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PhD Thesis

Gine Ørnholt Johansson

January 2017

DTU Food
National Food Institute

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TITLE SHEET

Title: Process analysis and data driven optimization in the salmon industry / Procesanalyse og datadreven optimering af lakseindustrien.

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Partners: Skagerak Salmon A/S and Danish Seafood Association.

PREFACE

This PhD project was carried out at the National Food Institute, Technical University of Denmark. Associate Professor Stina Frosch was until May 2016 principal supervisor followed by Associate Professor Bo Munk Jørgensen. The project was funded by Danish AgriFish Agency under the GUDP programme, and the Technical University of Denmark.

This PhD project is part of a larger project called Bop-Fisk - *Follow the fish – Sustainable and optimal resource utilization in the Danish fish industry* (J. nr. 34009-12-0469) with the vision of securing the Danish fishery sector sustainable and optimal resource exploitation. Therefore, several industry partners have contributed to this work and I would like to thank Skagerak Salmon A/S for supplying raw materials and the Danish Fishery Association for the dissemination of the results.

I would also like to thank my supervisors, Stina Frosch, Bo Munk Jørgensen and María Guðjónsdóttir for endless discussions and much appreciated guidance making this PhD possible for me. Thanks to Heidi Olander Petersen for her never-ending assistance in the lab, your help was invaluable. Moreover, I would like to thank Nina Gringer and Julie Maria Aagaard for reviewing my thesis, yet again.

My great colleagues at DTU both former and present deserve the greatest of thanks for their numerous views on my scientific work, for their laughs, support and encouragement. I could not have done it without them.

Finally, I would like to express my greatest appreciation towards my family for their support and patient ears, to Naia for her laughs and crave for attention, and to Søren for his listening, remembering, and thoughts on my work – I would not have wanted to do this without you.

Gine Ørnholt Johansson

Snekkersten, January 2017

SUMMARY

Aquaculture supplies around 70% of the salmon in the World and the industry is thus an important player in meeting the increasing demand for salmon products. Such mass production calls for systems that can handle thousands of tonnes of salmon without compromising the welfare of the fish and the following product quality. Moreover, the requirement of increased profit performance for the industry should be met with sustainable production solutions. Optimization during the production of salmon fillets could be one feasible approach to increase the outcome from the same level of incoming raw material.

Today a lot of data is gathered in the different links of the value chain regarding raw material characteristics and processing parameters. Yet, even though traceability systems that allow for information transfer are available, this type of information does not follow the fish. This means that valuable information is gathered, but not exploited, and that data from for example the slaughtering companies cannot be included in decision processes related to the further processing of the fish or vice versa. Therefore, the overall aim of the present project has been to investigate if comprehensive collection and analysis of data from the salmon industry could be utilized to extract information that will support the industry in their decision-making processes. Mapping of quality parameters, their fluctuations and influences on yield and texture has been investigated. Additionally, the ability to predict the texture category of the salmon based on protein profile has been explored. The potential effect of the current project was expected to result both in a higher share of products of the highest possible quality, and allocation of products to match raw material to optimal product recipe (for example fillet, portion, smoked etc.). These measures could ensure the industry a higher price for the products, and will have a direct impact on the profit of the filleting companies.

The initial work comprised a process analysis of the process line at the collaborating partner Skagerak Salmon A/S where data was gathered on an individual level during filleting. A model was built based on the gathered data enabling prediction of yield after filleting. Moreover, during analysis of the headed salmon it was observed that 78% of the salmon had a larger right side fillet compared to the left side, while all heads had more meat on the left side compared to the right. The heading procedure was identified as the one responsible for the weight difference of the fillets with a potential for increasing the recovery of high value meat i.e. fillet. The difference in fillet size amounted to 23 g per fish, and if recovered 61 tonnes of additional meat a year with a value of 2 million Danish kroner. Furthermore, throughout the project data was gathered covering a total of 11 months in order to investigate the variation in quality parameters. A significant negative correlation between sea temperature at the rearing region and protein content was observed. To the best of my knowledge, no study has reported this previously, and this observation thus segregates from the commonly accepted statement that protein content is a stable parameter in farmed salmon muscle. In the work related to the texture of salmon a model that can predict peak force, and thus texture category, based on protein profile, was built. A total of 16 proteins were required for this prediction, and five proteins; serum albumin, dipeptidyl peptidase 3, heat shock protein 70, annexins, and a protein fragment believed to be titin, were identified.

In conclusion, the present project shows how process analysis and extensive data analysis can be used in the salmon industry in the attempt to increase yield. Knowledge of slaughter yield for a certain batch may facilitate optimal planning of the production of salmon fillets by ordering and assigning the right batch to the right product category to obtain an optimal yield and quality. Moreover, it is contemplated that the identification of proteins significant for the measured texture, will contribute to the further understanding of texture. Although more research is still needed in this area, the perspectives extending from the present work may challenge the industry to restructure the information flow of the value chain. This may incorporate an approach that enables all links to receive data that can be used in optimization of processes, and by that achieve an optimal exploitation of the resources in the future.

RESUMÉ

Akvakultur leverer omkring 70% af laks i verden og industrien er derfor en vigtig aktør når den stigende efterspørgsel på lakseprodukter skal efterkommes. En sådan masseproduktion kræver systemer, der kan håndtere tusindvis af tons laks uden at kompromittere velfærden for fisken og den efterfølgende produktkvalitet. Samtidig bør behovet for øget fortjeneste blandt branchens aktører blive mødt med bæredygtige løsninger. Her kunne optimering i selve produktionen af laksefileter være en mulig fremgangsmåde til at øge udbyttet fra den samme mængde indkommende råmateriale.

I dag indsamles mange data om råvareegenskaber og parametre med betydning for forarbejdningen i de forskellige led i værdikæden. På trods af at sporbarhedssystemer, der giver mulighed for informationsoverførsel, er tilgængelige, følger denne type data ikke fisken. Det betyder, at værdifulde oplysninger indsamles, men forbliver uudnyttede, og at data fra for eksempel slagtevirksomhederne ikke kan indgå i beslutningsprocesser relateret til den videre forarbejdning af fisken eller omvendt. Derfor har det overordnede formål med dette projekt været at undersøge om omfattende indsamling og analyse af data fra lakseindustrien kan udnyttes til at uddrage information, der vil støtte industrien i deres beslutningsprocesser. Kortlægning af kvalitetsparametre, deres udsving og indflydelse på udbyttet og teksturen er blevet undersøgt. Derudover er muligheden for at forudsige teksturkategorien baseret på proteinprofilen af laksen blevet udforsket. Den tænkelige effekt af det dette projekt var forventet at resultere i både en højere andel af produkter af den højest mulige kvalitet, og allokering af produkter således at råmateriale bliver matchet til den mest optimale produktopskrift (f.eks. filet, portion, røget osv.). Disse foranstaltninger kan sikre branchen en højere pris for produkterne, og vil have en direkte indvirkning på fortjenesten hos fileteringsvirksomhederne.

Det indledende arbejde omfattede en procesanalyse af proceslinjen hos den samarbejdende partner Skagerak Salmon A/S, hvor data blev opsamlet på individniveau under filetering. En model, der muliggør prædiktion af udbyttet efter filetering, blev bygget på grundlag af de indsamlede data. Endvidere blev det observeret under analyse af de afnakkede laks, at 78% af laksene havde en større højre filet i forhold til den venstre filet, mens alle hoveder havde mere kød på venstre side end højre. Afnakningsprocessen blev identificeret som der, hvor vægtforskellen på fileterne blev introduceret, og åbner derved muligheden for at øge udvindingen af kød med høj værdi dvs. filet. Forskellen i filetstørrelse udgjorde 23 g pr fisk, og hvis genvundet, 61 tons ekstra kød om året til en værdi af 2 millioner danske kroner. Derudover er der igennem projektperioden løbende blevet indsamlet kvalitetsrelaterede data, i alt dækkende 11 måneder, for at undersøge variationen i kvalitetsparametre. En signifikant negativ korrelation mellem havtemperaturen ved opdrætsfylken og protein blev observeret. Til min bedste viden, har ingen studier rapporteret dette tidligere, og denne observation adskiller sig således fra den almindeligt accepterede fremstilling af proteinindholdet som en stabil parameter i opdrættet laksemuskel. I arbejdet med teksturen af laks blev der bygget en model baseret på proteinprofilen, der kan prædiktere den maksimale modstand musklen vil yde mod tryk, og dermed teksturkategorien. I alt 16 proteiner var nødvendig til denne prædiktion, og fem proteiner blev identificeret: serumalbumin, dipeptidylpeptidase 3, heat shock protein 70, annexiner og et proteinfragment, der menes at være titin.

Afslutningsvis har dette projekt vist, hvordan procesanalyse og omfattende dataanalyse kan bruges i lakseindustrien i forsøget på at øge udbyttet. Viden omkring slagteudbyttet for et specifikt batch kan fremme en optimal planlægning af produktionen af laksefileter gennem bestilling og tildeling af det rette batch til den rette produktkategori for at opnå det optimale udbytte og kvalitet. Yderligere forventes det at identifikationen af proteiner, der er signifikante for den målte tekstur, vil bidrage til en yderligere forståelse af teksturen. Selvom yderligere forskning stadig er nødvendig kan perspektiveringerne, der strækker sig fra dette projekt, udfordre industrien til at omstrukturere informationsstrømmen i værdikæden. Dette kan omfatte en tilgang, der muliggør at alle led i værdikæden modtager data, der kan benyttes til at optimere processerne og på den måde at opnå optimal udnyttelse af ressourcerne i fremtiden.

LIST OF PAPERS

In the thesis, papers will be designated by their roman number.

I. Ørnholt-Johansson, G., Gudjónsdóttir, M., Nielsen, M.E., Skytte, J.L. & Frosch, S. Analysis of the production of salmon fillet – prediction of production yield.

Research paper, submitted to Journal of Food Engineering.

II. Ørnholt-Johansson, G., Jørgensen, B.M. & Frosch, S. Variation in some quality attributes of Atlantic salmon fillets from aquaculture related to geographic origin and water temperature.

Research paper, submitted to Aquaculture.

III. Ørnholt-Johansson, G., Frosch, S., Gudjónsdóttir, M., Wulff, T. & Jessen, F. Muscle protein profiles used for prediction of texture of farmed salmon (*Salmo salar* L.) fillets.

Research paper, submitted to Journal of Agricultural and Food Chemistry.

Paper made in relation to this PhD study, and which I have co-authored.

A. Skytte, J. L., Johansson, G. Ø., Frosch, S. Fat content prediction of farmed Atlantic salmon using multispectral imaging and automated image analysis.

Research paper, submitted to Journal of Agricultural and Food Chemistry.

ABBREVIATIONS

ATP	Adenosine Triphosphate
ATPase	Adenosine Triphosphatase
CO_2	Carbon dioxide
DTU	Technical University of Denmark
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GUDP	Grønt Udviklings- og Demonstrations Program
HSP	Heat Shock Proteins
K factor	Condition factor
PLS	Partial Least Squares
PYD	Hydroxylysyl Pyridinolin
RMSECV	Root-mean-square error cross-validation
TGase	Transglutaminase

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1 INTRODUCTION AND OBJECTIVES

Aquaculture supplies around 70% of the salmon in the World (Marine Harvest, 2015) and the industry is thus an important player in meeting the increasing demand for salmon products. Mass production calls for systems that can handle thousands of tonnes of salmon without compromising the welfare of the fish and the following product quality. Moreover, in order to develop the industry, further increased profit performance is required. In times of increased attention on sustainability, this should be met with sustainable production solutions in which water resources and climate changes, amongst other factors, are considered (Godfray *et al.* 2010). A sustainable solution could be to direct the focus on reducing losses after the fish leaves the farm, rather than just expanding the farming facilities (Ytrestøyl *et al.* 2015). This would require optimization along the value chain when the process of harvesting the fish has ended. The concept "value chain" was first described in 1985, by Michael E. Porter in his work "*Competitive Advantage: Creating and Sustaining Superior Performance*" (Porter, 1985). The value chain defines a system of subsystems that together transforms a specific raw material, adds value to a product, and involves all processes, inputs and outputs at each level in the system (IfM, 2016). The value chain for farmed Atlantic salmon thus encompass all subsystems related to hatching, rearing, harvest, slaughter, processing, and all transportation steps in between. In relation to this, optimization in food engineering can be defined as identifying a set of conditions that will lead to for example increased output while taking into consideration a number of constraints, which could be product quality (Banga *et al.* 2003). In the context of the current PhD thesis, optimization may cover any procedure along the value chain for farmed salmon – from hatching and rearing to primary and secondary processing – that would result in better utilization of each fish by minimising losses during the production while maintaining an eye on the product quality and price.

