Gaping and loss of fillet firmness in farmed salmon (*Salmo salar* L.) closely correlated with post-slaughter cleaning of the abdominal cavity

Ása Jacobsen[1], Hóraldur Joensen[2] & Jonhard Eysturskarð[1]

[1]Fiskaaling, Aquaculture Research Station of the Faroes, Hvalvík, Faroe Islands

[2]Department of Science and Technology, University of the Faroe Islands, Tórshavn, Faroe Islands

Correspondence: Á Jacobsen, Fiskaaling, Aquaculture Research Station of the Faroes, Við Áir, Hvalvík 430, Faroe Islands. E-mail: asa@fiskaaling.fo

[The copyright line for this article was changed on 4 October 2015, after original online publication.].

Abstract

This study analysed the effect of cleaning intensity of the abdominal cavity and storage temperature from slaughter to the end of processing on the quality of farmed salmon (*Salmo salar* L.) fillets. These two parameters were manipulated in an experimental setup using in total thirty salmon with an average weight of 4.2 kg. The experiment was designed to imitate realistic scenarios in a normal production process in the Faroe Islands. The salmon stored at low temperatures had an average muscle temperature of 4.65°C, whereas the salmon stored at ambient temperature had an average muscle temperature of 11.27°C. After the salmon were gutted to remove all viscera except the kidney, the abdominal cavity was either rinsed lightly or meticulously cleansed of kidneys, all blood and bodily fluids. A wide range of quality and production parameters were measured either straight after cleaning or after the salmon had been stored in chipped ice at 1.5°C for 7 days. All measured parameters were analysed for possible correlations by principal component analysis (PCA). Blood and remains left in the abdominal cavity were shown to have a significant negative effect on fillet firmness ($P < 0.01$) and gaping ($P < 0.01$). The different storage temperatures between slaughter and gutting, tested in this experiment, did not significantly affect fillet firmness or gaping. However, the fillet colour showed significant negative correlation ($P < 0.01$) with the storage temperatures applied.

Keywords: fillet quality, fillet gaping, fillet firmness, Atlantic salmon

Introduction

Quality issues related to farmed Atlantic salmon (*Salmo salar* L.) fillets have been studied quite intensively over the last few decades. There are many quality parameters to take into account, but some of the main concerns are gaping and loss of fillet firmness, which according to Mørkøre and Rørvik (2001) are not inherently interrelated. Gaping is the tearing of the connective tissue between muscle layers and hence causes holes and slits in the fish fillet. This leads to the downgrading of the food product, and hence economic loss, because of the rejection by consumers due to its unappealing appearance. This also causes limitations for specialized food production (Pittman, Grigory & Brandebourg 2013). Fillet firmness is measured manually by pressing a finger on the fillet to estimate the elasticity and firmness. This should not be confused with fillet texture, which is measured mechanically by a Texture Analyser. Loss of fillet firmness poses the same problems as gaping for the industry due to difficulty in further processing, downgrading of the product, and consumer rejection is also a very important sensory criterion (Veland & Torrisen 1999; Torgersen, Koppang, Stien, Kohler, Pedersen & Mørkøre 2014). Relating these quality issues to one specific reason has proven to be difficult. Although the extensive research has shed light on several key elements and optimization possibilities, there are still contradictory results, which indicate that the underlying causes are not yet fully understood, especially so for gaping. The most recognized cause of gaping and soft fillets is the increased

level of stress caused by handling prior to and at slaughter (Roth, Slinde & Arildsen 2006; Bahuaud, Mørkøre, Østbye, Veiseth-Kent, Thomassen & Ofstad 2010). This is believed to be linked to a decrease in pH (Lavety, Afolabi & Love 1988; Skjervold, Fjæra, Braarød & Einen 2001). Acidic conditions also cause an increased activity of Cathepsin L in the muscle tissue, which degrades collagen and is linked to the softening of the fillets (Bahuaud, Gaarder, Veiseth-Kent & Thomassen 2010). However, stress cannot always be correlated with gaping and loss and fillet firmness (Kiessling, Espe, Ruohonen & Mørkøre 2004). Fillet firmness has also been shown to be heritable (Bahuaud, Gaarder et al. 2010) whereas gaping, on the contrary, does not seem to be genetically determined (Kause, Quinton, Airaksinen, Ruohonen & Koskela 2011). On the other hand, gaping has been shown to vary with season, although a consistent pattern seems difficult to deduce (Mørkøre & Rørvik 2001; Espe, Ruohonen, Bjørnevik, Frøyland, Nordtvedt & Kiessling 2004). In addition, location of catch has been shown to have an effect (Margeirsson, Jonsson, Arason & Thorkelsson 2007). However, it is clear that the loss of strength in the connective tissue, which reveals itself as gaping, is connected to a higher percentage of soluble collagen in the extracellular matrix (ECM) compared to the fillets with intact connective tissue (Espe et al. 2004). The location of tearing has been found to be in the sarcolemma, the cell membrane of the muscle cells, which connects the muscle fibres to the myocommatal sheets of the ECM, and in the innermost layer of the myocommata, the endomysium. The myocommatal–muscle fibre interface has been shown to have an increased degree of disconnection with increased gaping (Bremner & Hallett 1985; Hallett & Bremner 1988; Ofstad, Egelandsdal, Kidman, Myklebust, Olsen & Hermansson 1996; Fletcher, Hallett, Jerrett & Holland 1997). Both in the extracellular matrix and in the sarcolemma, the components: collagen, proteoglycans (PGs) and glycosaminoglycans (GAGs) interact to form a structural network, which greatly influences the textural properties of various connective tissue types. Collagenases present in the tissue can cause harmful proteolytic degradation of the collagen but in sound connective tissues PG and GAG chains protect the collagen (Geng, McQuillan & Roughley 2006). In living tissue, the expression, activation and inhibition of these collagenases is controlled to maintain tissue homoeostasis including necessary turnover and restructuring of collagen (Birkedal-Hansen, Moore, Bodden, Windsor, Birkedal-Hansen, DeCarlo & Engler 1993). The increased amount of soluble collagen in gaping tissue therefore suggests that these normal conditions of equilibrium are somehow disturbed. Dr Nielsen, QC Manager at Hiddenfjord (Faroe Islands), states that apart from handling stress, two production parameters seem to be critical factors responsible for the gaping severity of farmed Atlantic salmon fillets. One factor is the initial storage temperature from the time the salmon are killed and until they are further processed. In their experience, chilling the salmon immediately after slaughter seems to have a positive effect on the quality of the fillets. The other factor is the thoroughness of the post-mortem cleaning of the abdominal cavity. Leaving parts of the organs or intestines, blood and/or other bodily fluids in the abdominal cavity was suspected to induce gaping. Changing procedures to meticulously cleaning the abdomen reduced occurrences of gaping (personal communication). This article reports the results of an experiment focusing on these two production parameters, which we will attempt to relate to the corresponding firmness and gaping level of Atlantic salmon fillets. A broad range of other production and quality parameters were also included in the study, and principal component analysis (PCA) was performed to reveal possible correlations.

