proteins. During ice storage, however, Hultmann and Rustad (11) indicated that WHC in salmon muscle seems to be unrelated to muscle pH due to small variations in pH during storage of *post-rigor* fillets.

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Previous research has pointed to the fact that seasonal variations influence the drip loss. A study with *pre-rigor* filleted Atlantic salmon, stored for 13 days at 4 °C, observed the highest drip loss in October and the lowest drip loss in April (12). In addition, the Norwegian salmon industry has observed differences in water-holding properties of salmon fillets related to a north-south gradient along the Norwegian coast. In addition, a relatively high drip loss has been observed in *pre-rigor* filleted salmon during the first days when exported in ice (0 °C).

Even though considerable work has been done in the last few years to describe the WHC of muscle foods, little if any work has been done to study WHC of Atlantic salmon muscle in *pre-rigor* state. In the presented study, we studied the WHC of both *pre-* and *post-rigor* muscle from whole head on gutted (HOG), and *pre-* and *post-rigor* muscle from fillets of Atlantic salmon. The study was moreover performed at two different localities in a north-south gradient along the Norwegian

coast, both in spring and in autumn. The aim of the study is to gain more knowledge about factors affecting WHC and water content of Atlantic salmon fillets, and subsequently the drip loss during storage. Investigated factors were: season, Norwegian coast (north and south), filleting (whole fish *versus* fillets) and time of filleting (*pre-* *versus* *post-rigor*).

MATERIAL AND METHODS

Experimental animals and design

In May 2013, November 2013 and May 2014, a total of 160 farmed Atlantic salmon (*Salmo salar*) with mean value±standard deviation (S.D.) mass of (4.4±1.2) kg were sampled during commercial slaughtering at two different plants in Norway. A full factorial design (Fig. 1) with four factors was designed; season (spring/autumn), location (north/south), *rigor* status (*pre/post*) and filleting (HOG/fillet) resulting in 16 different groups where