In 2012, traceability in the fishery sector was made mandatory by European law (COMMISSION IMPLEMENTING REGULATION (EU) No 404/2011). Besides transferring the information required by law, the development within systems used for traceability of food products has enabled the exchange of data between different links of the value chain (Frosch *et al.* 2008; Randrup *et al.* 2008). Despite of the development within traceability systems the situation is often that little information follows the fish, except what is required by law. Traditionally, information regarding a batch of fish is sent one step forward and includes the information provided one step backward in the existing value chain as depicted in Figure 1a. Information flow in the traditional value chain, as in Figure 1a, is always forward. This means that there will be no economical incitement at farm level to for example increase the amount of data collection to pass on to the subsequent links of the value chain. As an example, several studies have already investigated the incorporation of cheaper ingredients in the feed to minimize the cost and maintain or optimize the growth rate and flesh quality (Pratoomyot *et al.* 2010; Refstie *et al.* 2001; Waagbø *et al.* 2013; Øverland *et al.* 2009). Approximately 50% of the cost of rearing is related to the cost of the feed. Therefore, optimization at this level, in the top of the existing value chain, will lower the production cost and thus increase the profit down the entire value chain. However, if optimization takes place further down the value chain, the economical gain will only filtrate down the value chain from the step where the optimization has taken place. Consequently, the further down the value chain the optimization takes place, the less economical impact it will have on the preceding levels of the value chain.

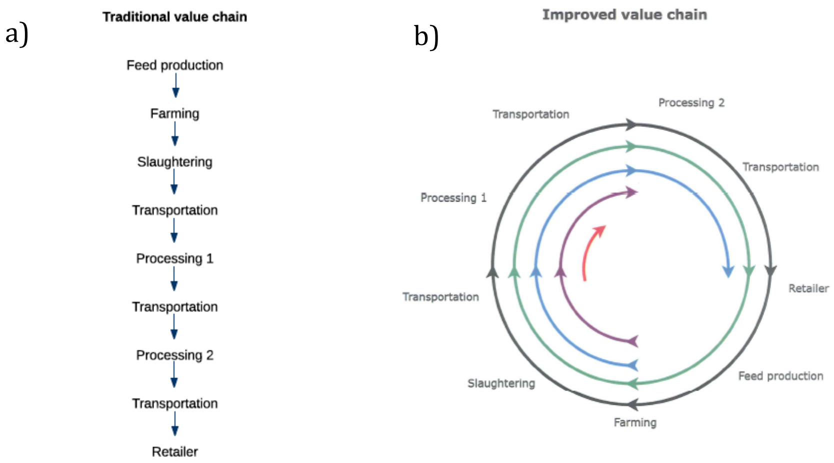

Figure 1 Illustrations of two representations of the information flow in the value chain for farmed Atlantic salmon. Figure a) shows the traditional perception of a top-to-bottom value chain, while b) depicts a circular value chain working as a feedback loop to ensure optimal information transfer between all links of the value chain.

Contrary to the traditional value chain, an improved value chain might be regarded as a feedback loop, as seen in Figure 1b, where all parties of the value chain communicate and share information. This requires that all information follows the fish forward, and all data gathered during the production, whether it be at slaughter level or during secondary processing, is distributed back to the preceding levels in the value chain. In this way, information gathered during optimization at slaughter level would be sent back, as well as forward, in the value chain and thus feed producers would be able to consider the experience gained at slaughter level to improve feed, as an example. This thought-scenario would require a total restructuring of traditions and information transfer including the handling of big data. Yet, the potential for increased earnings and better utilization of resources could be vast.

The potential effect of the current project was expected to result both in a higher share of products of the highest possible quality, and allocation of products to match raw material to optimal product recipe (for example fillet, portion, smoked etc.). These measures could ensure the industry a higher price for the products, and will have a direct impact on the profit of the filleting companies. The secondary processing was the starting point of the research in the current project. The anticipation was that the findings could aid the filleting companies in making decisions based on incoming data and by that deciding the optimal production route based on product characteristics. This would mean that products with deviations in for example texture could be directed to a more suitable manufacturing process thereby reducing loss. As an example, prediction of texture category would enable the producer to direct salmon with soft texture into a process in which the final product could be salmon mince rather than fillets for smoking. In this way, the texture category could be used to define the set-up of the processing line making it more suitable for handling a specific product type. By adapting the process line to the product it can be hypothesized that downtime could be reduced saving both time and money for the company. Moreover, aiming for a more flexible process line could facilitate that a higher percentage of product could be sold as the primary product by adjusting the process line to handle for example firm fillets. Achieving a 1% increase in yield at the filleting company would, in 2012, result in a direct economical gain in the area of three million Danish kroner when considering a production of the size of the one seen at Skagerak Salmon A/S.

Even though the aquaculture and processing companies accumulate data regarding raw materials, production and final products, these data are rarely passed on and used analytically for optimizing the production. This means that valuable information is gathered but not exploited and that data from for example the slaughtering companies cannot be included in decision processes related to the further processing of the fish or vice versa. The overall hypothesis, and thus basis for this work, has been that:

Increased information transfer between and over the links in the value chain can strengthen the chain as a unity with increased outcome on several levels, including yield, profit and quality, as a result.

This hypothesis entails the complete value chain from egg to consumer to be investigated, and even though the results would become more conclusive it has not been possible to conduct such an extensive analysis. Therefore, information on feed, farming conditions, slaughtering time, temperature, transport and handling of raw material has not been included in the present PhD project. However, the extensive amount of available literature regarding salmon aquaculture in all its aspects combined with the results generated in the current project has allowed for some degree of (theoretical) extrapolation of the results in the effort to provide an answer to the overall hypothesis.

The overall hypothesis has been the driving force behind the present PhD project, and as the work progressed it became evident that several parameters could and would influence the yield, for example soft texture. Consequently, the focus of the project was divided into work packages (WP1-3) and the connections between these are presented in Figure 2.

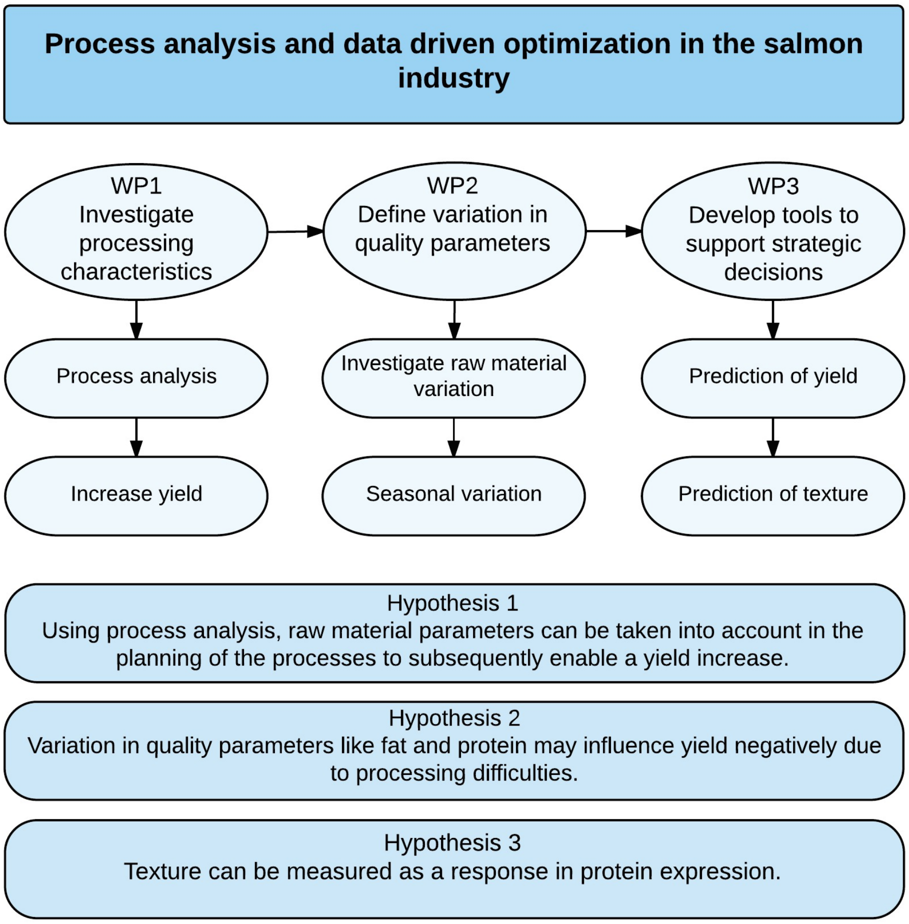

Figure 2 Schematic overview of the work of the present PhD thesis. Three work packages (WP1-3) are presented together with three specific research hypotheses that were tested in the experimental part of this PhD project.

The initial work (WP1) of the present project was directed towards optimizing yield. This was achieved by using process analysis in which the production line at Skagerak Salmon A/S was at our disposal and we could follow each step the salmon went through from whole gutted fish to packed fillets. Such an approach has, to the best of my knowledge, not been reported in the scientific literature in relation to the salmon filleting industry. The work was combined with the aim of work package 3 (WP3), which was designed to develop tools to support strategic decisions, by investigating whether the measured variables could be combined in a way that enabled the prediction of yield.

In the second work package (WP2) raw material variation was examined with a focus on investigating to what extent seasons affect especially fat and protein content. The findings are presented as a literature study in Chapter 4. Moreover, variation in raw material composition was investigated by performing measurements on salmon fillets supplied to DTU Food from March 2014 to November 2015. Albeit previous experiments have gathered seasonal data from farmed salmon, none of these have collected data with as close an interval as we have, and our data set thus comprise data from 11 months. The results formed the basis for the research presented in Paper II in which the temperature dependent variation in quality attributes of Atlantic salmon fillets from aquaculture were investigated.

In the beginning of the project period it was presumed that soft texture was one of the biggest problems in the value chain of farmed Atlantic salmon fillets. With the aim of securing the industry the highest possible price, a method to categorize salmon according to texture, was sought. The protein profile constitutes an overall characterization of the properties of the muscle, including texture (Morzel *et al.* 2006), and thus information on the protein profile of the fish can be used to describe the texture and be used to measure or predict texture. Accordingly, the focus of the third work package (WP3) was to investigate correlations between protein composition and texture in attempt to add to the existing knowledge and find a solution to categorize the salmon according to texture category.

1.1 OUTLINE OF THE THESIS

The work made in connection with this PhD project has been to investigate several aspect of the filleting industry. Process analysis, prediction of yield and the influence of seasonal differences in proximate composition received the initial attention. However, textural issues were often highlighted at the processing facility, by other industry contacts and in the literature, and thus became the common denominator for the project. The literature reports many attempts to establish the cause of soft texture; still, no exact conclusions have been drawn. This lack of precise understanding highlights the need to identify the causes of this significant problem, and to develop solutions that will lead to improved profitability for the processing industry.

The scope of the present work has mainly revolved around texture and the background for the present thesis is provided through a review of literature to support this. Chapter 2 focuses on production methods and yield to provide the reader with a general understanding of the industry. Chapter 3 presents a definition of texture together with an exposition of the causes of soft texture in relation to the different production stages presented in Chapter 2. Chapter 4 presents a literature study in which what factors that might influence fat and protein content in Atlantic salmon are investigated. Chapter 5 introduces production facilities at Skagerak Salmon A/S as the collaborating partner of the project, and offers a discussion on the textural methods chosen for the experimental part of the project. Moreover, a presentation of the use of prediction models is included. Chapter 6 presents a summarising discussion of the results presented in the papers made in connection with the present PhD project. Chapter 6 will also discuss the hypotheses presented in Figure 2. Chapter 7 highlights the conclusions of the present work followed by an outlook towards further optimization of the salmon filleting industry.

Whereas Chapter 2, 3 and 4 represent the work by others, and will thus not include results from this project, the papers made in connection with this thesis can be found in Chapter 9.

2 FROM EGG TO CONSUMER

The salmon value chain include the production of broodstock and eggs, hatching, smoltification, transfer to and growth in sea pens, slaughtering, distribution, primary processing, distribution and secondary processing either by the consumer or by other industries in order to increase the value of the final product. Primary processing includes slaughtering and gutting, whereas secondary processing covers the transformation of slaughtered salmon to value-added products by filleting, trimming, portioning (in different cuts), smoking, or ready-to-eat meals. This chapter will provide a brief overview of the different steps in the processing of farmed Atlantic salmon with focus on the production conditions employed in Norway, and under the assumption that harvest and slaughter takes place at one site followed by transport to other production sites where further processing takes place.