Materials and methods

Material background and experimental setup

Thirty Atlantic salmon, with an average size of 4.2 kg, were kindly provided by the aquaculture farming company Hiddenfjord (Faroe Islands). All individuals measured were taken from the same net pen on an aquaculture site located in Sørvágur, Faroe Islands. On the day of sampling, 7 July 2011, the seawater temperature in the area was 9.8°C. Fish from the same net pen are usually of the same age and all the biotic and abiotic factors have been identical from fry to slaughter. None of the salmon used in the experiment had reached maturity. The standard process of slaughter laid emphasis on avoiding handling and crowding stress as much as possible. The net pen with salmon was tugged very carefully from the on-growing site in the fjord to the slaughter

facility by the shore. They were subsequently pumped up to the slaughter processing line, where they were killed by a blow to the head in an automated system. The gills were thereafter slit open, before the salmon were released from the automated system to an inspection table. All individuals were inspected manually to ensure the gills had been cut well enough for the salmon to bleed out properly. The first fifteen specimens to be used in the experiment were taken off the inspection table at this stage. They were carefully held with the head angled down until having bled out and thereafter stored at ambient temperature, approximately 11°C. All other individuals were submerged in iced seawater in a transport container. At the time of the experiment, the cooling system unfortunately was not functioning optimally, and the temperature of the ice water in the container was approximately 3.5°C. When full, the container was moved to another facility where the salmon were processed further. The time span between slaughtering and gutting was approximately 2 h. At the first stage on the processing line all salmon were gutted. Intestines and organs were removed, though the kidney was not cut/scraped out completely. The fifteen salmon taken earlier off the inspection table were, at the same time as the others, put through the same process of gutting. At the second stage the salmon were rinsed and their abdominal cavity cleaned thoroughly, removing all traces of organs, intestines, blood and bodily fluids. Ten of the fifteen salmon previously stored at ambient temperature were cleaned well, according to standard procedure, whereas the other five were only rinsed lightly, not cleaned properly. All 15 salmon, previously stored at ambient temperature, were thereafter stored in transport boxes with chipped ice. Fifteen of the salmon that had been stored and transported in the iced seawater were also taken to be used in the experiment. Ten of these 15 individuals were only rinsed lightly, not cleaned properly, whereas the other five were rinsed and the abdominal cavity thoroughly cleaned. Again, all fifteen individuals were subsequently stored in transport boxes with chipped ice. Table 1 provides an overview of the various treatments.

Quality measurements and evaluations

Three hours after the beginning of the slaughtering process, all thirty salmon taken aside for the experiment were stored in transport boxes with

Table 1 The various experimental treatments and number of salmon subjected to investigation

	Well cleaned	Not cleaned	Total
Low temp.	5	10	15
High temp.	10	5	15
Total	15	15	30

chipped ice. At this time a few measurements were taken. The objective measurements were length, weight and muscle temperature. Sensory evaluations included stage of rigour mortis, skin condition, colour of gills and cleaning of the abdomen. The sensory evaluations were each categorized into groups 0–3, where 0 was the best condition and 3 the worst. Following the measurements, every individual was again stored in a transport box with chipped ice, and all were subsequently transported to a cooling facility for storage at 1.5°C for 7 days.

On the 7th day, standardized quality measurements and evaluations (Table 2) were carried out by trained personnel, unaware of the experimental changes made to the initial storage temperature and cleaning of the abdominal cavity. The salmon were filleted by hand, and additional sensory evaluations were conducted categorizing each individual according to appearance in the same manner as on day one. These evaluations included the Quality Index Method (QIM) for salmon, developed by Sveinsdottir, Hyldig, Martinsdottir, Jorgensen and Kristbergsson (2003). The number of holes and slits observed in the fillet determined the gaping score. Fillet firmness score was determined by pressing with the forefinger on the loin right in front of the dorsal fin. Objective measurements performed on day seven included analysis of a standard section of the fillet, the Norwegian Quality Cut (NQC – Norwegian standard procedure – NS 9401 1994). The fillet texture was measured as breaking strength with a TA.XT plus Texture Analyser (Stable Micro System Ltd., Surrey, UK), equipped with a flat-ended cylinder (12.5 mm diameter, type P/0.5). The trigger force was 0.1 N and the test speed was 1 mm s^{-1}. The force–time graph was recorded by a computer, equipped with the Texture Expert software for Windows (version 1.15, SMS, Surrey, UK). Three recordings per fillet were made perpendicular to the muscle fibres, and mean values, expressed as the force (N) required puncturing the surface of the sample, were calculated.