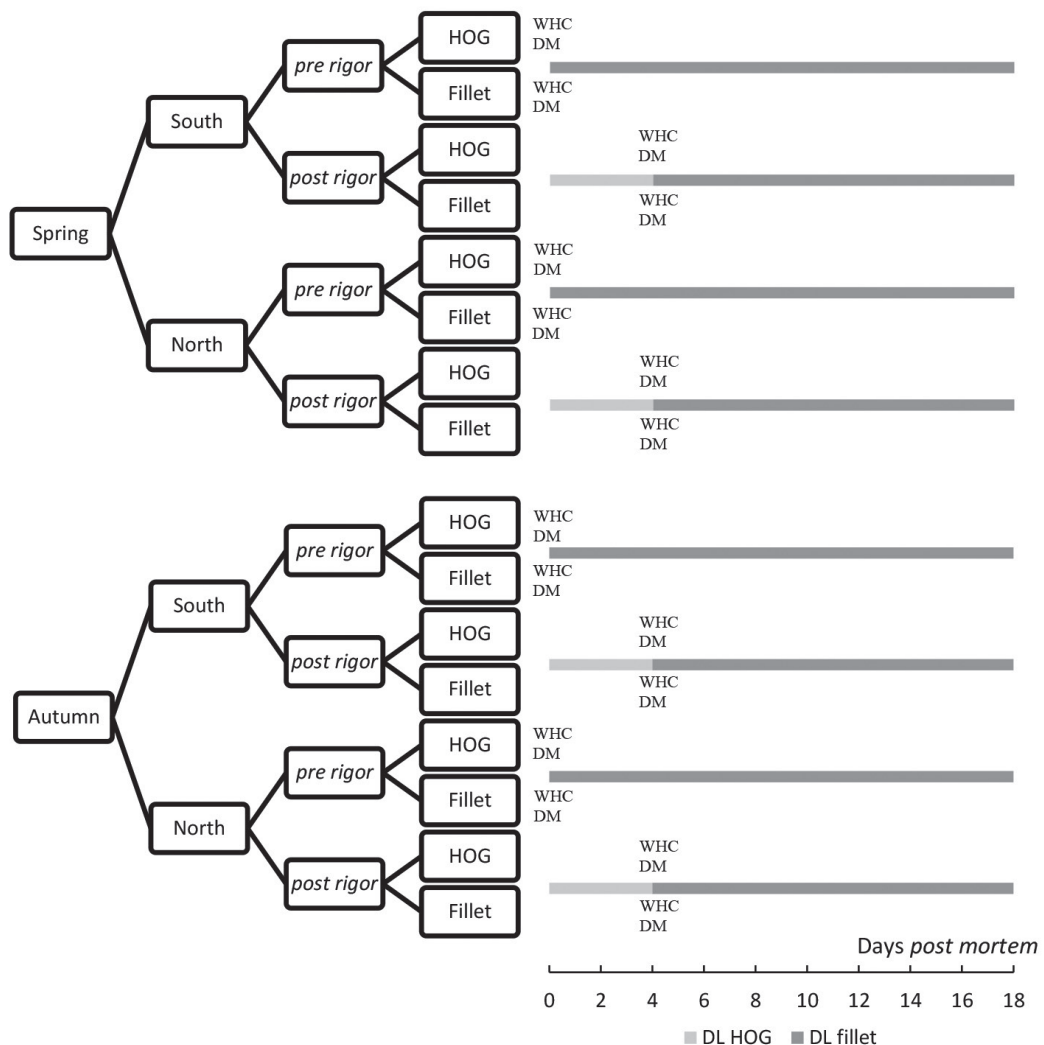


Fig. 1. Schematic illustration of the experimental design, where spring and autumn refer to May and November, respectively, south and north to N58-59° and N63-64°, respectively, *pre-* and *post-rigor* to point of filleting, and head on gutted (HOG) and fillet to when the analysis was conducted (before and after filleting, respectively). The time scale shows when the specific analyses were conducted for each group. WHC=water-holding capacity, DM=dry matter, DL=drip loss

the effect on water-holding capacity (WHC) and water content was investigated. In addition, 160 fillets with mean \pm S.D. mass of (1.6 \pm 0.4) kg from the entire design apart from the filleting were stored on ice for 14 days to quantify the drip loss during storage and to determine the composition of the drip loss (Fig. 1).

Fish from the north site was farmed around N63-64°, while the fish from the south site was farmed around N58-59° along the west coast of Norway. Spring part of the trial was performed in May, while the autumn part was performed in November. The sea temperature was 9.5 and 5.0 °C at the south location, while the north location had 9.5 and 6.0 °C in November and May, respectively. Before sampling, the fish were slaughtered according to the commercial practice at the plants; the fish was crowded, pumped in a double-chamber vacuum pump, live chilled up to 45 min, stunned with percussive captive bolt and gills were cut before bleeding in a bleeding tank for up to 60 min. The fishes were then gutted to end up as head on gutted (HOG), and all the fish were measured (mass, length and pH), tagged and randomly subjected to *pre-rigor* analysis and WHC or storage trial, respectively. The fish subjected to *pre-rigor* analysis were analyzed consecutively, while fish subjected to *post-rigor* analysis were stored in expanded polystyrene boxes on ice in a chilled room (0-4 °C) for four days to go through *rigor mortis*.

pH, temperature, condition factor and drip loss

pH and temperature were measured right after gutting in the anterior dorsal muscle close to the gills, using a Mettler Toledo SevenGo Pro™ pH-meter (Mettler Toledo Inc., Columbus, OH, USA) connected to an Inlab puncture electrode (ibid). In addition, *post-rigor* fish were measured after 4 days of storage. During storage of fillets, muscle pH and temperature were measured on the cranial part of the fillet.

Before analysis, the fish were dried off and weighed, and the fork length was measured. Fulton's condition factor (CF) (13) was calculated for gutted fish according to the following formula:

$$CF = m/l^3 \cdot 0.1 \quad /1/$$

where m is gutted mass (kg) and l is fork length (m).

In the storage trial, HOG subjected to *post-rigor* filleting were weighed after being dried off on day 0 and day 4 to quantify the drip loss. Fillets for the storage trial were machine filleted, dried off, weighed and packaged in a closed bulk polyethylene bag inside expanded polystyrene boxes with top icing. Thereafter, all samples were transported within 24 h to the laboratory where the fillets were stored in chilled rooms (0-4 °C) for 14 days. During storage, melting water was removed through weep holes in the bottom of each box, while drip loss was gathered. Drip loss (DL/%) from the fillets was measured as the difference in fillet mass between day 0 and day x of both the right and left fillet:

$$DL = \left(\frac{m_0 - m_x}{m_0} \right) \cdot 100 \quad /2/$$

where m_0 is fillet mass (g) on day 0, and m_x is fillet mass (g) on day x .