2.1 REARING AND GROWTH AT SEA

Rearing of farmed fishes will for most species start by selecting parent fish, called broodstock, with respect to desirable traits like growth efficiency, delayed onset on maturation and disease resistance, to mention a few (Glover *et al.* 2009). Selective breeding is employed in order to enhance broodstock with such traits (Gjedrem, 2010; Taniguchi, 2003), and genetically modified strains comprise 97% of the farmed Atlantic salmon in the World (Gjedrem, 2010).

In the fall, eggs are stripped from the broodstock and fertilized. Following fertilization eyed (fertilized) eggs are incubated until hatching (Marine Harvest, 2015), which happens in the spring (Mangel & Satterthwaite, 2016). The newly hatched egg feeds on the yolk sack, which sustains them until their first feeding. Hereafter, the juvenile salmon (fry) will be transferred to fresh water tanks and as they grow in size they prepare for the transformation from freshwater to saltwater in a process called smoltification, or the parr-smolt transformation. This takes place in tanks where the salinity is gradually raised until it mimics that of seawater and the smolts are ready to be transferred to sea. For a more in depth description of the smoltification process please see for example Björnsson *et al.* (2011). Prior to transfer, the smolts are all vaccinated to be able to withstand the most common diseases. Following this, transfer takes place in well boats that sail the fish to the net pens and pump them into these. The salmon will grow at sea until they reach the desired market size. Still, some factors may influence the time of harvest, as for example onset of sexual maturation. Sexual maturation is not desired as it alters the flesh and colour of the salmon making it a less valuable product or even an inedible product (Quinton *et al.* 2005).

The production from egg to the point of transfer to sea takes about 10-16 months followed by another 14-24 months of growth at sea, adding up to a production time from egg to consumer of 24 to 40 months (Marine Harvest, 2015). In order to be able to meet the market demands for Atlantic salmon, smolts in Norway are produced both during spring and fall. Smolts transferred to seawater in the spring following their hatching are termed 1+, whereas smolts transferred to seawater out-of-season, typically in fall and early winter, are designated 0+ (Alne *et al.* 2011; Duncan *et al.* 1998; Marine Harvest, 2015; Roth *et al.* 2005). The production cycle can be accelerated by up to 6 months by the use of light manipulation of the juvenile salmon. Light manipulation, or changes in photoperiod, can accelerate the smoltification process and thereby accommodate the annual demand (Duncan *et al.* 1998).

2.2 PRIMARY PROCESSING

The primary processing of Atlantic salmon from aquaculture describes the processing steps involved from the time at which the salmon are transferred from the sea cages until they are packed on ice. The flowchart presented in Figure 3 illustrates the processes involved in the transformation of whole, live salmon to bled and gutted products.

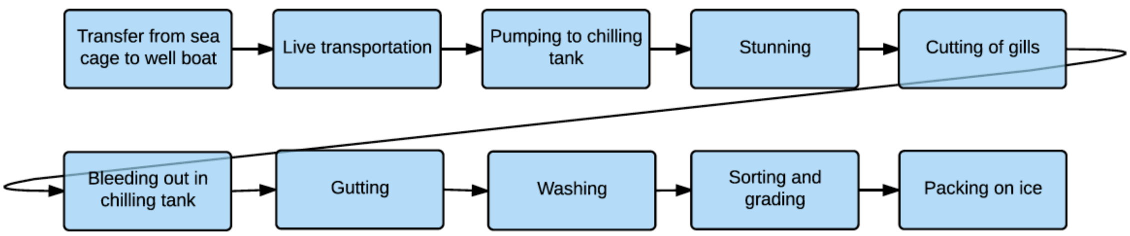

Figure 3 Flow chart of the primary processing steps depicting the processes involved from the time of transfer from sea cages to the final packing of salmon.

The salmon are pumped from the sea cages to a well boat that allow for a live transfer of the fish to the shore (Erikson *et al.* 1997). The well boat holds the fish in the hull and takes in fresh seawater while sailing and by that ensuring good seawater quality. From the well boat the salmon are transferred either to a holding cage or directly to the chilling tank (Erikson, 2008). The chilling tank contains seawater at around 1 °C, which cools the fish rapidly, and has a sedative effect. This delays the onset of rigor, as well as the resolution, and has a positive influence on the shelf life and quality of the final product (Skjervold *et al.* 2001a). After the live chilling the salmon are stunned. Several methods for this exist and the result should be immediate unconsciousness for the method to be accepted as humane (EFSA, 2009). In 2008, the use of carbon dioxide (CO_2) for stunning was banned in Norway and now the most used methods are percussive stunning (sharp blow to the head), and electric stunning (the application of an electrical potential across the brain) (EFSA, 2009). The stunning leaves the fish unconscious so that they do not feel pain or fear prior to cutting of the gills for exsanguination, or bleeding. This takes place in chilling tanks in order to ensure the shelf life and quality of the product (EFSA, 2009). The final steps before packing include gutting in which the viscera is removed, washing to remove blood, slime and loose scales, and a quality step where the salmon are graded and sorted accordingly. Salmon are sold in weight classes and mainly divided into 3-4 kg, 4-5 kg, 5-6 kg and 6-7 kg. According to the Norwegian industry standard for fish, a Norwegian salmon can be quality graded either as being superior, ordinary or production. Superior represents salmon with no considerable defects such as damaged skin and significant loss of scales. Moreover, they must be void of bruises, damaged belly or musculature. The classification "ordinary" can comprise fish with limited external or internal faults while not to an extent that makes processing difficult. If the salmon cannot be classified either as superior or ordinary they fall under the classification "production". Fish in this category may for example show signs of sexual maturation, deformities, or sores, or processing faults like substantial loss of scales. According to the standard these fish must be supplied without heads (NBS, 1999).

Grading of the slaughtered salmon often takes place by dividing them into different quality grades by calculation of their condition factor, the K factor. The K factor describes the length-weight relationship of the fish and provides an estimate of the fatness, or condition, of the fish (Ricker, 1975). It is given as the weight multiplied 100 and divided by the cubed length (Hoar, 1939; Richter *et al*. 2000). The primary assumption for using the K factor is that growth is isometric, meaning that an increase in weight does not change the proportions of length, height and thickness in fish showing similar conditions (Richter *et al*. 2000). This assumption has, however, been challenged as fish growth is not always isometric. Fish show differential growth rates (Richter *et al*. 2000) and growth is a result of the combined effect of hyperplasi and hypertrophy. Hyperplasia describes the recruitment of new muscle fibres whereas hypertrophy is the increase of muscle cell size, and the combination of the two result in a life long growth of most fish species (Matschak *et al*. 1995; Mommsen, 2001). Bremnes Seashore has together with Marel, leading producer of processing equipment for the salmon industry, developed a method to grade the salmon based on the K factor and by that allocating specific grades of salmon to the most suitable processes (Marel, 2016).

2.3 SECONDARY PROCESSING AND YIELD

In the current project primary and secondary production took place at different production sites, as this is the case for the collaborating partner of the project, Skagerak Salmon A/S. However, both primary and secondary processing may in some cases take place in immediate continuation of each other.

Secondary processing comprises the processes after the fish has been slaughtered and gutted. Generally, and regardless of the production systems employed, the removal of head and tail will take place first. Filleting, where the fillets are removed from the skeletal frame, follows this. Trimming of the fillets encompass removal of parts of the fillet not desired in the final product, as well as cosmetic corrections that are prerequisites of delivering appealing products to the consumers. Skinning and an additional step including further trimming and quality inspection can be included prior to packing the products. Secondary processing also encompass all production of value-added products including for example smoking, however, the smoking process is not the main focus of the present project and will therefore not be reviewed.

Norwegian farmed salmon are primarily exported to Europe, which is the largest market for value-added products (Marine Harvest, 2015). The product range of Atlantic salmon from Norway includes the following, and is an extract from the Norwegian Seafood Council's portal for market insight (Sjømatrådet Innsikt, 2015):

- Fresh/chilled whole salmon, fillet and meat
- Frozen whole salmon, fillet and meat
- Live
- Smoked (both whole and fillet)
- Prepared and preserved

The fresh and frozen products make up most of the export, whereas prepared and preserved salmon products only contribute marginally to the overall export (Sjømatrådet Innsikt, 2015). Export of processed fish from Norway to the European Union (EU) is subjected to a 13% sales tariff, whereas the export of fresh and frozen whole salmon is only subjected to 2%. Hence exporting processed salmon from Norway is unprofitable making the market for secondary processing in EU large (Reuters, 2016).

In Table 1, an overview of the average percentagewise yield and by-products from the production of fillets at Skagerak Salmon A/S is provided for three weight classes of salmon: 3-4 kg, 4-5 kg, and 5-6 kg. Caroline Gundorph Møller has made the calculations as part of a special course on her Master education at DTU Food, 2013. One batch consists of a minimum of 1000 kg whole salmon.

Table 1. Average values for yield (%) of the bled and gutted salmon including standard deviations during the production of salmon fillets at Skagerak Salmon A/S. The data are extracted for fish in the three weight classes; 3-4 kg, 4-5 kg, and 5-6 kg, and are from 2013. One batch contains a minimum of 1000 kg of whole Atlantic salmon. Courtesy of Caroline Gundorph Møller, who performed the analysis during a special course in relation to her Master education.

Body part	3-4 kg[a]	4-5 kg[b]	5-6 kg[b]
Head %	13.1 ± 0.8	11.0 ±0.7	11.1 ± 1.0
Skeletal frame %	13.2 ± 0.6	12.6 ± 0.6	11.6 ± 0.4
Belly flap %	10.5 ± 0.6	10.2 ± 0.6	9.8 ± 0.5
Trim % (auto)	1.7 ± 0.4	1.5 ± 0.4	1.4 ± 0.4
Trim % (manual)	1.6 ± 0.3	1.2 ± 0.5	1.2 ± 0.4
Skin %	8.8 ±0.6	9.7 ±0.2	10.4 ± 0.7
Fillet %	51.3 ±2.0	53.6 ± 1.0	54.6 ± 1.4

[a] Based on three batches

[b] Based on four batches

According to Table 1, the average fillet yield is just above 50% of the whole, gutted and bled salmon that arrives at the processing facilities. The filleting yield increases with increasing weight of the incoming raw material. The removed body parts comprise the rest and can be utilized for human consumption, or further processed into for example fishmeal or fish oil. In an economical aspect, the best would be to ensure that as much of the meat as possible could be sold as high value products like the fillets. Nonetheless, other products can be produced from the remaining body parts. Ghaly *et al.* (2013) reviewed the uses of fishery by-products as valuable sources of protein, amino acids and oils. Currently, the most common products from fish by-products are silage, which basically is fish liquefied by enzymes and added acid (Ghaly *et al.* 2013). The oils present in fatty fish like salmon must be removed prior to silage production due to the subsequent oxidation of these oils making the silage unsuitable for animal feed (Ramírez, 2007). Fishmeal, fish sauce, gelatine and surimi are other value added products that can be produced from the salmon by-products (Ramírez, 2007).

At Skagerak Salmon A/S the by-products are sold primarily to Asia for human consumption as for example soup (heads and tails) or sushi (belly flap), and although not constituting the main part of the profit, it is still money earned rather than money spent on waste removal.

3 COMMON QUALITY FAULTS

An analysis made by Michie (2001) identified two types of common quality faults, soft textured salmon and salmon with gaping, which together accounted for up to 40% of all downgraded products during secondary processing. Another 40% of the downgraded[a] products were due to pale or uneven colour. Even though the analysis made by Michie (2001) is not recent, it outlined the initial argumentation of this PhD project as to why it was so important to reduce these faults during secondary processing.

However, from personal communication with one of the market leaders within aqua-cultured salmon (confidential) gaping and soft texture now (in 2016) only constitutes around 6% of the downgraded products suggesting a large improvement in the treatment and processing of the fish. Even though the country of origin for the mentioned study is different than Norway it was also suggested by a representative from Marel in Norway that soft texture and gaping account for less downgraded products than previously. It was mentioned that downgrading primarily was a result of bruising and maturing fish followed by melanin and blood spots, color, and then the textural faults. This indicates that significant improvements has been implemented in the production practices, which could include better temperature control and better understanding of the effect of stress prior to harvest. Melanin spots in the meat is seen as black spots, which is strongly suggested to be the result of vaccination (Koppang *et al.* 2005). The presence of melanin spots may result in a redirection of raw material to a portion cutter, as these fillets cannot be sold whole due to the lack of consumer acceptability (Mathiassen *et al.* 2007).