Fat content and pigmentation (astaxanthin concentration) in the NQC was analysed using PhotoFish^MT (Nofima, Ås, Norway). An overview of all measurements and parameters categorizing the salmon is given in Table 2.

Statistical analysis

Fisher's exact test for Count data (Fisher 1922) was used for testing differences in gaping and firmness scores between the groups with differing cleaning and storage conditions (Table 1). The Fisher's exact test was applied because it is useful for categorical data and, contrary to approximation tests, is valid for small sample sizes. Strictly speaking, the test requires both the column and row totals in the data matrix to be fixed in order for it to be exact. In cases where not all of the row

Table 2 Parameters included in the study

Day	Parameters	Units/Grades
Sensoric evaluations		
1	Loss of scales	0–3
1	Rigour status	0–3
1	Cleaning of the abdominal cavity	0–3
1	Gill colour	0–3
7	Fillet – gaping	0–3
7	Fillet – firmness	0–3
7	Fillet – colour	*
QIM		
7	Skin – appearance	0–2
7	Skin – slime	0–2
7	Skin – smell	0–3
7	Skin – firmness	0–2
7	Eye – pupils	0–2
7	Eye – shape	0–2
7	Gills – colour	0–2
7	Gills – slime	0–2
7	Gills – smell	0–3
7	Abdomen – blood	0–1
7	Abdomen – smell	0–3
Objective measurements		
1	Weight	kg
1	Length	cm
1	K-factor	†
1	Temperature	°C
7	Temperature	°C
7	NQC colour – PhotoFish	‡
7	NQC pigmentation – PhotoFish	mg kg^{-1}
7	NQC fat content§ – PhotoFish	%
7	Fillet texture – Texture Analyzer	N

*A *Salmo*Fan™ Ruler was used for measuring.
†K-factor was calculated as K = $10^N W/L^3$, N = 5.
‡PhotoFish measures expressed as SalmoFan values.
§Group average.

or column totals are conditioned, the Fisher's exact test instead provides a conservative P-value. The 4 × 4 factorial matrixes, four treatment groups and four categories of either gaping or firmness, were analysed. The Fisher exact tests were also applied to two groups at a time, in order to compare groups with only one of the treatment parameters varying. If a treatment parameter was shown to have no significant effect on either gaping or firmness scores, the groups, which differed in only that parameter, were combined. The Fisher exact test was thereafter performed on the 2 × 2 factorial matrix for the other treatment parameter. The tests were performed in the statistical software package R (http://www.r-project.org).

For multivariate analysis the data matrix with results from the extensive sensory analysis was scaled first by normalization and secondly by log-transformation. The number of variables and samples was 28 and 30 respectively. Normalization is obligatory for a subsequent proper comparison of the sample data, and this part of the pre-processing was carried out by expressing each variable as a percentage of the sum of values for each sample. The normalized data were then log-transformed to avoid domination of the variables with comparatively large entries. The data were since subjected to Principal Component Analysis, PCA (Wold 1976) using the software package SIRIUS (Kvalheim & Karstang 1987). In the PCA process the samples or objects are placed in a 28-dimensional vector-space, i.e. one coordinate for each variable and new orthogonal vectors or principal components, PCs, are generated through the centroid of all the objects in the multidimensional space. The courses of the new PCs are in the direction of the largest and second largest variation of the objects. In this manner the dimensionality is reduced from 28 dimensions to two without loosing much of the total variance. The relation among the objects is displayed by projecting them on the plane spanned by the two PCs, that is PC1 and PC2 describing the largest variation and next largest variation respectively.

Results and discussion

Cleaning of the abdominal cavity

The Fisher's exact test applied to the 4 × 4 factorial matrixes, four treatment groups and four categories of either gaping or fillet firmness, showed

highly significant differences between the groups; $P < 0.01$ for both gaping and firmness. However, this does not state what effect each of the treatment parameters, temperature and level of cleaning, had on the quality estimates. The Fisher exact tests applied to two groups at a time, with the same cleaning treatment but different initial storage temperatures, were not significant for neither gaping nor fillet firmness (data not shown). The Fisher exact tests applied to the groups with different cleaning treatments kept at same temperatures, on the other hand, were significant in most cases. Concerning gaping, the Fisher's exact test of the high temperature groups showed significant differences between the two cleaning treatments ($P < 0.01$), but not for the low temperature groups ($P = 0.077$), whereas the tests for fillet firmness were significant for both high and low initial storage temperatures ($P < 0.01$). Because the temperatures tested in the experiment did not seem to have different effects, the salmon were grouped according to how well the abdominal cavity was cleaned only, for further analyses.

The salmon with well-cleaned abdominal cavity and the salmon, which were not cleaned, had different group distributions of gaping scores (Fig. 1a). The salmon that were not cleaned ($n = 15$) had a very high occurrence of gaping with 14 individuals having the highest gaping score. In the group of salmon with well-cleaned abdominal cavity ($n = 15$), there were equally many with gaping scores 1 and 3. Fisher's exact test for Count data showed a significant difference between the distribution of gaping scores of the two groups ($P < 0.01$).

Fillet firmness did not vary much, as only scores 1 and 2 were detected. This indicated a more moderate effect, but still, the level of fillet firmness in the group of salmon with well-cleaned abdominal cavity was markedly different from that in the group of salmon with not cleaned abdominal cavity (Fig. 1b). Thirteen of the 15 individuals, cleaned well, had a firmness score of 1, whereas all but one of the not cleaned individuals had a firmness score of 2. Again Fisher's exact test for Count data showed a significant difference ($P < 0.01$) between these two groups.