Drip loss was assessed on days 7 and 14 after filleting (*i.e.* day 7 and 14 for *pre-rigor*, and day 4 (HOG), day 11 and 18 for *post-rigor*). In addition, drip loss of *post-rigor* fillets is reported as cumulated drip loss including drip loss as HOG. An average of both fillets was used for statistical analysis.

Water-holding capacity and water content

Water-holding capacity was measured in the dorsal muscle. Samples for determination of WHC and water content of whole fish were first sampled from the left side before the fish was machine filleted, and samples from the right fillet were taken approx. 45 min later. Samples were cut in triplicates (diameter 31 mm, height 20 mm) from the cranial part on the dorsal back and backwards, above the lateral line on identical locations on all fishes using the white muscle tissue (Fig. 2). Three portions from each sample, approx. 5 g were made, and the first two (Fig. 2c, I and II) were weighed and dried at 105 °C for 16 to 24 h (14) for estimating the dry content of the muscle and hence the water content. The last sample (Fig. 2c, III) was weighed and placed into metal carriers (Hettich Lab Technology, Tuttlingen, Germany) and centrifuged (Rotina 420 R; Hettich Lab Technology) for 15 min at 4 °C, using a free swing rotor at 530 \times g. WHC was calculated from the following formula (15):

$$WHC = \left(\frac{w - \Delta w}{w} \right) \cdot 100 \quad /3/$$

where

$$w = \left(\frac{m_w}{(m_w + m_D)} \right) \cdot 100 \quad /4/$$

and

$$\Delta w = \left(\frac{\Delta m_w}{(m_w + m_D)} \right) \cdot 100 \quad /5/$$

where m_w is the mass (g) of water in the muscle sample, m_D is the mass (g) of dry matter in the muscle sample, and Δm_w is the mass of the liquid separated from the sample during centrifugation.

Composition of drip loss

During storage, the fillets were stored in closed bags to gather the drip loss and to prevent contamination from the melting ice. The amount of water, protein, fat and astaxanthin were quantified in triplicate according to the design.

Nitrogen content was measured on a Tecator Kjeltex system (model 2020 digester and 1026 distilling unit; Tecator, Höganäs, Sweden) (16). Protein content was calculated from nitrogen measurements using the formula:

$$w(\text{protein}) = w(\text{nitrogen}) \cdot 6.25 \quad /6/$$

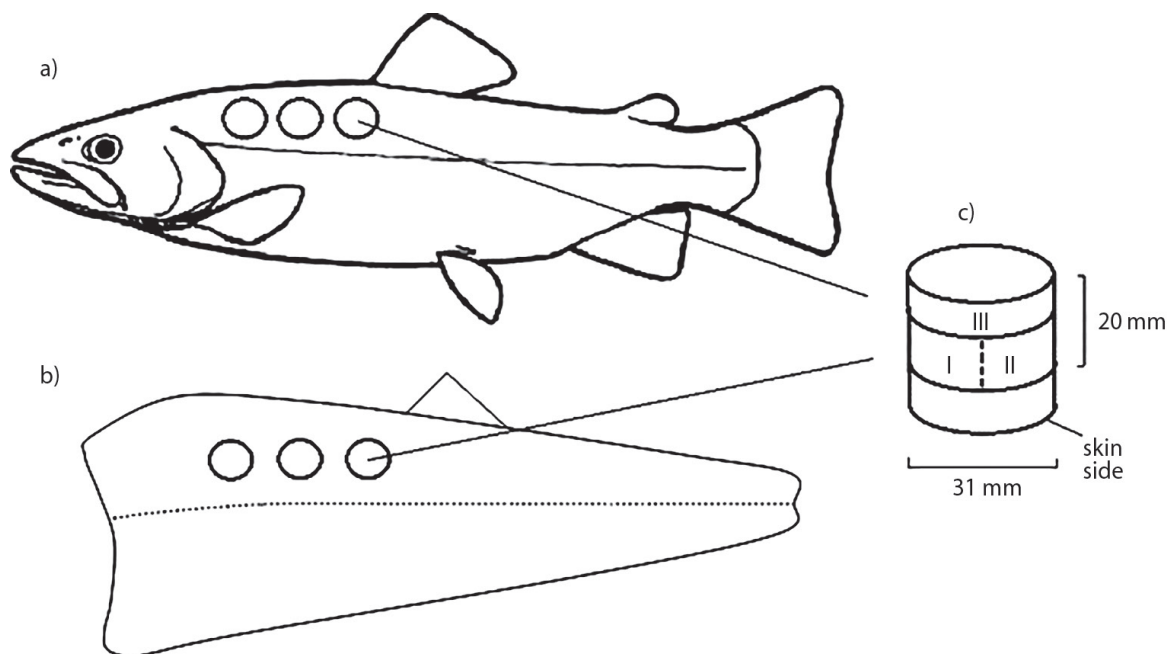


Fig. 2. Schematic illustration of the: a) head on gutted and b) fillet areas used for determination of water-holding capacity (WHC) and water content (WC). In addition, insert c) shows the splitting and use of the sample, where two parts were used for WC (I and II) and one part for WHC (III) determination

Fat and astaxanthin were extracted by a method after Bligh and Dyer (17). Total contents of fat were calculated and total contents of astaxanthin were quantified by HPLC using a H_3PO_4 -modified silica gel column (17) and all-E-astaxanthin (A-9335; Sigma-Aldrich, Merck, St. Louis, MO, USA) as an external standard. The eluent was acetone/*n*-hexane (86:14) and the flow was 1.2 mL/min. Detection wavelength was set at 470 nm. The employed molar absorption coefficients, $\epsilon_{1\text{ cm}}$ (1 %), at 470 nm in hexane containing 4 % (by volume) $CHCl_3$ were 2100 for all-E-astaxanthin (18), and 1350 and 1750 for 13Z- and 9Z-astaxanthin, respectively. The $\epsilon_{1\text{ cm}}$ (1 %) for 13Z- and 9Z-astaxanthin were estimated from the HPLC response factors of Schüep and Schierle (19). Water content was measured as described previously.

Statistics

All data were analyzed by general linear model (GLM), regression (R) and Pearson's correlation coefficient (*r*), using Minitab v. 17 statistical software (20). All results are given as mean value \pm S.D., unless stated otherwise.

RESULTS AND DISCUSSION

Biometrics

Comparing different populations of fish during commercial conditions certainly poses many challenges, as the fish used in this trial was sampled during a period of one year at two different locations. Even if they differed significantly ($p < 0.002$) in gutted mass between the two locations (north

(4.1 ± 1.1) kg, south (4.7 ± 1.2) kg), none of the design factors varied significantly in initial pH ($p > 0.084$) or condition factor ($p > 0.065$). Average initial pH was 6.9 ± 0.3 , while condition factor was 1.1 ± 0.1 .

Water-holding capacity, water content and pH

Water-holding capacity is known to be related to pH, but including pH as a covariate in the general linear model did not give any significant result ($p = 0.645$). The WHC was measured at three different positions on the fillet (Fig. 2), but no effect of position on the fillet ($p = 0.295$) was detected, so the average of the three measurements was used in the following analysis of the results.

The water content in the muscle was not affected by season, but there was a significant difference between the two localizations ($p < 0.001$, $F = 14$) (Table 1), and a significant ($p < 0.001$, $F = 147$) interaction between localization and season was detected (Fig. 3). In addition, pH was significantly affected by localization ($p < 0.001$, $F = 78$) and season ($p = 0.001$, $F = 12$) (Table 1) together with an interaction effect between localization and *rigor* ($p < 0.001$, $F = 29$, data not shown). No other significant ($p > 0.190$) effects on pH were detected.