Irrespective of the less significant importance of textural faults during secondary processing reported by the industry, a substantial amount of literature has been found in relation to the texture of salmon. This is included in both the current thesis and the papers made in connection with it. A non-exhaustive list could include:

- Farming conditions
- Feed composition and the implementation of new ingredients
- Production conditions
- Proximal composition and yield

Despite this, no definite cause or conclusion for the origin of soft texture or gaping has been uncovered.

[a] Downgrading means to reduce a product to a lower grade implicitly meaning the product will be less valuable.

Even though the causes of downgrading today are different than what was assumed when the project began, texture and gaping still have a significant impact on the consumer acceptability of the final products (Rasmussen, 2001). Because of this and in combination with the difficulties experienced during the production of salmon fillets, as observed at the collaborating company, this PhD project has tried to contribute to the understanding of the texture of farmed Atlantic salmon from Norway. Hence, this chapter will present the main findings from the literature of the possible causes of textural issues of raw salmon.

3.1 DEFINING TEXTURE

In 1963, Szczesniak reviewed the definition of texture and stated that texture was a combination of; 1) mechanical characteristics of a food matrix, 2) how the food matrix was organized with respect to for example size, shape and orientation of the food particles, and 3) the physiochemical properties of the food matrix, as for example fat content and moisture. For the general understanding of what texture describes, this definition serves the purpose well and will be used for the remainder of this work. Several parameters are used to describe the quality of Atlantic salmon, including texture, and studies have shown that a firm texture is important with respect to the eating experience (Rasmussen, 2001), implicitly stating that soft texture is an undesired trait. In the present project the main focus has been soft texture and this phenomenon will thus receive the primary attention. However, for the purpose of understanding the issues related to textural faults, a brief description of gaping is provided. Whereas soft texture can often be seen as mushy areas in the muscle, gaping can be described as areas in the muscle where slits arise. Gaping is thus a result of how well the muscle fibres interact and maintain the structure of the fish meat. Gaping and soft texture are often, but not always, correlated (Mørkøre & Rørvik, 2001a), but common for the two textural faults are that they are undesired traits as they lead to a downgrading of the products as well as a decrease in yield (Einen *et al.* 1999; Michie, 2001). The time from harvest to filleting influence the severity of gaping (Skjervold *et al.* 2001b), possibly by affecting the pH to such an extent that it causes the tissue to weaken by breaking the myosepta (Concollato *et al.* 2014). Moreover, Espe *et al.* (2004) found that gaping was correlated to the amount of soluble collagen, which is supported by the findings of Pittman *et al.* (2013) who also concluded that collagen plays an important role in the incidence of gaping, especially with respect to the ratio between the connective tissues and muscle.

Texture is not a permanent state of the muscle as it develops *peri-mortem*. Soft texture prevails right after slaughter, however, onset of *rigor mortis* changes this making the muscle blocks hard during the rigor stage. Rigor resolution makes the muscle relax again and the texture becomes softer (Careche *et al*. 2003). In the following sections a more in-depth description of the underlying causes of soft texture in Atlantic salmon from aquaculture, will be provided.

3.2 CAUSES OF SOFT TEXTURE

Many investigations have been undertaken to identify the causes of soft texture, yet the results are still inconclusive. Martinez *et al.* (2011) studied the differences in skeletal muscle proteins expression in normal and soft textured salmon fillets, but could not find any systematic differences between the two groups of fish. While some have found that the texture of the meat is directly correlated to the amount and type of muscle proteins (Montero *et al.* 2003; Pittman *et al.* 2013; Skaara & Regenstein, 1990), Moreno *et al.* (2012) found no correlation between the amount of collagen and texture. Rather, the authors discovered that soft salmon exhibited a less stable collagen structure compared to firmer salmon. Several authors have confirmed the relationship between firm texture in salmon and the concentrations of the collagen cross-link called hydroxylysyl pyridinolin (PYD) (Johnsen *et al.* 2011; Johnston *et al.* 2006), indicating the importance of the stability of the collagen. Martinez *et al.* (2011) found that higher levels of gelatinases were present in the softer textured salmon, which confirms the previously mentioned relationship between soft texture and collagen, as gelatine is prepared from collagen. Larsson *et al.* (2014) observed that a diet supplemented with the amino acid glutamate enhanced the fillet firmness after storage of commercially reared salmon. The results, however, did not show correlations between collagen content and firmness, thus contradicting the findings of others. In another study, it was observed that soft textured muscle contained detached muscle fibres and accumulated glycogen (Torgersen *et al.* 2014), but in the study by Larsson *et al.* (2014), the glycogen deposition was lower in the glutamate fed group despite of this group displaying firm texture. In an earlier study the structures in salmon related to changes in hardness and loss of rigor were investigated (Taylor *et al.* 2002). The authors observed that the development of soft texture was determined by breaks in the attachment between muscle fibres, and consequently that loss of fillet texture was a response to a series of structural changes occurring between muscle fibres and connective tissue. The combination of these findings infers that texture, and the understanding of the development of softness, is yet to be fully understood, although the progress within the field is advancing.

Development of softness in fish muscle is affected by both ante and post-mortem processes (Hyldig & Nielsen, 2001). According to the industry the causes of textural issues in the salmon can be divided into three different categories; the fish itself, harvest and slaughter (primary processing), and secondary processing. In this section, textural problems and especially soft texture will be reviewed in order to provide the reader with an overview of at least some of the factors that influence the textural quality of Atlantic salmon from aquaculture.

3.2.1 The fish

The life and product history of the fish as described previously comprise of many steps, procedures and treatments, which together may influence the prevalence of soft texture and gaping. Just the simple fact that there are textural differences between wild caught and reared salmon demonstrates how aquaculture production influences the composition of the fish and with that, quality parameters like texture. Montero *et al.* (2003) observed that farmed Atlantic salmon had a firmer texture compared to wild caught salmon. This was explained by higher collagen contents and lower incidences of protein aggregation in the farmed salmon resulting from a higher fat content. Hence, production practices including feeding intensities, feed composition, and photoperiod, which are all parameters that can be controlled in aquaculture, have a strong influence on fat content and growth, and thereby also the texture of the fish meat.

The industry experience an increased number of claims regarding soft texture in the fall, which are speculated to be a result of the increased growth rate that the fish experienced during summer when temperatures are high and the days long. Yet, even though the relationship between growth rate and development of soft texture and gaping has been extensively investigated, the results are inconclusive and do not strongly support the observations from the industry. Johnston *et al.* (2004) studied how smolts, which were reared at different photoperiods and transferred to seawater out-of-season, performed with respect to growth. They found that the growth rate was higher when the fish were exposed to continuous light compared to fish held at ambient light. Moreover, both the number of muscle fibres and the fibre diameter were greater in continuous light reared fish indicating that these fish experience more hypertrophic growth than their ambient reared counterparts. Nevertheless, the authors did not find any correlation between growth rate and the final texture of the muscle (Johnston *et al.* 2004). In a study from 2007, Johnston and co-workers confirmed their previous results as they found no correlation between growth rate and the prevalence of soft texture and gaping (Johnston *et al.* 2007). These findings indicate that the higher prevalence of soft texture during fall might be a result of something that is not related to fast growth. Martínez *et al.* (2011) proposed a theory that softness might arise due to a period of muscle re-modelation (degeneration, regeneration and growth) prior to slaughter, which is also supported by observations by farmers stating that soft texture is not a permanent state of the muscle tissue but something the fish recovers from. Muscle re-modelation could include the fusion of myoblasts into new muscle fibres – a process that involves the protease system containing calpain and calpastatin (Nemova *et al.* 2010). Calpain is a Ca^{2+} dependent protease similar to palpain, which is commercially used to tenderize meat, and its inhibitor calpastatin. Some studies have related the activity of calpain and calpastatin to the post-mortem development of soft texture in salmon (Gaarder *et al.* 2012) and sea bream (Salmerón *et al.* 2013). Hence, the possibility of the calpain/calpastatin system being active in the live salmon, and by that influencing the texture via proteolytic degradation of myofibrillar proteins, might be a reasonable theory to pursue.

In a study by Johnston *et al.* (2002), a relationship between muscle fibre density and susceptibility to gaping was discovered. Fillets with lower fibre densities were more prone to gaping, yet no explanation for this relationship was found. Fibre density has information in both fibre number and their size (Vieira *et al.* 2007). Vieira *et al.* (2007) showed that selection for low fat in breeding programmes could also affect the texture of salmon flesh by increasing the amount of muscle fibres. High fibre numbers are correlated with high fibre density, and thus increased firmness of the muscle (Johnston *et al.* 2000). Breeding programmes are already responsible for at least some of the success of the Norwegian aquaculture by producing stocks that grow faster than their wild counterparts and are better adapted to life in confinement (Gjerde *et al.* 2007), hence, further work on breeding salmon with an optimized texture is considered reasonable.

Disease is another factor that may affect the texture. As an example, pancreatic disease is a major problem in relation to texture (Larsson *et al.* 2012; Taksdal *et al.* 2012). Lerfall *et al.* (2012) investigated different quality attributes of raw and smoked salmon that had been attacked by pancreatic disease. The results showed that fish recovering from pancreatic diseases had lower levels of protein and was in poorer condition, probably due to a reduced ability to utilize the nutrients from the feed. Moreover, firmer texture was seen in these fish, which was explained by increased amounts of connective tissue due to the disease.

In the light of the reviewed literature, it is evident that numerous factors, while the fish is alive, can influence the textural quality of Atlantic salmon. In the sections below, the effects of handling during harvest and slaughter and the subsequent secondary processing will be reviewed for their influence on texture.

3.2.2 Primary processing (harvest and slaughter)

Several studies have investigated the effects of harvest and slaughter on the subsequent textural quality of Atlantic salmon fillets. Stress is unavoidable when fish are transferred from the sea cages to the well boats for transportation to the shore, and the following slaughter. Transportation involves pumping, transport, unloading and stocking (Ashley, 2007), which are all procedures that have a negative affect on the fish. Salmon are often starved for a period of time prior to slaughter. This is done to reduce the metabolic rate in an attempt to lower the effect of the stress factors the salmon are subjected to during the handling (Einen & Thomassen, 1998), for example early onset of rigor. Onset of rigor is affected by the amount of glycogen present in the muscle cells. The glycogen is used to produce ATP needed in order to break the cross-bridges between actin and myosin formed during contraction of the muscle. Stress, vigorous movement and escape patterns (due to fear) all stimulate an earlier onset of rigor as these processes will result in a faster depletion of glycogen and ATP in the muscle compared to rested and unstressed fish (Erikson *et al.* 1997; Skjervold *et al.* 2001a). Erikson (2008) observed that even after live chilling most of the fish at the investigated facilities were stressed but not exhausted. Exhaustion is critical to obtain in order to slow down the depletion of glycogen and ATP associated with stress. However, Skjervold *et al.* (2001a) observed that short-time stress resulted in softer texture, whereas firmer texture was observed after long-term stress, which might be explained by low glycogen stores and thus a high pH level after long-term stress. Several studies have examined how different slaughtering methods affect texture (Erikson, 2008; Kiessling *et al.* 2004; Roth *et al.* 2002; Roth *et al.* 2009) and Roth *et al.* (2006) concluded that the textural quality of Atlantic salmon was more influenced by the stunning method than pre-slaughter conditions. However, in a study by Mørkøre *et al.* (2008), the authors found that starved salmon showed a slowed metabolic rate in the muscle resulting in a slower utilization of ATP and thus a slower rigor development. This can for example be utilized in pre-rigor filleting, as a delayed onset of rigor is needed to perform these process steps.

Following slaughter, factors like storage temperature and handling also influence texture. Pittman *et al.* (2013) concluded that gaping was associated with factors like temperature at slaughter and muscle pH. This underlines the importance of the live chilling step presented in Section 2.2 serving both as sedation to keep the fish calm and as a product cooling. Moreover, softening after slaughter is connected to changes in the connective tissue (Moreno *et al.* 2012; Taylor *et al.* 2002), and degradation of the muscle proteins. Bahuaud *et al.* (2010) showed that the breakdown of proteins in the connective tissue and muscle fibres would soften the fillet after slaughter and that firmer fillets had a tendency for higher activity of protease inhibitors, thus reducing the protein degradation. In relation to this, Jacobsen *et al.* (2015) observed that poor cleaning of the abdominal cavity after slaughter resulted in increased occurrence of gaping and loss of firmness. Both blood and viscera contain proteolytic enzymes that will digest the proteins causing for example the cross-bridges between actin and myosin to disintegrate with a loss of firmness in texture as a result.