This experiment reveals that bodily fluids, remnants and blood left in the abdominal cavity during storage have a highly negative effect on the fillet quality of Atlantic salmon. The strong correlation detected between not cleaned abdominal
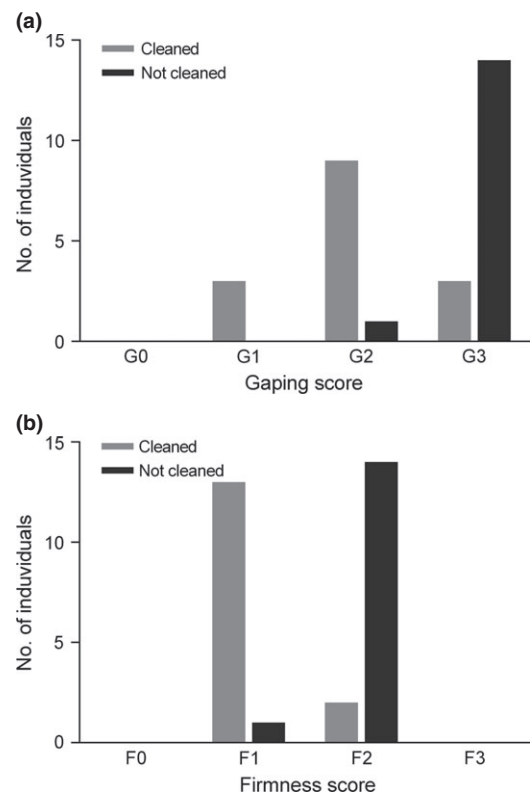
Figure 1 (a,b) Fillet gaping and firmness. Distribution of (a) gaping scores and (b) firmness scores of the individuals with either cleaned or not cleaned abdominal cavities. Gaping score is determined by number of holes and slits in the fillet. G0: no gaping, G1: < 5 small, G2: < 10 small and/or 5 large, G3: > 10 small and/or 5 large. Small slits: < 1 cm, large slits: > 1 cm. Firmness score is determined by pressing with the forefinger on the loin right in front of the dorsal fin. F0: firm and elastic, F1: firm but not elastic, F2: soft, F3: very soft.

cavities and a higher occurrence of gaping and loss of fillet firmness has to our knowledge not been previously reported. However, in a review by Borderias and Sánchez-Alonso (2011) it was recommended to clean gutted fish thoroughly in order to remove traces of blood and intestinal content, as this has been shown to reduce the microbial load (Erkan 2007). For the same reason starvation was highly recommended in order to reduce the amount of digestive enzymes produced by bacteria in the intestines. In fresh, chilled fish the bacteria generally do not invade the muscle tissue, as their activity is mainly on the surface. On the other hand, the bacterial enzymes diffuse from the surface into the muscle tissue, releasing compounds into fluids and watery substances,

which diffuse outwards (Huss 1995). It is also possible for the bacteria to degrade the collagen network in the ECM with their collagenases. These differ from vertebrate collagenases, as they exhibit broader substrate specificity (Peterkofsky 1982; Birkedal-Hansen 1987). They can attack almost all collagen types, and are able to make multiple cleavages within triple helical regions (Mookhtiar & Van Wart 1992). Bacterial collagenases have shown the ability to disrupt the extracellular matrix of arterial walls in vertebrates, where collagenases present in the blood did not cause such degradation (Rosenberg, Estrada, Kelley & Kornfeld 1993). Brown, Hook and Tragakis (1972) reported the same phenomenon, where corneal collagen was protected by proteoglycans from degradation by collagenases present, but was degraded by bacterial collagenases, as they also broke down the proteoglycans. However, bacterial activity is not the only possible explanation for the ECM degradation detected in this experiment, as polymorphonuclear leucocytes in the blood contain collagenases, that are also able to degrade proteoglycans and hence the collagen structure. These lysosomal collagenases, also named neutrophilic collagenase or Matrix Metalloproteinase 8 (MMP-8), have been connected to various diseases, where the collagen structure is damaged (Harris, Faulkner & Brown 1975; Gangbar, Overall, McCulloch & Sodek 1990; Herman, Sukhova, Libby, Gerdes, Tang, Horton, Kilbride, Breitbart, Chun & Schönbeck 2001). Matrix Metalloproteinase 8 is mainly produced in polymorphonuclear granulocytes and preferentially degrade collagen type I. Matrix Metalloproteinase 8 degrades collagen type I much more effectively than the other interstitial collagenases, MMP-1 and MMP-13, which preferentially degrade collagen types III and II respectively (Herman *et al.* 2001). As type I collagen is a major constituent of the ECM in salmon, MMP-8 could very likely cause damage to the connective tissue resulting in gaping of the fillet. Coagulation of the blood also induces an enhanced release and activation of MMP-8 (Jung 2008; Manello 2008), and the remains of the blood present in the abdominal cavity will thus contain a high concentration of activated MMP-8, capable of degrading the ECM in the salmon fillet. As mentioned in the introduction some lysosomal cysteine proteases also have the ability to disrupt the collagen network, but their activity is generally thought to be restricted to the lysosomes, as they are inhibited by the neutral pH

and oxidative extra lysosomal environment. However, cathepsin L, which previously has been associated with post-mortem autolysis of fish muscle, has fairly recently been detected in blood cells of certain fish species (Ahimbisibwe, Inoue & Aoki 2010). Blood with lowered pH, caused by stress prior to and at slaughter, left in the abdominal cavity could, thus contain active forms of cathepsins, capable of degrading the ECM structure. Degradation by both bacterial and neutrophilic collagenases can also be enhanced by stress. Hansen, Rødbotten, Eie, Lea, Rudi and Mørkøre (2012) showed that crowding stress had a significant impact on the bacterial load in salmon fillets. Others have discovered that stress causes an increase in polymorphonuclear leucocytes in the blood (Sasagawa, Matsubara & Satow 1993; Suzuki, Totsuka, Nakaji, Yamada, Kudoh, Liu, Sugawara, Yamaya & Sato 1999). Several components may also operate in concert, as various bacteria have the ability to activate host MMPs and neutrophil interstitial procollagenases as well as inactivate proteinase inhibitors (Lähteenmäki, Kuusela & Korhonen 2001).