Furthermore, a significant difference ($p < 0.001$, $F = 148$) between HOG and fillet was detected in WHC (Table 1), where fillet had a decrease of 2.6 % compared to HOG. The water content in the muscle was significantly affected by filleting ($p < 0.001$, $F = 78$), where HOG had approx. 2 % more water than the fillet, measured on the same fish. Filleting opens up the muscle structure of the fish and removes amongst other the

Table 1. Effect of *rigor* status (*pre/post*), filleting (fillet/head on gutted (HOG)), season (spring/autumn) and localization (north/south) on water-holding capacity (WHC), water content and pH of the muscle of Atlantic salmon (*Salmo salar*)

	WHC/%	w(water)/%	pH
<i>Rigor</i> (N=80)			
<i>Pre</i>	95.8±2.2	64.8±3.0	6.9±0.3
<i>Post</i>	90.4±3.2	64.9±3.3	6.29±0.06
p-value	<0.001	>0.05	<0.001
Filleting (N=80)			
HOG	94.4±3.7	65.9±3.0	6.6±0.4
Fillet	91.8±3.6	63.8±3.0	6.6±0.4
p-value	<0.001	<0.001	>0.05
Season (N=80)			
Spring	93.0±3.8	64.7±3.6	6.6±0.4
Autumn	93.2±4.0	65.9±2.7	6.6±0.4
p-value	>0.05	>0.05	<0.001
Localization (N=80)			
South	93.2±3.93	65.3±3.1	6.7±0.4
North	93.0±3.85	64.4±3.2	6.5±0.3
p-value	>0.05	<0.001	<0.001

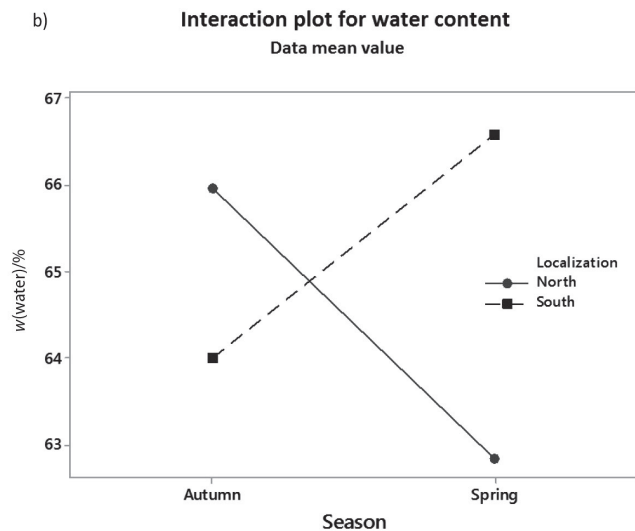
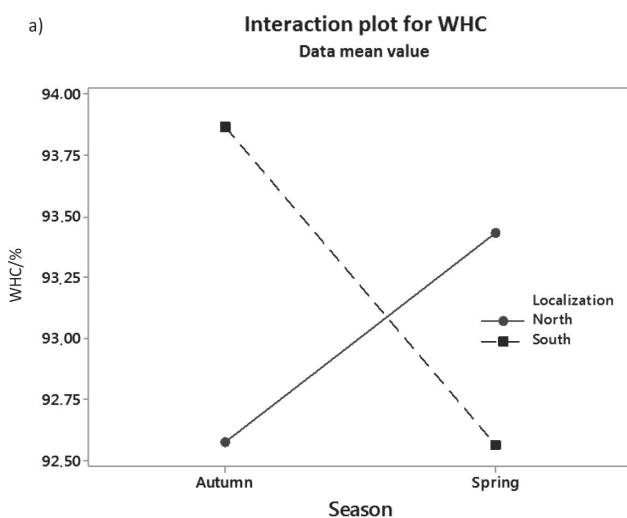
The results are expressed as mean value±S.D., ranked by ANOVA (general linear model)

peritoneum. In addition, it is likely that the filleting machine induces micro-ruptures in the muscle structure. These expose the muscle and physically decrease its water-holding property and the water content. Jørpeland *et al.* (21) found the same effect in farmed cod, where whole fish had 0.5 % more water than hand-filleted cod. Filleting by machine as done in the present study might give the muscle increased pressure compared to hand filleting. This can have squeezed more water out of the fillet, hence the increased difference than that observed in farmed cod.