After slaughter, exsanguination and gutting the salmon are packed on ice in expanded polystyrene boxes, packed on a cooling truck and transported to their destination. During transport the salmon will undergo rigor and be ready for further processing upon arrival at the filleting facilities.

3.2.3 Secondary processing (value-added products)

During secondary processing the biological processes that were initiated at slaughter, still prevail. Temperature control is thus of the uttermost importance as high temperatures will speed up the degradation process in the meat. In Norway, the salmon are most often packed in expanded polystyrene boxes on flake ice. However, Piñeiro *et al.* (2004) reviewed the use of slurry-ice systems to obtain the same degree of cooling. Slurry-ice is a two-phase system containing ice and a liquid, and it possesses a higher heat-exchange capacity compared to for example flake ice. Therefore, it can cool the fish faster. The simplest systems use water and are available as commercial equipment (Piñeiro *et al.* 2004). Transport of the salmon from the primary processing plant to the secondary processing facilities can be done in a slurry ice, and is a method practiced by Marine Harvest Scotland (Marine Harvest, 2013). Another cooling option that is emerging, is superchilling. In this process, the surface of the product is frozen, and the formed ice accumulates heat from the interior of the product (Magnussen *et al.* 2008). In the traditional flake ice cooling the total weight of each box is increased due to the amount of ice required to maintain a cold product during transportation (Gallart-Jornet *et al.* 2007). The idea behind superchilling is, besides enhancing the quality of the fish, to reduce the weight and thereby the transportation costs. Quality deterioration processes are slowed down compared to flake ice cooling, but too long storage with this method might have a negative impact on the texture (Gallart-Jornet *et al.* 2007). In summary, it is of paramount importance to cool the salmon fast during harvest and slaughter, and maintain this cooling degree also during transport and storage prior to secondary processing. Moreover, the time from primary to secondary processing is also critical: The longer the time period between the fish being slaughtered and filleted, the more prevalent textural issues become (Skjervold *et al.* 2001b; Skjervold *et al.* 2001c).

During secondary processing several issues also prevail. This part of the thesis is mainly built on information acquired during several meetings at Skagerak Salmon A/S in combination with information from the equipment producers, BAADER DANMARK A/S and the fish processing section of Marel.

Salmon with soft texture and gaping may cause downtime in the production due to problems during for example filleting resulting in a considerable loss (Michie, 2001). On the other hand, the equipment may also be responsible for especially gaping as it can tear the meat. Moreover, the lay-out and design of the process line can also influence the final product as gaps between conveyor belts or shifts in direction (due to restricted space) forces the product to be shifted and pushed, and by that putting a force on the muscle blocks that may cause them to tear apart and cause gaping. Additionally, the manual trimming of the fillets require that the fillets be lifted from the high-speed conveyor belt to a table in front of the person doing the trimming. This lift can cause severe damage to the fillet if not performed gently.

The salmon fillets at Skagerak Salmon A/S are all filleted post-rigor, as up to 5 or 6 days can pass before the truck makes the trip from northern Norway to the processing facilities in Hirtshals in the northern part of Denmark. However, pre-rigor filleting is gaining grounds due to the increased market demand for fresh fish (Skjervold *et al.* 2001b) and the suggested positive influence on quality characteristics like colour and texture (Veiseth-Kent *et al.* 2010). Due to the diminished prevalence of especially gaping in salmon when filleted before onset of rigor, some processing facilities in Norway experiment with pre-rigor filleting (Skjervold *et al.* 2001b). According to Marel (personal communication), around 5% of the salmon produced in Norway are currently being filleted pre-rigor. Processing salmon pre-rigor might result in products of higher value due to the texture or even enhanced colour. Nevertheless, the removal of pin bones is an almost impossible process that would tear the meat if forced. However, in a Norwegian study supported by The Norwegian Seafood Research Fund several methods for the removal of pin bones in pre-rigor filleted salmon has been investigated. Focus of the study has been weakening of the bone attachments by ultrasound and new approaches for pulling the bones but the results are yet to be published (FHF, 2015).

Although pre-rigor filleting is not yet standard practice, the method offers many advantages. Firstly, the increasing consumer demand for fresh fish can more readily be met as the salmon can be processed and packed even before onset of rigor followed by transportation to the consumer. Secondly, the use of pre-rigor filleting reduces the meaningless transportation of waste products as the fish is already filleted prior to transport and thus skeletal frame, head and skin not transported (Bahuaud *et al*. 2008). Yet, selling the already processed salmon (when processed in Norway) will increase the price of the product when sold to the European market due to the before mentioned 13% sales tariff.

Conclusively, even though the price of the products may be reduced due to the lower transportation costs, the products will be of higher quality (compared to post-rigor processed salmon) and thus of higher value. It can be speculated that the resulting higher prices might push farmed salmon to another segment of the market dominated by consumers with stronger buying capacity thereby making it a luxury product.

All in all, many factors may have a negative impact on the final product illustrating the complexity of the quality control along the value chain. It has already been established that the composition of the fish muscle of Atlantic salmon affects the texture, which in turn affects the mechanical handling during production and the final product quality. In the next chapter, a literature study investigating variation in fat and protein content of Atlantic salmon from aquaculture will be presented in order to further elucidate the complexity of producing salmon products.

4 VARIATION IN FAT AND PROTEIN CONTENT

As part of the work of the current PhD thesis raw material composition was examined with focus on seasonality and temperature dependence of some quality characteristics like fat and protein content. As previously mentioned, the quality of the raw material has a strong influence on processing where traits like soft texture and gaping are undesired as they lead to downgrading of the products, as well as a decrease in yield (Einen *et al*. 1999; Michie, 2001). Salmon caught at different seasons have been reported to differ in hardness and it has also been found that there are differences in the effect of seasonality, which could be explained by water temperature variation between seasons and years (Mørkøre & Rørvik 2001a; Merkin *et al.* 2014).

In the comprehensive amount of literature regarding Atlantic salmon from aquaculture, limited literature has been found containing continuous measurements of fluctuations in fat and protein content over a full one-year period. Most literature found mainly covers other variables and not seasonality, such as effects of feeding regime on growth performance and flesh quality (e.g. Johnsen *et al.* 2011; Johnsen *et al.* 2013), lipid metabolism (e.g. Nordgarden *et al.* 2003), or effect of feed composition on growth (Hemre & Sandnes, 2008). Seasonality of fat and protein content has been reported by Berg & Bremset (1998) for wild salmon and trout, which is different from aquaculture as it is subjected to nature. However, even though aquaculture is considered a standardized production method compared to wild fish, variation in the raw material and outcome is also seen there (Mørkøre *et al.* 2001). Because of this, this chapter will provide the reader with a literature study of reported fat and protein content of Atlantic salmon, and the possible causes of the observed variations. A discussion of the findings in relation to the hypotheses presented in Figure 2 is to be found in Chapter 6.2.

4.1 FACTORS AFFECTING FAT CONTENT OF ATLANTIC SALMON

A wide range of studies, exploring different aspects of fat content in salmon, are available, however, priority has been given to studies including farmed salmon from the Norwegian aquaculture industry. An overview of the reviewed studies is presented in Table 2. Data is listed together with the month in which the sampling was performed, number of fish included (samplings, n), percentage lipid with standard deviation where available, weight of the fish (kg), time of sea transfer (due to differences in growth profiles for smolt transferred to sea at different times of year), analytical method used and the respective references.

Table 2. List of studies with data on fat content (%) in Atlantic salmon. The table comprises sampling month, number of samples (n), average fish weight (kg), time of sea transfer, the analytical method used and the reference. Standard deviations have been added when available from the references.

Month	**n**	**Lipid%**	**Fish weight (kg)**	**Sea transfer**	**Method**	**Reference**
January	145	15±3	4.3±0.03	Fall	Bligh and Dyer, 1959	Refsgaard *et at.* 1998
January June	9 8	10-13 8-12	3.3 2.3	Fall	Soxleth by AOAC (1984)	Hillestad *et al.* 1998
January	12	18.1±0.3	4.9	Spring	Ethyl acetate extraction	Sveier & Leid, 1998
January May	2x10	11.4 11.3-14.2	2.35 3.55-4.01	Spring	Ethyl acetate extraction	Mørkøre & Austreng, 2004
February June September	3x51	14.8±0.5 8.9 12	3.0 1.7 6.5	Spring	Ethyl acetate extraction	Espe *et al.* 2004
January April May October October November	105 30 75 105 30 75	14.5 16.7 16.8 17.9 21.8 19.4	3.4 4.0 3.6 4.1 3.9 4.0	N/A	Computerized Xray tomography	Mørkøre *et al*. 2001
November	4x3	19.4±0.9 19.3±0.2 18.3±0.2 17.4±1.0	2.82±0.01 2.79±0.01 2.61±0.00 2.22±0.00	August	Soxtec system HT 1043	Hevrøy *et al.* 2013
February April August October	4x36	16.9 16.1 16.9 15.9	5-6	N/A	Digital camera with RGB signals from each pixel. Transcribed to correspond to fat content.	Mørkøre *et al.* 2010

Month	**n**	**Lipid%**	**Fish weight (kg)**	**Sea transfer**	**Method**	**Reference**
January	4x45	12.4±0.3 13.3±0.4 14.4±0.4 12.9±0.4	1.1 1.7 1.7 1.9	April May May May	Ethyl acetate extraction	Roth *et al.* 2005
June	4x45	13.0±0.3 13.1±0.3 13.5±0.3 13.0±0.3	2.1 2.6 3.0 3.0	April May May May		
October	4x45	15.8±0.4 15.1±0.4 15.3±0.4 14.8±0.4	4.8 5.2 5.4 4.9	April May May May		
January March May July September November	6x10	18.6 17.1 17.3 19.2 11.4 16.7	2.7 3.0 3.9 5.1 1.1 2.1	October	Ethyl acetate extraction	Mørkøre & Rørvik, 2001b
January March May July September November	6x10	12.8 13.4 14.7 17.1 9.4 14.3	1.3 1.5 2.1 3.3 0.6 1.1	May		
January April	2x3	18.8 18.4	6.3 4.4	June	Ethyl acetate extraction	Einen *et al.* 1998
January April	2x30	16.7 15.4	6.3 4.4	June		

The fat contents listed in Table 2 ranges between 8.0% and 19.9%, which is comparable to data reported by Gjerde *et al.* (2007), who gave a normal fat range for farmed salmon between 6% and 22%. The two sample types included in the literature study (fillet and Norwegian Quality Cut (NQC), which is a standardized cut used for sampling) are expected to contain different amounts of fat due to the location on the fish at which they are taken, since different tissues contain different amounts of fat (Aursand *et al.* 1994). While there is a difference in fat content, with the fillet having higher levels of fat compared to the NQC, the general picture of seasonality is not largely affected, since data from the two sample types are spread out over the months and cannot readily be distinguished.

The collected data infer a very large variation, which can be accounted to several factors, including different diets, age, size and season, as well as the fact that we are working with biological material having an inherent variation. In the following sections (Section 4.1.1-4.1.5) the relevant references will be discussed in relation to these factors. The included figures are based solely on data accessible from the available literature.

4.1.1 Season

Fat content is positively correlated to the weight of the fish (Shearer *et al.* 1994) with larger and older salmon containing more fat than younger and lighter salmon. The data presented in Figure 4 confirms this relationship.

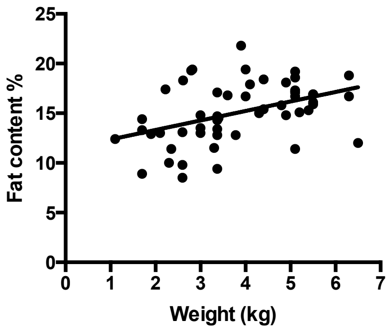

Figure 4. Relationship between weight (kg) of the sampled fish and fat content (%). Illustration of data extracted from references presented in Table 2.

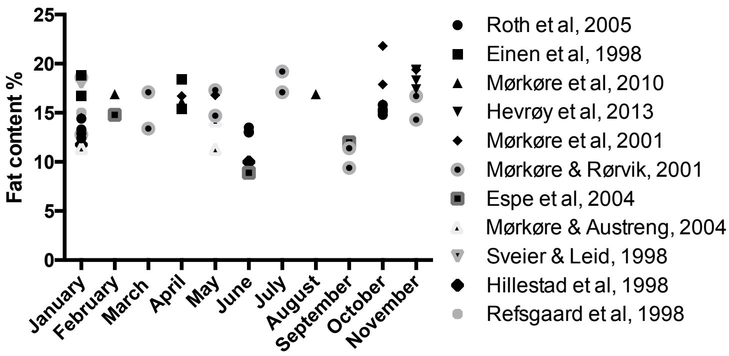

Figure 5. Fat content (%) in Atlantic salmon divided between sampling months. Illustration of data extracted from references presented in Table 2 alongside respective references.