Further research is required to describe the degradation process properly and determine the enzymes involved in the loss of fillet firmness and increase in fillet gaping.

## Temperature between slaughter and gutting

In contrast to the cleaning of the abdominal cavity, the storage temperature between slaughter and gutting did not show an effect on gaping or fillet firmness (data not shown), and Fisher's exact test for Count data was not significant ($P > 0.05$). The muscle temperature in the salmon stored at ambient temperature was on average 11.27°C, SD = 0.08, measured straight after gutting and cleaning. Under optimized conditions, the salmon are stored in iced seawater between slaughter and gutting in order to keep the salmon chilled at all times. However, at the time of sampling, the cooling system was not operating at full effect due to a malfunction. Muscle temperatures measured straight after gutting and cleaning of the fifteen salmon which were chilled was on average 4.65°C, SD = 0.65. This is slightly higher than the desired threshold of max 4.0°C, and the variation within the group is fairly large. These limitations in the comparisons of temperature differences between the various experimental groups, caused

by relatively high and varying muscle temperatures in the low temperature group, have to be taken into account, when considering the results. As the results show no difference in the occurrence of gaping or loss of fillet firmness between the two initial storage temperatures tested, two options are indicated. One option is that the muscle temperature has to be above 11.3°C, during an approximately two to three hour period in the production process before proper storage, to induce gaping or loss of fillet firmness. The other option is that the muscle temperature has to be kept below the approximate 4.5°C, measured in this experiment during initial storage and processing, in order to avoid temperature-induced gaping and loss of fillet firmness. If this latter option would be the case, then only a small elevation of initial storage temperature could have an effect on quality. Purely speculative, the overall high gaping scores found in this study might be an indication of this. Furthermore, it should be kept in mind that many of the possible mechanisms behind the specific tissue degradation that causes gaping or loss of fillet firmness are temperature dependent.

Multivariate analysis

A comprehensive simultaneous evaluation of the samples and variables was obtained by multivariate analysis, PCA, visualized in a PC-plot (Fig. 2), which illustrates the relationship between the manipulated variables, temperature (Temp) and cleaning of the abdominal cavity (Abd) and the sampled individuals as well as their mutual relationship. The reciprocal relationship between all relevant quality variables measured is also illustrated in the plot. The variables centred around origo and a few others without relevancy to the plot were eliminated for clarity.

It is evident that the two manipulated variables, Temp and Abd, have the largest influence on the positioning of both the sampled individuals and the other variables in the PC plot. First, all the other measured variables are much closer to the origo and second all the individual salmon samples are neatly grouped according to temperature and cleaning treatment of the abdominal cavity, positioned in relation to the manipulated variables. Individuals stored at low and high temperatures, respectively, are separated along the axis connecting Temp and origo and the cleaned and not cleaned individuals are separated along the axis
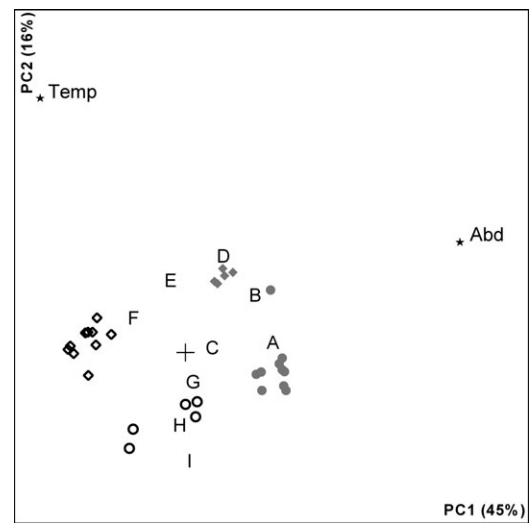

Figure 2 PC plot of variables and samples. The represented variables are: temperature in the abdominal cavity between gutting and storage (* Temp), remains left in the abdominal cavity (* Abd), fillet gaping day 7 (A), fillet firmness day 7 (B), fillet texture day 7 (C), rigour status day 1 (D), smell of skin day 7 (E), abdominal cavity temperature day 7 (F), fillet colour day 7 (G), and colour of gills day 7 (H) and day 1 (I). The samples are salmon with well-cleaned abdominal cavity and kept chilled between slaughter and gutting (○), well cleaned but not chilled (◇), not cleaned but chilled (●), and not cleaned and not chilled (◆). The odd one out sample near variable B had a relatively high temperature (>6°C). The first principal component (PC1) and the second principal component (PC2) describe 45% and 16% of the variance respectively.

connecting Abd and origo. The odd one out had a relatively high storage temperature compared to the other salmon from the same treatment group.

The quality parameters gaping (A) and firmness (B) are drawn towards Abd, which means higher scores are positively correlated with the lack of cleaning of the abdominal cavity. Gaping seems to be slightly more affected as it is drawn a bit closer towards Abd and at a slightly narrower angle.

In order to see how well gaping was correlated with the level of cleaning, a new calculation was carried out including the not cleaned individuals only. The resulting biplot has Abd scores as abscissa and gaping score as ordinate (Fig. 3a).