Rigor status had a significant ($p<0.001$) impact on WHC, with a decrease of 5.5 % on average from *pre-* to *post-rigor*, and explained a major part of the observed variation ($F=656$)

(Table 1). No effect of *rigor* was detected ($p>0.234$) on the water content of the muscle. Assessing WHC of *pre-rigor* Atlantic salmon has as far as we know never been done before. Several papers report WHC of *pre-rigor* filleted salmon, but the time of measurement was at least 1 day after filleting (22,23). However, a study of ground rainbow trout (*Oncorhynchus mykiss gairdneri*) did not reveal any differences in WHC measured immediately or 24 h *post mortem* (24). The observed decrease in WHC may be caused by several mechanisms related to the development of *rigor mortis*. WHC is known to be affected by pH, as pH close to the isoelectric point is known to lower the WHC (4), while some other researchers have stated that there is no strong connection between the pH and WHC in salmon muscle due to small variations during storage (11). Even though pH was not detected as a covariate to WHC, a positive correlation between pH and WHC was found ($r=0.66$, $p>0.001$). This connection was most obvious when comparing *pre-* vs. *post-rigor* fish, as both pH ($p<0.001$, $F=1589$) and WHC were significantly decreased in *post-rigor* fish compared to *pre-rigor*. pH had an average drop of approx. 0.6 pH units from *pre-* to *post-rigor*, ending up at $pH=6.29$. This drop is caused by the conversion of glycogen to lactate *post mortem* (25). In addition, the muscles form actomyosin during *rigor*, leaving less space for water (26), and together with changes in the salt ion balance (27), these factors might explain the observed drop in WHC between *pre-* and *post-rigor* samples.

Higher water content in the muscle implies more loosely bound water that is easier to remove, hence lower WHC and increased drip loss. Both WHC and water content have a significant interaction between localization and season in this trial, where increased amount of water in the muscle was accompanied by decrease in WHC. Water content and fat content are proved to have a strong negative correlation in salmon (28), so the observed difference in water content might imply increased fat content in the fish and that the amount of fat in the fish affects both WHC and drip loss, which might

**Fig. 3.** Interaction plot between localization (north: N63–64°, and south: N58–59°) and season (spring and autumn) for: a) water-holding capacity (WHC; $p<0.001$, $F=24$) and b) water content in the muscle ($p<0.001$, $F=147$) ($N=80$)

explain the observed differences of WHC and pH during the season. Observed differences in pH between the two localities might be explained by stress before slaughter.

Drip loss during storage

Storage time is a continuous variable and pH is known to have an influence on water-holding capacity (4), so incorporating both storage time (days from slaughtering) and pH as covariates into general linear model revealed increased drip loss with both increasing storage time ($r=0.846$, $p<0.001$, $F=877$) and increasing pH ($r=0.222$, $p<0.001$, $F=14$).

Season affected the drip loss significantly ($p<0.001$, $F=16$) (Table 2). Significant interactions between localization and season ($p<0.001$, $F=22$, data not shown) and localization and *rigor* ($p=0.027$, $F=5$, data not shown) were detected. No other significant effects were detected ($p>0.169$). Season is known to affect both quality and composition of salmon, where fat, texture and drip loss increase after the strong growth in the summer (12,29). The growth of farmed salmon is strongly linked to the temperature of the sea (30), where the fish increase growth up to a certain point, before it drops (31). Both photoperiod and water temperature are different along the long Norwegian coast, where the south part has higher water temperature than the north, but north has longer daylight in the summer. Salmon slaughtered in the spring had 0.27 % increased drip loss compared to those slaughtered in the autumn, while localization had no significant effect ($p>0.05$) on either drip loss or WHC in our trial. In addition, WHC, drip loss and water content had a significant interaction with localization and season, where increased WHC was accompanied by decreased drip loss and decreased water content and *vice versa*. This is in contradiction with the findings of Mørkøre *et al.* (12), who found an increased drip loss in October compared to April in salmon, while Roth *et al.* (32) found no effect of season on turbot.

The drip loss was moreover significantly affected by the *rigor* status ($p<0.001$, $F=49$) (Table 2). By splitting the data set in