The correlation between fat content and weight emphasizes the necessity to consider the weight of the sampled fish when analysing the fat content. Figure 5 depicts the variations in fat content according to month and is based on data obtained from the included literature (in Table 2). Large variations are observed in January, not only in fat content but also in the sampled fish weight. From a mean value for January of 14.7%±2.6% (n=13) the fat content increases until April with an average of 16.7%±1.3% (n=4). In the same period the weight data show a range in fish size between just below 2 kg and up to almost 6 kg. Due to the correlation between fat and weight, larger variations in fat content for these months would be expected, yet this is not the case. The entries for June show relatively low levels of fat (11.9%±2.0%) (n=6) in combination with sampled fish of low weight and it is therefore difficult to conclude whether the decrease in fat content is due to seasonality or the weight of the fishes.

Only sparse data have been found, and thus included in the literature study, covering the fat content in salmon in July (18.5%±1.5%) (n=2), August (16.9%) (n=2), and September (11.1%±1.1%) (n=3), making it difficult to provide a reliable picture of this part of the year. The data from September conflicts with the increasing fat content otherwise seen with increasing weight. The data are from two separate studies (Espe *et al.* 2004; Mørkøre & Rørvik, 2001b), and show a low fat content compared to the remainder of the year. The sampled fish have both the highest and lowest weight in the collected data, thus contradicting the fat-weight relationship present in the data.

In the months stretching from September to October (16.8%±2.3%) (n=8), and November (18.0%±1.8%) (n=8), the fat content tends to increase although the weight of the sampled fish decreases from October to November from an average of 4.8 kg to an average of 2.5 kg, respectively. From November it would be expected that the level of fat would decrease further with decreasing water temperature by influencing appetite (Elliott, 1982). Other studies have also reported decreasing fat levels during winter in farmed adult salmon (Einen *et al.* 1999).

Based on the reviewed literature, while there is a positive correlation between fat content and weight, the variations in fat content observed in the studies presented in Table 2 cannot solely be contributed to differences in weight of the sampled salmon, and hence, seasonal variation amongst the presented data is established.

4.1.2 Water temperature

Water temperatures in Norway are at their lowest in winter and spring, slowly rising during summer to decrease again in the fall (Sea Temperature, 2016). Immature salmon increases their feed intake as water temperature rises in spring in contrast to sexually maturing salmon where decreasing appetite has been observed, starting in early June with the lowest level of feed intake in July (Kadri *et al.* 1997). As the data comprises fish from commercial aquaculture, the tendency to increase fat content during the summer supports the observations by Kadri *et al.* (1997). Commercial aquacultures want low incidences of pre-harvest maturation due to the anorexic behaviour that leads to decreasing body weights, poor flesh quality and poor skin colour (Quinton *et al.* 2005) making the final product not marketable. The decrease in fat content in the salmon during winter conforms to the general belief that the appetite of Atlantic salmon is temperature dependent (Elliott, 1982). The decrease in appetite is correlated to a lower metabolic demand during winter as the activity level of the salmon is reduced, due to lower water temperatures (Hiscock *et al.* 2002). Increased appetite correlates to the higher temperatures of the Norwegian coastline in the fall (an average of 11-13 °C in the months of August to October (Akvafakta, 2015)), and decreased appetite as the water temperatures decrease in winter (down to around 5 °C in February (Akvafakta, 2015)).

In a controlled temperature trial, Hevrøy *et al.* (2013) investigated how raising the temperature from 13 to 15, 17 and 19 °C, respectively, when rearing in indoor tanks, affected the growth process of the salmon and found a significantly reduced growth rate over the experimental period at elevated temperatures. Hence the final weight was observed to decline linearly with increasing temperature and the highest growth rate and fat content was observed at 13 °C. Mørkøre *et al.* (2010) harvested salmon in February, April, August and October with seawater temperatures of 8, 6, 15 and 11 °C, respectively. Although the groups were fed the same feed the fat content reported by the authors was significantly higher in February and August (both with 16.9%) compared to October (15.9%) and April (16.1%). The authors gave no explanation of biological origin for these differences, but contributed the observed differences to the overall variation in the measurements rather than season. Mørkøre *et al.* (2001) sampled salmon from four different locations six times during a period of almost two years trying to estimate the effect of season but although the reported fat content differed between sampling months, the authors did not deduce whether this difference was due to season or geographical location of the farm. Refsgaard *et al.* (1998) considered the lipid distribution in 145 farmed salmon fed a commercial diet and measured a mean value for fat content of 15%±3% for salmon harvested in January. This study illustrates that the biological variation of fish from the same origin, grown under the same conditions is large, and thus it is very likely that the differences observed by Mørkøre *et al.* (2010) can be explained by this.

4.1.3 Diet, feeding states and feeding regimes

Sveier & Leid (1998) used feed composed of 38.0% protein and 32.2% fat, which is comparable with the commercial feed used in the study by Einen *et al.* (1998), containing 42.8% protein and 32.1% fat. Hillestad *et al.* (1998) tested two different feeds, a low fat with 43.8% protein and 22.8% fat and a high fat consisting of 39% protein and 30.3% fat. Mørkøre & Austreng (2004) used a commercial dry feed with 43% protein and 39% fat and moist feed with 28% protein and fat, respectively. Each of these studies reports different compositions of the feed, which can account for the differences in fat content seen amongst the used feeds. The feeds were all commercial or composed to have similar content as a commercial feed and containing fish meal, fish oil and a wheat source, except the moist feed used by Mørkøre & Austreng (2004), which contained mackerel offal and wheat. The results from these studies show that the fat content of the fish is related to the fat content of the feed (Mørkøre & Austreng, 2004), but also that the feed ratio and intensity influence the fat content greatly (Einen *et al.* 1998; Hillestad *et al.* 1998; Sveier & Leid, 1998). Hence, it is reasoned that the observed differences cannot be contributed to the feed alone, but has to be considered together with feeding intensity and feed ratio. However, these are parameters that some companies vary according to the season.

Several researchers have studied the effects of different feeding regimes on fat content amongst other parameters. Sveier & Leid (1998) investigated the effects of 1h or 22h feeding regimes, respectively. In the first case salmon were fed to the point of satiation once a day, whereas in the second case feed was supplied continuously. The two groups did not differ with respect to body composition and both groups had a final fat content of 18.1%±0.3%. Mørkøre & Austreng (2004) fed salmon two types of feed, dry and moist, with the dry feed having a higher energy content (39% fat) than the moist feed (28% fat). They found that the fat content was significantly higher in the dry feed group (14.2%) compared to the moist feed group (13.3%) when fish were sampled in May. The difference in energy content between the feeds likely explains the difference in fat content as salmon increase their feed intake in the spring and hence, the dry feed group would have a higher energy intake than the moist feed group. In a study by Hillestad *et al.* (1998), different feeds were tested together with different feeding rates in order to investigate the effect of high-fat diets on growth, feed conversion and carcass quality. Fat content in the cutlet increased with an increased feeding rate, while the fat content of the feed did not have an influence on growth. Moreover, they only found a tendency for increased fat in the carcass when the fat content of the feed was increased from 22% to 30%. The fat content of the cutlet ranged between 10 and 13% when measured in January and between 8 and 12% when measured in June. The difference between the two groups cannot be explained by increased feeding intensity during spring, as the June samples displayed lower levels of fat. More likely, the difference can be explained by a difference in weight gain, as the salmon sampled in January had a higher final weight compared to the fish sampled in June. In a starvation study by Einen *et al.* (1998), the initial fat content of whole fillets and the NQC, were 18.8% and 16.7%, respectively. Even though the two sample types were expected to contain different amounts of fat due to the differences between tissue types included in the samples, no significant effect of starvation was seen with respect to changes in fat content in these two sample types (Einen *et al.* 1998). This indicates that starving salmon might utilize other depots for energy, for example the fat stored in the belly flap, rather than using the fat stored internally in the muscle.

4.1.4 Age at sea transfer and harvesting time

Roth *et al.* (2005) studied the effect of body size and age at sea transfer on different quality variables including fat content of growing salmon. The authors found no significant differences in fat content between the different sizes of smolt but observed a significant increase in fat content during growth due to season, with an average fat content of 13.0% in January and 13.2% in June rising to 15.4% in October. Consequently, the size of the smolt did not influence the final fat content, but the season, at which the smolts were released, did. Espe *et al.* (2004) investigated the effects of harvesting time on the meat quality and found that the fat content increased from June to September (from 8.9% to 12.0%), and from September to February (from 12.0% to 14.8%±0.5%), respectively, most likely due to the size, and accordingly age, of the fish when harvested.

In order to be able to meet the market demands for Atlantic salmon, smolts are produced both during spring (1+ smolts transferred to seawater in the spring following their hatching) and fall (0+ smolts typically transferred to seawater out-of-season in fall and early winter) (Alne *et al.* 2011; Roth *et al.* 2005). In Table 2, 12 entries stem from out-of-season smolts, 10 from in season smolts, and two studies do not mention time of sea transfer. Eight months had entries from both smolt types and the tendency was that fish transferred as 1+ smolt had lower or equal fat content compared to 0+ smolts. Several studies have confirmed that the growth of 0+ and 1+ smolts is strongly affected by the large changes in temperature and photoperiod that are seen e.g. in Norway (e.g. Mørkøre & Rørvik, 2001a; Nordgarden *et al.* 2003). Duncan *et al.* (2002) compared the performance of 1+ and 0+ smolts when subjecting them to different photoperiods and found that in spite of different growth profiles, the overall growth was similar between the two types of smolt. As the aquaculture industry is a business, like every other, it can be speculated that the difference in growth profiles is of limited interest to the farmers as long as the final weight conforms to what is expected. In a release study by Espe *et al.* (2003) smolts at three different ages were released to seawater in late spring, early autumn and autumn, and harvested in January after 18, 16 and 14 months, respectively. The authors found only slight differences in fat content between the three different smolt types with an average of around 15% measured in January. In summary, based on the reviewed literature it is unlikely that the differences in fat content seen amongst the included references stem from the time of sea transfer of the smolts, and thus is more likely to be a seasonal effect.

4.2 PROTEIN LEVELS THROUGHOUT THE YEAR

Several studies have been conducted on protein content in salmon from aquaculture, and it seems widely accepted that the protein content does not vary or fluctuate much over the year (Shearer *et al.* 1994). Shearer *et al.* (1994) investigated the protein level in Atlantic salmon from 25 g to harvest size and found a nearly constant protein level in immature fish larger than 100 g. Einen *et al.* (1999) found that the protein content decreased 1% after 110 days of starvation, and although this decrease was not significant they concluded that starving salmon must utilize proteins as an energy source. In Table 3 data on protein content in Atlantic salmon are listed together with sample size (n), sampling month, method and reference.

Table 3. List of references with data on protein content (%) in Atlantic salmon together with standard deviation where possible. The table comprises sampling month, number of samples (n), the method used and the reference.

Month	**n**	**Protein%**	**Method**	**Reference**
November	3x4	17.1-18%	Kjeldhal	Hevrøy *et al.* 2013
February June September	51x3	19.7%±0.17%	Calculated assuming proteins contain 16%N	Espe *et al.* 2004
January	6	17%	Nitrogen gas analysator from Perkin Elmer	Sveier & Leid, 1998
January	4	23%	Kjeldahl	Aidos *et al.* 1999
N/A	6	18.6±0.8%	Kjeltec Auto System	Birkeland *et al.* 2004
May	16	20%	Kjeldahl with N*6.25 Kjeltec Auto System	Skjervold *et al.* 2001c
January	N/A	19%	N determined after total combustion. Crude protein calculated as 16%N	Espe *et al.* 2003

The protein content presented in Table 3 ranges between 17 and 23% (with an average of 19.1%±1.9%), which is in good correlation with Shearer *et al.* (1994), who reported an average of 18.5% for fish from 125g up until they reached maturity.