With one sample excluded, as it turned out to be an outlier, the two variables were perfectly correlated ($R^2 = 1.000$). The exact correlation is of course influenced by the fact that the variables are discrete data, prior to normalization and
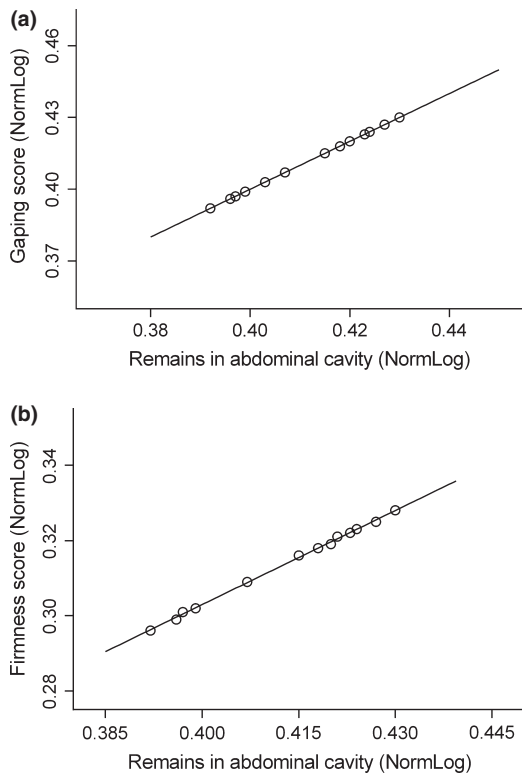
Figure 3 (a,b) Biplot of gaping scores (a) and firmness scores (b) correlated with level of remains in the abdominal cavity after normalization and log-transformation. Not cleaned individuals only.

Log-transformation. In the same way, an analysis of fillet firmness as a function of cleaning of the abdominal cavity was conducted. Again the variables were perfectly correlated ($R^2 = 0.999$) (Fig. 3b), when another different outlier sample was excluded.

Mørkøre and Rørvik (2001) found out that gaping could well occur even though firmness was high. This assessment that there is no linear relationship between gaping and fillet firmness is not disputed by our results. However, it is clearly demonstrated that both quality parameters are negatively affected by a not cleaned abdominal cavity during storage; something researchers should have in mind for future experiments including these parameters.

In the PC plot (Fig. 2) the mechanical measurement of fillet texture (C) is also drawn towards Abd, albeit not as far as gaping and firmness, but in an almost straight line, a clear indication of correlation. As a consequence, the not cleaned salmon thus have fillets with higher breaking

strength at the same time as they have more gaping and loss of firmness. The lack of intuitive correlation between texture and gaping has been demonstrated previously by Kiessling et al. (2004). Likewise, Stien, Hirmas, Bjørnevik, Karlsen, Nortvedt, Bencze Rørå, Sunde and Kiessling (2005) did not find any significant effect of stress on fillet texture in cod, when measured by a texture analyser. Also rigour (D) is affected by the manipulated parameters, as it is positioned close to the samples neither cleaned nor cooled, which are positively correlated with both Temp and Abd. The correlation between rigour and storage temperature is consistent with previous findings (Borderias & Sánchez-Alonso 2011), but a mutual relationship between rigour and lack of post-slaughter cleaning of the abdominal cavity has to our knowledge not been reported previously.

Along with the high temperature groups, the skin smell (E) and temperature (F) on day 7 are drawn towards Temp (Fig. 2), and thus positively correlated with the initially elevated storage temperatures. The colour of the fillet and gills (G, H, I) on the other hand seems to be negatively correlated with the elevated temperature and not affected by the cleaning of the abdominal cavity, as these are at an almost 90 degree angle to Abd and 180 degree angle to Temp from the Origo. The difference in fillet colour for salmon stored at high and low temperature between slaughter and gutting is illustrated in a boxplot (Fig. 4) with the SalmoFan values (NormLog). A Wilcoxon test,
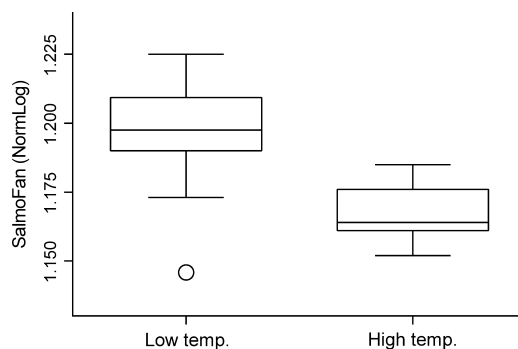
Figure 4 Fillet colour. Boxplot of normalized and log-transformed values of fillet colour comparing salmon chilled (4.65°C average) with salmon kept at ambient temperature (11.27°C average) from slaughter to end of gutting and cleaning. Fillet colour was measured using a SalmoFan™ Ruler and values ranged from 25.5 to 29.0.

performed in Excel, showed a significant difference between the two groups ($P < 0.01$). Fillet colouration is generally caused by carotene deposition in the muscle pre-mortem, but it has been shown to be affected by slaughtering methods (Robb, Kestin & Warris 2000; Erikson & Misimi 2008). However, the effect of temperature differences on fillet colour straight after slaughter has to our knowledge not been reported previously.

Fat content and harvest weight showed no connection to either gaping or loss of fillet firmness (data not shown). This is consistent with previously published findings (Andersen, Strømsnes, Steinsholt & Thomassen 1994; Andersen, Thomassen & Rørå 1997; Johnston, Bickerdike, Li, Dingwall, Nickell, Alderson & Campbell 2007; Kause et al. 2011).