post-rigor and *pre-rigor* and performing a linear regression with time (Fig. 4), the drip loss of *post-rigor* fillets increased on average 0.14 % each day ($R^2=0.945$), whereas the drip loss of *pre-rigor* fillets increased on average 0.18 % each day ($R^2=0.985$), reaching (2.50 ± 0.46) % on day 18 and (2.62 ± 0.50) % on day 14 respectively. The drip loss of HOG after 4 days of storage was (0.14 ± 0.34) %. Previous studies on the effect of *rigor* status during filleting on drip loss are inconclusive. Skjervold *et al.* (33) and Rosnes *et al.* (34) found higher drip loss in *pre-rigor* fillets after 14 and 16 days of storage on ice than in *post-rigor* fillets, while Einen *et al.* (25) and Skjervold *et al.* (22) found no effect of filleting time on drip loss. Fillets in the present study had a drip loss of 2.5–2.6 % at the end of storage, while in the cited studies it ranged from 0.9–3 %. All these reports related the drip loss to the time from filleting, not the time from slaughtering. In the present study, however, the HOG had almost ignorable drip loss, where some of the HOG even gained mass. One could expect less drip loss in *pre-rigor* filleted fillets since *pre-rigor* fillets have higher WHC. The drip loss was however higher in *pre-rigor* filleted samples than in those filleted *post-rigor*. During filleting the muscle is exposed, which increases the flesh surface and subsequently the potential for drip loss. In addition, WHC in *pre-rigor* filleted salmon measured *post-rigor* showed the same WHC as *post-rigor* filleted salmon (22,23). It is important to remember that the major variation of drip loss in the present study was explained by storage time. If the drip loss in the present study was related to the time of filleting, *post-rigor* would have 0.18 % drip loss on average each day, almost identical to *pre-rigor* (data not shown).

Composition of drip loss

Storage time was a significant ($p<0.001$) covariate for all the four analyzed constituents, where the amount of water ($r=-0.94$, $p>0.001$, $F=271$), fat ($r=-0.85$, $p>0.001$, $F=92$) and astaxanthin ($r=-0.68$, $p<0.001$, $F=27$) decreased significantly with storage time (Table 2). Protein, on the other hand, increased with storage time ($r=0.91$, $p<0.001$, $F=205$).

Table 2. The effect of *rigor* status during filleting (*pre/post-rigor*), growth season (spring/autumn) and location (south/north) on drip loss and the composition of drip loss

	Composition of drip loss				
	Drip loss/%	w(water)/%	w(fat)/%	w(protein)/%	γ (astaxanthin)/(mg/L)
<i>Rigor</i> (N=80)					
Pre	1.9±0.9	85.7±2.3	0.03±0.01	13.6±2.7	0.15±0.04
Post	1.4±1.1	86.4±1.3	0.035±0.008	12.6±1.4	0.15±0.02
p-value	<0.001	0.003	>0.05	0.002	>0.05
Season (N=80)					
Spring	1.8±1.1	86.0±1.8	0.03±0.01	13.4±2.2	0.1±0.03
Autumn	1.5±1.1	86.1±2.2	0.03±0.01	12.8±2.2	0.2±0.04
p-value	<0.001	>0.05	>0.05	0.014	>0.05
Localization (N=80)					
South	1.6±1.1	85.8±1.9	0.03±0.01	13.2±2.2	0.14±0.04
North	1.7±1.0	86.3±2.2	0.03±0.01	13.0±2.3	0.15±0.03
p-value	>0.05	0.011	>0.05	>0.05	>0.05

The results are expressed as mean value±S.D., ranked by ANOVA (general linear model)

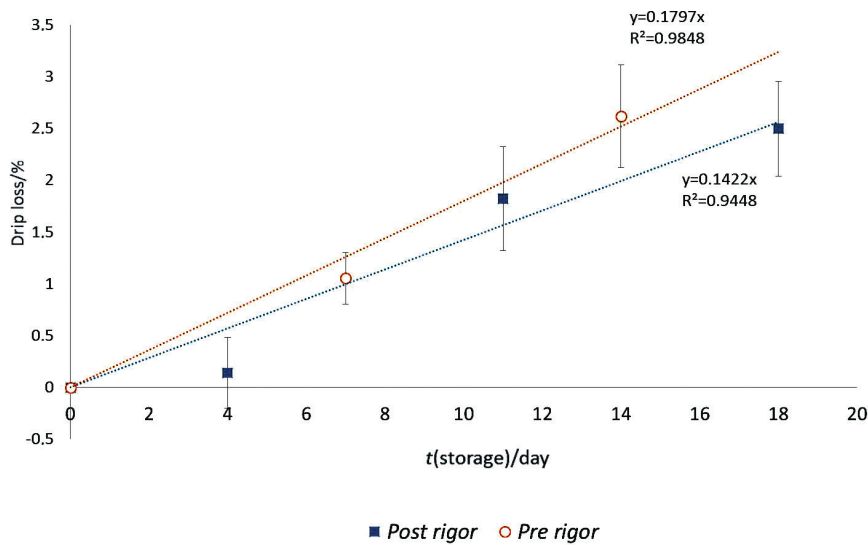


Fig. 4. Accumulated drip loss of *pre-rigor* (circle) and *post-rigor* (square) fillets stored on ice, where the *post-rigor* ones were stored as head on gutted for the first 4 days ($N=80$)