Hevrøy *et al.* (2013) reared salmon at elevated water temperatures (from 13 to 15, 17 and 19 °C) and found small but significant linear elevations in protein content with increasing temperature from 17.1%±0.4% at 13 °C to 18.0%±0.1% at 19 °C. In contrast, Espe *et al.* (2004) found that salmon sampled in June, September and February displayed a stable protein content of around 19.7±0.2%. This is in spite of the different seawater temperatures that are expected for the three sampling months, with September commonly having the highest temperatures compared to January and June. Sveier & Leid (1998) found no differences on protein content (17%) between two different feeding regimes (1h or 22h), whereas Aidos *et al.* (1999) reported a protein level of commercially reared salmon of 23%. These two studies, both with samples taken in January, exemplify that although stable protein levels are an accepted fact, the range of the measured levels is not constant. Supporting this, Birkeland *et al.* (2004) measured protein levels in the range of 17.0-19.6%, Espe *et al.* (2003) found a protein content of 19%, and Skjervold *et al.* (2001c) reported levels in the area of 20%.

In a study by Refstie *et al.* (2001) no effect of differing levels of dietary protein (40 and 45% protein in feed) on final protein content of the fish was found. Øverland *et al.* (2009) tested the replacement of animal based protein in the feed with a protein source derived from treated pea hull. They found no differences between the control feed group and the experimental group on growth performance and final body composition. However, other findings suggest that supplementing an animal based protein feed source with a plant based, has a negative effect on growth, as well as increasing the fat deposition in the fish, but without documenting the effect on final protein level in the muscle (Espe *et al.* 2006; Sveier *et al.* 2001). Therefore, some effect of diet composition on the final protein content in the fish is expected while the degree of this is unknown. Based on this, it is reasonable to conclude that seasonality of protein content in reared Atlantic salmon is minimal, but that protein content may be affected by the feed.

5 MATERIALS AND METHODS

The specific methods that have been employed during the related studies are presented in the respective papers. Consequently, this chapter presents some of the prerequisites for even performing the research. First and foremost, the production at Skagerak Salmon A/S is highlighted and presented here, as it is one of the cornerstones of the current PhD project. Secondly, because texture, and thus textural measurements, is a main part of the project the thoughts behind the employed methods are presented. Thirdly, the building of prediction models has been used extensively to assess the data, and to explore correlations and possibilities of utilising data to increase the outcome. Hence, some background on the use of prediction models is provided.

5.1 PRODUCTION PRACTICES AT SKAGERAK SALMON A/S

The secondary processing comprises the part of the value chain after the fish has been slaughtered and gutted. In the present PhD study, some of the work has been performed in collaboration with Skagerak Salmon A/S representing this part of the industry, and this section will therefore describe their production site and methods.

At the production site at Skagerak Salmon A/S, two production lines are used; one semi automatic and one full automatic. Experiments have only been conducted on the full automatic line hence it is only this line that will be described. A flow chart representing this production line can be seen in Figure 6.

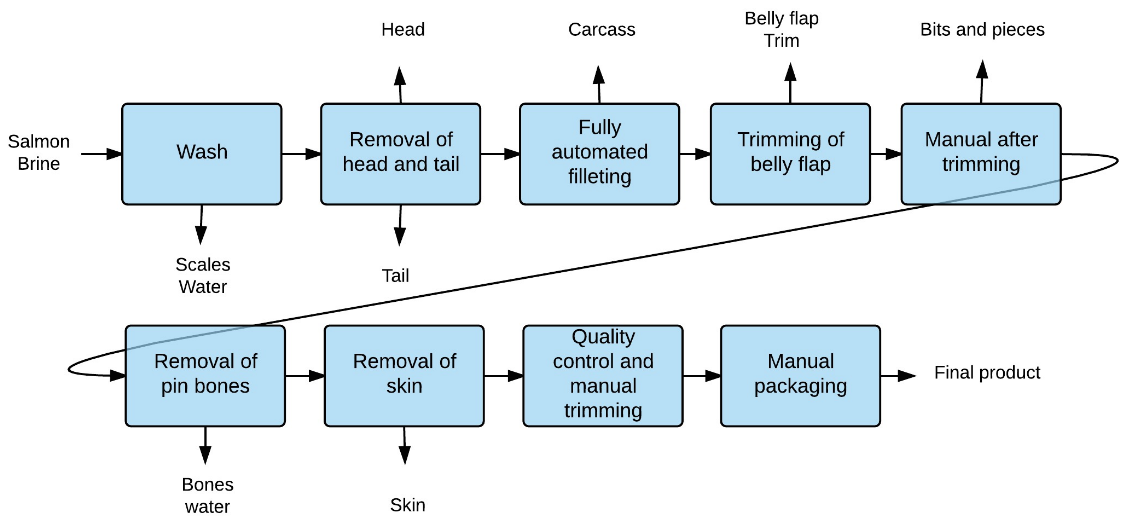

Figure 6. Flow chart showing the processes involved in the production of fillets from Atlantic salmon at Skagerak Salmon A/S. Salmon and brine enters the production line and by-products are shown at the respective process steps before the final product is packed.

Skagerak Salmon A/S purchases around 99% of their salmon from Norway. The salmon are transported to the factory by truck from Norway. Upon arrival the boxes are unloaded, weighed and given a batch number. A manual quality control is taken comprising five salmon from each batch, which are given a score based on their quality. Once the quality has been approved, the batch can progress to the production. Styrofoam boxes containing ice and the gutted salmon are emptied out on the conveyor belt, which allows the ice to fall down. The salmon are then washed to remove the slime layer and loose scales. From here the salmon are placed in the heading machine that places a U-cut behind the gills to remove the head, and a cut to remove the tail. The headed fish can now be placed in the filleting machine. Although filleting is automatic, manual labour is needed to ensure the correct placement of the fish on the saddle, tail first. The filleting machine cuts out the carcass and two fillets emerge. The fillets are then transported to the automated trimming machine where they are trimmed according to the recipe chosen for a specific product requested by a specific customer. This trimming includes the removal of the belly flap and fins. Manual inspection and further trimming is required to obtain products of the right quality. The fillets are then passed through a pin-boning machine, which removes the pin bones, and a skinning machine if the customer requires skin removal. Manual inspection and quality control follow where any leftover pin bones may be removed, or the fillets discarded if there are faults on them that downgrade the quality. If the fillet is regarded as sale material it will be packed or transferred to a new process line for portioning. Skagerak Salmon A/S produces salmon fillets or portions, fresh or frozen, and with or without marinade.

5.1.1 Sample handling

The seasonal experiment presented in Paper II investigates the raw material variation in relation to temperature. Skagerak Salmon A/S, who received the whole salmon and processed them as described above, supplied samples of salmon fillets. Even though the law of traceability dictates that information regarding for example rearing site must follow the fish, this information was lost in the system and thus not made available to us. Hence, it was not possible to include transportation time, which largely depends on rearing and slaughter site, or post-mortem age in the analysis of data.

During transportation the salmon are packed in expanded polystyrene boxes and covered with ice. During the many visits at the production of Skagerak salmon A/S it was always noted that ice was still present when the boxes were tipped on to the conveyor belt for processing. Hence, it was assumed that the salmon always were stored at no more than 2 °C.

At best the packaging number was always provided. The packaging number is an individual and unique tag that couples a company to a region, albeit without supplying the exact location in the region. Raw material history has thus been sparse, yet all the available information has been included when required.

5.2 TEXTURE MEASUREMENTS

The definition of texture given in Section 3.1 infers that texture is a quite complex measure. This is also supported by Vincent (1997) who took on the application of materials science in the measurement of fish texture. Texture is most often measured on a texture meter and the sampling method as well as the choice of equipment will influence the result (Jonsson *et al.* 2001; Veland & Torrissen, 1999). Cheng *et al.* (2014) reviewed the literature in order to describe the different methods for performing textural measurements on fish products. The common denominator for these methods is that they are destructive for the muscle fibres. For example, studies investigating the breaking strength of the muscle do so by exerting 60% compression on the sample as a measure for firmness (Bahuaud *et al.* 2010; Einen & Thomassen, 1998; Kiessling *et al.* 2004), consequently breaking the muscle fibres. The textural measurements employed in the present PhD study thus differ from what is frequently reported in the literature. The reason for this was that the analyses made in connection with this study required intact samples. Hence, in the present PhD project, a much lower compression degree was used in order to obtain a measure for the texture while not breaking the muscle.

Texture measurements performed using high compression force can be speculated to also include information on the strength of the connective tissue in combination with the tissue surrounding the point of measurement. Salmon muscle has a rather brittle structure with sheets of connective tissue separating the myotomes (Dunajski, 1979). Careful handling of the products is required not to compromise the texture (Kiessling *et al*. 2006) and consequently, the cohesiveness of the muscle blocks. Food products are highly complex biological matrices with a combination of chemical and physical factors, which all together define the product characteristics (Rahman, 2005). The inherent variation in these factors such as fat, protein and size, result in a natural raw material variation that influences the processing of the product. Thus, similar to the method described by Kiessling *et al.* (2006) it was chosen that during the seasonal experiment (presented in Paper II) texture would be measured on the whole salmon fillet in order to reduce the effects of handling, for example by cutting the samples. Additionally, in the received samples the pin bones were not removed. In that way pin bone removal was performed by hand and thus not under the force of a machine, hopefully minimising the destruction of texture. However, this approach was only employed in the seasonal experiment. The fillets used for determining the relationship between texture and proteome were fully trimmed and skinned and thus presumed already largely affected by the handling procedures.

During the initial studies on texture in 2012,15% compression with a spherical probe was employed. The data suggested a relationship between proteomic response and the measured peak force. Thus, when repeating the trial in 2014, the same degree of compression was chosen in order to be able to compare the results. However, prior to starting the study on seasonal effects of several parameters including texture, an initial trial for probe selection and compression degree was conducted. Here it was found that 10% compression with a spherical probe would influence the fillet the least. Hence, this degree of compression was used in the seasonal study. At no point in the current PhD project has data from the two studies been compared.

5.2.1 Experimental set-up

Texture was in all experiments measured using the TA.XT2 Texture Analyzer (Stable Stable Micro Systems, Surrey, England), equipped with a 30 kg load cell, using a flat-ended cylinder with a diameter of 35 mm. Single compressions were used and texture was registered as peak force (measured in Newton), which was recorded automatically by the software TextureExpert32 (Stable Micro Systems, Surrey, England). The sample was placed under the probe and the probe travelled at a pre-test speed of 1 mm/s. When the probe came into contact with the sample, the software automatically recorded the thickness of the fillet. The probe continued at a constant speed a fixed percentage of the sample thickness. Two different compressions were employed:

- Seasonal experiment: 10% compression, eight sampling positions along the whole fillet, above the lateral line.
- Texture prediction: 15% compression, four sampling positions, samples blanked from the fillet, above the lateral line.

Further details on the experimental set-up can be found in Paper II and III in Chapter 9.

5.3 PREDICTION MODELS

In the food industry, many types of data are accumulated and the information that can be extracted from these data can be of significant importance in the attempt to understand mechanisms and processes better. Mathematical methods combined with computers can handle these data providing a whole new level of managing design, control and manufacturing processes (Bruin & Jongen, 2003). Bro (1998) thoroughly describes how multivariate data analysis can handle the complicated data analysis that is inherent when attempting to analyze data of different origins. One method is the Partial Least Squares regression (PLS) in which the independent data are modeled in a way where the variation can be used to predict the dependent variable (Bro, 1998). According to Shmueli (2010) a predictive model encompasses a method that will produce a prediction irrespective of the methodology employed, whether it is parametric or nonparametric, data mining algorithms or statistical models, to mention some. Therefore, the PLS models presented in relation to this PhD project will often be described as predictive models.

Studies involving value chain analysis of the seafood sector often do so in a managerial perspective. For example, Duijn *et al*. (2006) conducted a value chain analysis on the Philippine seafood sector with the aim of identifying bottlenecks, with the aim of deciding whether to make investments in the sector. Few studies have modelled and analysed parts of, or the whole, value chain for seafood. However, Margeirsson *et al*. (2007) investigated how factors during catching cod could influence fillet yield, gaping, bruising and a number of nematodes. Multivariate regression analysis was used to explore the relationship between the independent and dependent variables. The authors found that, amongst others, time of year and catching grounds had a significant impact on the fillet yield. The data was used to set up a regression model to forecast the yield with the aim of aiding the cod industry to better production management (Margeirsson *et al*. 2007). Further analysis of the data was later provided in Margeirsson *et al*. (2010).

Although forecasting is of great interest it is temporal and uses the past to deliver a set of conceivable outcome in the future. Contrary to this, prediction models provide one estimate of a specific outcome at a given time point (Shmueli, 2010). Prediction models are widely used in shelf life prediction of food products (Emborg & Dalgaard, 2008; Mejlholm *et al.* 2010; Mejlholm *et al.* 2015), and several studies have investigated the use of for example imaging technology for the prediction of fat content in salmon (Skytte *et al.* 2016, submitted, Zhu *et al.* 2014) or quantified the gaping in smoked salmon (Merkin *et al.* 2013). However, only a few studies have been found covering the aspect of prediction of yield for a food product. In 1982, Alverez *et al.* proposed a yield prediction model for sugarcane. As much as 25 variables were identified to influence the yield including a weather variable. The sugarcane growers had knowledge of most of the remaining variables and the models were useful in the prediction of yields on a field basis. Døving & Måge (2001) investigated the influence of weather conditions on strawberry fruit yield. In this case the models revealed that weather conditions during the initial phases of flower induction were more important than the conditions during the actual flowering and ripening.