Conclusion

In conclusion, the results show that the severity of gaping as well as loss of fillet firmness is significantly increased by leaving blood and/or other remains in the abdominal cavity after slaughter and gutting. The multivariate analysis also verifies that gaping and loss of fillet firmness is not connected to any of the other quality parameters. Furthermore, the experiment shows that an elevated storage temperature of the salmon, for just two to three hours following slaughter, causes loss of fillet and gill colour.

Acknowledgments

References

Ahimbisibwe J.B., Inoue K. & Aoki T. (2010) Detection of cathepsin L in red cell membranes from fish blood. *Fisheries Science* **76**, 155–159.

Andersen U.B., Strømsnes A., Steinsholt K. & Thomassen M.S. (1994) Fillet gaping in farmed Atlantic salmon (*Salmo salar*). *Norwegian Journal of Aquaculture Sciences* **8**, 165–179.

Andersen U.B., Thomassen M.S. & Rørå A.M.B. (1997) Texture properties of farmed Rainbow trout (*Oncorhynchus mykiss*): effects of diet, muscle fat content and time of storage on ice. *Journal of the Science of Food and Agriculture* **74**, 347–353.

Bahuaud D., Gaarder M., Veiseth-Kent E. & Thomassen M. (2010) Fillet texture and protease activities in different families of farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture* **310**, 213–220.

Bahuaud D., Mørkøre T., Østbye T.-K., Veiseth-Kent E., Thomassen M.S. & Ofstad R. (2010) Muscle structure response and lysosomal cathepsin B and L in farmed Atlantic salmon (*Salmo salar* L.) *pre-* and *post-rigor* fillets exposed to short and long-term crowding stress. *Food Chemistry* **118**, 602–615.

Birkedal-Hansen H. (1987) Catabolism and Turnover of Collagens: *Collagenases. Methods in Enzymology* **144**, 140–171.

Birkedal-Hansen H., Moore W.G.I., Bodden M.K., Windsor L.J., Birkedal-Hansen B., DeCarlo A. & Engler J.A. (1993) Matrix metalloproteinases: a review. *Critical Reviews in Oral Biology and Medicine* **4**, 197–250.

Borderias A.J. & Sánchez-Alonso I. (2011) First processing steps and the quality of wild and farmed fish. *Journal of Food Science* **76**, R1–R5.

Bremner H.A. & Hallett I.C. (1985) Muscle fiber–connective tissue junctions in the fish blue grenadier (*Macruronus novaezelandiae*). A scanning electron microscopy study. *Journal of Food Science* **50**, 975–980.

Brown S.I., Hook C.W. & Tragakis M.P. (1972) Presence, significance, and inhibition of lysosomal proteoglycans. *Investigative Ophthalmology* **11**, 149–152.

Erikson U. & Misimi E. (2008) Atlantic salmon skin and fillet color changes effected by perimortem handling stress, rigor mortis and ice storage. *Journal of Food Chemistry* **73**, C50–C59.

Erkan N. (2007) Sensory, chemical, and microbial attributes of sea bream (Sparus aurata): effects of washing and ice storage. *International Journal of Food Properties* **10**, 421–434.

Espe M., Ruohonen K., Bjørnevik M., Frøyland L., Nordtvedt R. & Kiessling A. (2004) Interactions between ice storage time, collagen composition, gaping and textural properties in farmed salmon muscle harvested at different times of the year. *Aquaculture* **240**, 489–504.

Fisher R.A. (1922) On the interpretation of χ^2 from contingency tables, and the calculation of P. *Journal of the Royal Statistical Society* **85**, 87–94.

Fletcher G.H., Hallett I.C., Jerrett A.R. & Holland A.J. (1997) Changes in the fine structure of the myocommata-muscle fibre junction related to gaping in rested and exercised muscle from king salmon (*Oncorhynchus tchawytacha*). *LWT – Food Science and Technology* **30**, 246–252.

Gangbar S., Overall C.M., McCulloch C.A. & Sodek J. (1990) Identification of polymorphonuclear leukocyte collagenase and gelatinase activities in mouthrinse samples: correlation with peridontal disease activity in

adult and juvenile peridontits. *Journal of Periodontal Research* **25**, 257–267.

Geng Y., McQuillan D. & Roughley P.J. (2006) SLRP interaction can protect collagen fibrils from cleavage by collagenases. *Matrix Biology* **25**, 484–491.

Hallett I.C. & Bremner H.A. (1988) Fine structure of the myocommata-muscle fibre junction in hoki (*Macruronus novaezelandiae*). *Journal of the Science of Food and Agriculture* **44**, 245–261.

Hansen A.Å., Rødbotten M., Eie T., Lea P., Rudi K. & Mørkøre T. (2012) The effect of crowding stress on bacterial growth and sensory properties of chilled Atlantic salmon fillets. *Journal of Food Science* **71**, s84–s90.

Harris E.D. Jr, Faulkner C.S. 2nd & Brown F.E. (1975) Collagenolytic systems in rheumatoid arthritis. *Clinical Orthopaedics and Related Research* **110**, 303–316.

Herman M.P., Sukhova G.K., Libby P., Gerdes N., Tang N., Horton D.B., Kilbride M., Breitbart R.E., Chun M. & Schönbeck U. (2001) Expression of neutrophil collagenase (matrix Metalloproteinase–8) in human atheroma. A novel collagenolytic pathway suggested by transcriptional profiling. *Circulation* **104**, 1899–1904.

Huss H.H. (1995) *Quality and Quality Changes in Fresh Fish*. FAO fisheries technical paper 348. FAO Press, Rome.

Johnston I.A., Bickerdike R., Li X., Dingwall A., Nickell D., Alderson R. & Campbell P. (2007) Fast growth was not associated with an increased incidence of soft flesh and gaping in two strains of Atlantic salmon (*Salmo salar*) grown under different environmental conditions. *Aquaculture* **265**, 148–155.