The water content was significantly higher in drip loss from *post-rigor* fillets ($p=0.005$, $F=8.5$), and in fillets from the north location ($p=0.001$, $F=4.6$), while the protein content was significantly lower in *post-rigor* fillets ($p=0.002$, $F=12$) and in the fillets from the autumn group ($p=0.014$, $F=5.6$). In addition, significant interaction effects between location and *rigor* status were found on both water content ($p<0.001$, $F=22$) and protein content ($p=0.003$, $F=11$) (**Fig. 5**). Degradation of protein is an effect of autolysis, where the degradation increases with time. Proteins lost in the drip loss are the water-soluble proteins. The composition of proteins in salmon muscle varies during storage, and water-soluble proteins decrease significantly from 5 to 14 days *post mortem* (11). *Post-rigor* filleted salmon was 4 days older at the time of assessment than the

pre-rigor salmon, and this time difference can explain lower amount of protein in the drip loss. In farmed cod, *pre-rigor* fillets lost more protein than *post-rigor* fillets (35), most likely due to the same effect. In addition, the amount of protein increased during storage, confirming the degradation of the fillet.

CONCLUSIONS

All the investigated variables had impact on different quality aspects on the salmon, where *rigor* status and filleting had the highest impact on water-holding capacity and water content in the muscle. In addition, storage time was the factor that had the highest impact on the drip loss during storage.

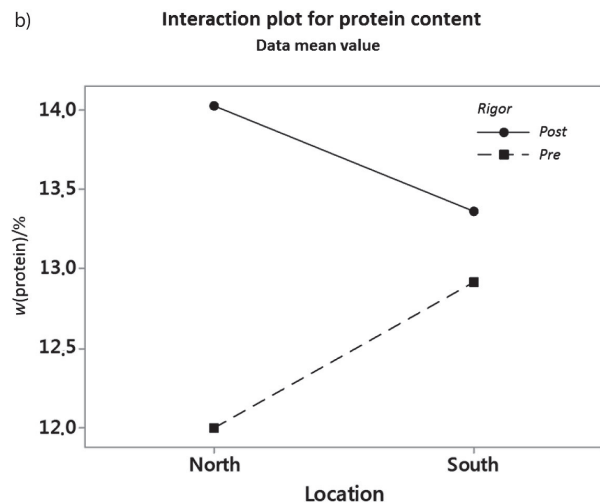
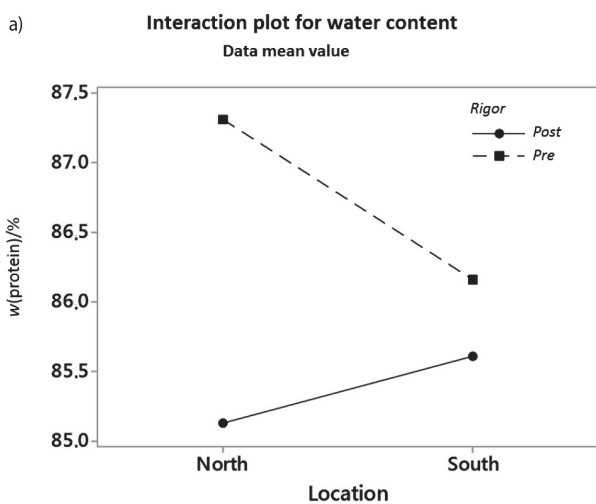


Fig. 5. Interaction plot between localization (north: N63-64° and south: N58-59°) and *rigor* (*pre* and *post*) status for: a) water content in the drip loss ($p<0.001$, $F=22$) and b) protein content in the drip loss ($p=0.003$, $F=11$) ($N=80$)

Based on this trial, it is concluded that *pre-rigor* filleted salmon have excellent water-holding properties and a great potential for early processing (*pre-rigor* processing). It was however difficult to find a clear connection between the drip loss and the water-holding capacity of the muscle.

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