Jung K. (2008) Measurements of matrix metalloproteinases and their tissue inhibitors in serum produces doubtful results. *The Journal of Infectious Diseases* **198**, 1722–1723.

Kause A., Quinton C., Airaksinen S., Ruohonen K. & Koskela J. (2011) Quality and production trait genetics of farmed European whitefish, *Coregonus lavaretus*. *Journal of Animal Science* **89**, 959–971.

Kiessling A., Espe M., Ruohonen K. & Mørkøre T. (2004) Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by pre-slaughter iso-eugenol or CO_2 anaesthesia. *Aquaculture* **236**, 645–657.

Kvalheim O.M. & Karstang T.V. (1987) A general purpose program for multivariate data-analysis. *Chemometrics and Intelligent Laboratory Systems* **2**, 235–237.

Lähteenmäki K., Kuusela P. & Korhonen T. (2001) Bacterial plasminogen activators and receptors. *FEMS Microbiology Reviews* **25**, 531–552.

Lavety J., Afolabi O.A. & Love R.M. (1988) The connective tissues of fish. IX. Gaping in farmed species. *International Journal of Food Science and Technology* **23**, 23–30.

Manello F. (2008) Serum or plasma samples? The "Cindarella" role of blood collection procedures. Preanalytical methodological issues influence the release and activity of circulating matrix metalloproteinases and their tissue inhibitors, hampering diagnostic trueness and leading to misinterpretation. *Arteriosclerosis, Thrombosis, and Vascular Biology* **28**, 611–614.

Margeirsson S., Jonsson G.R., Arason S. & Thorkelsson G. (2007) Influencing factors on yield, gaping, bruises and nematodes in cod (*Gadus morhua*) fillets. *Journal of Food Engineering*, **80**, 503–508.

Mookhtiar K.A. & Van Wart H.E. (1992) Clostridium histolyticum collagenases: a new look at some old enzymes. *Matrix. Supplement* **1**, 116–126.

Mørkøre T. & Rørvik K.-A. (2001) Seasonal variation in growth, feed utilisation and product quality of farmed Atlantic salmon (*Salmo salar*) transferred to seawater os 0 + smolts or 1 + smolts. *Aquaculture* **199**, 145–157.

Ofstad R., Egelandsdal B., Kidman S., Myklebust R., Olsen R.L. & Hermansson A.M. (1996) Liquid loss as effected by post mortem ultrastructural changes in fish muscle: cod (*Gadus morhua* L.) and salmon (*Salmo salar*). *Journal of the Science of Food and Agriculture* **71**, 301–312.

Peterkofsky B. (1982) Bacterial collagenase. *Methods in Enzymology* **82**, 453–471.

Pittman K., Grigory V.M. & Brandebourg T. (2013) Bridging the gap to sustainable salmon farming: overcoming the gaping problem. *Journal of Fisheries & Livestock Production* **1**, 104.

Robb D.H.F., Kestin C.S. & Warris P.D. (2000) Muscle activity at slaughter: I. Changes in flesh colour and gaping in rainbow trout. *Aquaculture* **182**, 261–269.

Rosenberg G.A., Estrada E., Kelley R.O. & Kornfeld M. (1993) Bacterial collagenase disrupts extracellular matrix and opens blood-brain barrier in rat. *Neuroscience Letters* **160**, 117–119.

Roth B., Slinde E. & Arildsen J. (2006) Pre or post mortem muscle activity in Atlantic salmon (*Salmo salar*). The effect on rigor mortis and the physical properties of flesh. *Aquaculture* **257**, 504–510.

Sasagawa S., Matsubara J. & Satow Y. (1993) Stress-related Induction of Hepatic Metallothionein Synthesis and Increase in Peripheral Polymorphonuclear Leukocytes in Mice. *Immunopharmacology and Immunotoxicology* **15**, 217–226.

Skjervold P.O., Fjæra S.O., Braarød Østby P. & Einen O. (2001) Live-chilling and crowding stress before slaughter of Atlantic salmon. *Aquaculture* **192**, 265–280.

Stien L.H., Hirmas E., Bjørnevik M., Karlsen Ø., Nortvedt R., Bencze Rørå A.M., Sunde J. & Kiessling A. (2005) The effects of stress and storage temperature on the colour and texture of pre-rigor filleted farmed cod (*Gadus morhua* L.). *Aquaculture Research* **36**, 1197–1206.

Suzuki K., Totsuka M., Nakaji S., Yamada M., Kudoh S., Liu Q., Sugawara K., Yamaya K. & Sato K. (1999) Endurance exercise causes interaction among stress

hormones, cytokines, neutrophil dynamics, and muscle damage. *Journal of Applied Physiology* **87**, 1360–1367.

Sveinsdottir K., Hyldig G., Martinsdottir E., Jorgensen B. & Kristbergsson K. (2003) Quality Index Method (QIM) scheme developed for farmed Atlantic salmon (Salmo salar). *Food Quality and Preference* **14**, 237–245.

Torgersen J.S., Koppang E.O., Stien L.H., Kohler A., Pedersen M.E. & Mørkøre T. (2014) Soft Texture of Atlantic Salmon Fillets Is Associated with Glycogen Accumulation. *PLoS ONE* **9**(1), e85551.

Veland J.O. & Torrisen O.J. (1999) The texture of Atlantic salmon (*Salmo salar*) muscle is measured instrumentally using TPA and Warner-Brazler shear test. *Journal of the Science of Food and Agriculture* **79**, 1737–1746.

Wold S. (1976) Pattern recognition by means of disjoint principal component models. *Pattern Recognition* **8**, 127–139.