In the current PhD project the most commonly used method to develop prediction models has been with the use of the software Unscrambler X (Camo ASA, Oslo, Norway). The approach, however, to each dataset has been unique and is described more in detail in the respective papers.

6 SUMMARISING RESULTS AND DISCUSSION

6.1 PREDICTION MODELS IN THE SALMON PROCESSING INDUSTRY

The two cases by Alverez *et al.* (1982) and Døving & Måge (2001) presented in Section 5.3 exemplify the use of prediction models albeit for processes that cannot be fully controlled and is as such not related to the secondary processing of food products. The models can be used in forecasting as the grower has an estimate on how much of his crop there will be to sell. The model for prediction of yield of salmon presented in Paper I in this thesis differs in that the developed model was built based on variables that can be measured pre-harvest to predict the yield after secondary processing. The aim was to gather information on how the processing would influence the individual fish and use this information to build the model. It was hypothesised that the final model could assist processing companies in their decision-making process when choosing where they purchase the raw material. The current practices are based on experience and gut feeling, and therefore require that the purchaser has a good sense of feeling and strong knowhow regarding how yield differentiates between companies or the effect of seasonal or regional differences on raw material composition. In Chapter 4 the seasonal differences in fat and protein content have been investigated while the study in Paper II revealed differences in raw material composition depending on which company the salmon was from, or in which region the salmon was reared. Hence, several factors apply when the choice of raw material origin/company is to be made. The suggested model can therefore be regarded as a useful tool. In the study presented in Paper I, 60 salmon were tracked through the production and the suggested model was based on the information gathered from these 60 salmon. Yield could be predicted based on Equation 1:

$$1)\ Yield\% = 52.95 + 0.293 \cdot W + 0.114 \cdot L + 0.241 \cdot T + 0.216 \cdot \frac{W}{LT} + 0.257 \cdot Kfactor - 0.211 \cdot shape\ ratio$$

Where W is the fish weight in grams, L is the fish length in cm, and T is the thickness over the dorsal fin in cm. The K-factor describes the condition of the fish and is defined by the weight divided by the cubed length. Shape ratio is defined as the length-to-thickness ratio. The beta coefficients are all weighted, meaning that they describe how much they change when the predicted value changes one standard deviation.

Despite of the promising results and the proven possibility of predicting yield, the industry operates on batch level. Therefore, an investigation on the possibilities of predicting yield of a complete batch based on parameters from a minimum amount of individuals, still needs to be explored. Additionally, while the proposed model offers an acceptable error margin (RMSECV of 0.68) in predicting the yield, it is still not fully implementable in a decision tool. For the model to become truly useful it needs to be imbedded in software that is readily available to the industry at a reasonable cost. Moreover, the parameters embedded in the model encompass the thickness over the dorsal fin – a measurement that is not made today. Based on personal communication with Jesper Hjortshøj (Manager of Strategy and Portfolio for Global Innovation, Marel), together with the insight gained during the work on this PhD project, the value chain for farmed Atlantic salmon does not seem to adapt quickly to external factors influencing the price of the final product. The price is influenced by supply and demand. Yet, a higher price of raw materials may reduce the demand due to changes in the customer segment. Moreover, even though exporters buy salmon at increasing prices it is not always possible for them to get their money back (iLaks, 2016). In this perspective, price is controlled from the bottom of the value chain. The exporters buy from, for example, the filleting companies and this can determine the price of the product based on the total availability of products. A higher price for the final products does not trigger a higher price for the gutted fish, or for that matter the price of the smolt prior to sea transfer. Still, price and quality is of course correlated with high quality products giving rise to higher prices.

In the light of this, any attempt to optimize the processing yield will only benefit the secondary processing. Thus, it is not feasible to demand the farmer to perform a measurement that will not bring him an additional income for the sake of increasing the profit further down the value chain. However, if further research, in which a large number of salmon are included in the model, shows that this particular measurement can aid in the prediction of yield, the processing companies can demand the farmers to supply the measurement. In this scenario, an agreement could be made where the processing company will pay a higher price for the products. For that, a cost analysis including this parameter is required to be able to evaluate the cost of performing the measurement compared to the additional yield increase and thus a higher price.

It is acknowledged that the data set of the current study is small. The results, however, indicate that it might be beneficial to incorporate data analysis into the acquisition phase in order to base purchasing on more than gut feeling and experience. The outlying samples of the study presented in Paper I were analysed separately in order to investigate how they deviate from the remainder of the data. In a larger data set this group of samples might not even be outliers but represent a separate group lying between the main data and the true outliers. The model itself cannot increase yield but can enable the company to perform a more sensible product management in which purchasing can be coupled to requirements of existing orders. The model is not meant to change fish dimensions in order to obtain the desired yield, but rather obtain an estimate of the yield of a particular batch before the processing company orders from the slaughterhouse. This will allow the company to match orders to not-yet-ordered batches. Today the salmon are ordered based on gut feeling and an idea of seasonal changes in demand. However, this practice results in salmon being stored for several days (up to 10 days) before a favourable match between raw material and product can be made. Hence, prediction of yield may assist the processing company in better raw material management and thus serve as a decision support tool in the acquisition phase of the salmon allowing the processing company to define their demands when ordering raw materials from the farms.

6.1.1 Prediction of texture

Prediction of yield is not the only model that would have an immense impact on the data driven resource optimization that is suggested in relation to the present work. In Paper III, the experimental work testing the third hypothesis presented in Figure 2, on the possibility of measuring or predicting texture based on protein profile, is presented. The aim of the study was to investigate the relationship between specific proteins and texture as a step towards developing a non-invasive, fast method of determining texture and by that help the industry optimize production and improve profitability. The hypothesis can be positively confirmed as 16 proteins were found to have a significant influence on the measured peak force and by that allowing peak force to be predicted based on the protein response.

The hypothesis tested in Paper III was further extended as it was speculated that products could be allocated to the product category best suitable for salmon of a certain texture, and by that increase the yield. However, this extension cannot be tested experimentally yet. At best, the extended hypothesis can theoretically be accepted based on the reviewed literature, and experience from the industry, on the relationship between yield and texture. In Figure 7 a schematic illustration of presumed connections between textural category and product type, is presented.

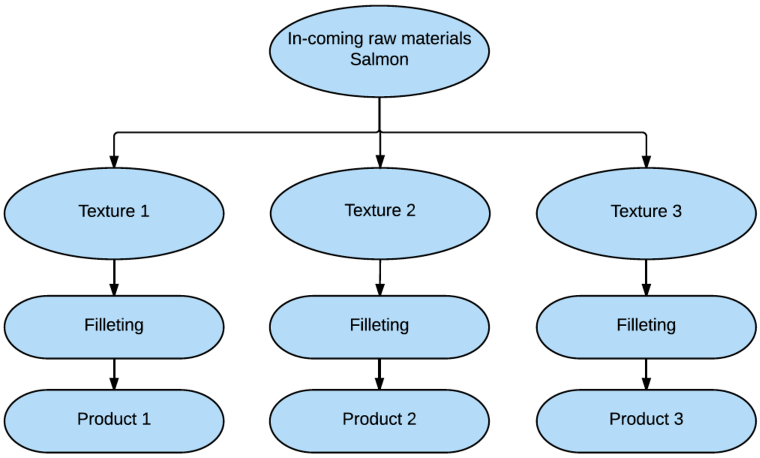

Figure 7 Illustration of the conceptual idea of connecting products with different categories of texture to different product types.

The prerequisite for a meaningful division of products, as presented in Figure 7, is that a developed method for detecting texture prior to processing should be able to handle detection on an individual level. Yet, this has long prospects, as the method would need to be non-invasive and fast with regards to both sampling and results. Some systems for fast identification of proteins have been developed proving that the technology is possible and that a result can be given within an hour (Ibáñez *et al.* 2007). However, for the processing of fish, a highly perishable product, the time from slaughter to market is critical and too slow a system would create a bottleneck in the value chain. A second aspect of dividing products into textural categories is that the production facilities subsequently can handle different products. Hence, both an optimized, fast method for measuring texture based on protein composition, and an optimized handling procedure for incoming raw material at the filleting company, need to be developed.

The work presented in Paper III has encompassed the correlation of a set of proteins to the measured texture i.e. predicting peak force based on protein composition. Thus, the actual development of softness has not been investigated in this study. Based on the correlation loadings plot of X and Y for the prediction model in Figure 8, it is possible to identify a group of proteins, which correlated highly to a high peak force, and a group of proteins that correlated to a low peak force.

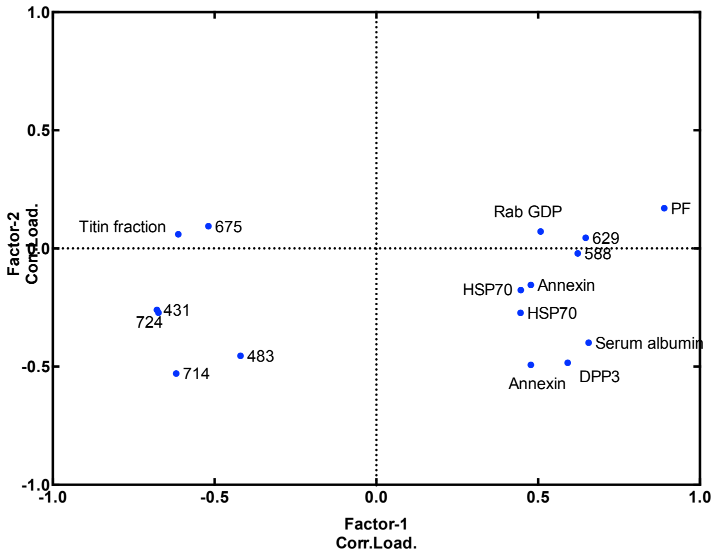

Figure 8 Correlation loadings of X and Y for the prediction model consisting of the 16 proteins significant for the measured peak force (PF). In the cases where the proteins have been identified, their names are provided.

This is the first step in splitting texture into two categories objectively and not by the subjective evaluation of the quality department of the processing facility. Hence, the prediction model can estimate the peak force of a sample, based on the combination of proteins that give rise to a specific texture, and thus provide a prediction of the texture category of a salmon. Work on developing the texture categories based on for example peak force in combination with the protein profile, is still needed in order to successfully obtain reference points for future measurements.

The idea of dividing products into categories could mean that less raw material would have to be discarded. If soft fillets are processed together with firm fillets, they might not remain intact during filleting at the set speed of the conveyor belt, and because of that it will stop the production. If the raw materials were divided, not only into weight class but also texture category, then soft fish could be processed at lower speeds and by that reduce the downtime of the production line. Moreover, raw material for salmon mince would require less care and the manual trimming could be reduced or excluded. Consequently, the thought of handling products based on texture seems feasible but may not happen for yet some time.

6.1.2 Implementing the use of models in the industry

Most of the literature highlighted in relation to the present thesis has been focused on one part of the value chain for farmed salmon, whether that being rearing conditions, feed composition or methods for slaughter, to mention a few. However, there has been encountered no literature that investigates the value chain at several levels with the focus on increasing information transfer, for example by the use of the existing systems implemented for traceability. In the future, if data are gathered and passed on between the links in the value chain, there will still be a need for systems to handle these data and extract the information. This can be obtained by the use of models. Empirical models have been developed for many applications in the food industry. And they are useful. For instance, it has been shown that they can be used to more accurately estimate how long the drying process of bay leaves will take in order to reduce energy costs and optimize economical gain (Gunhan *et al.* 2005). However, one thing is for research institutions to develop said models, another is for the industry to actually implement them. The food industry in for example Denmark can, rather frankly, be described as resistant towards change. It is business as usual and they might not be willing to change operating procedures because the old ones work. Although times are changing and food factories today are closer to the pharmaceutical industry than before, food is still biological matrices that greatly differs in composition in a way that in some cases will influence the actual processing. The skilled employee knows his product range and has a sense for which buttons to press for the desired outcome. However, the skilled worker will not be there forever, and new methods must take over. One way to facilitate the transition is to implement these models in the decision processes. They will assist in making the best choice every time and assure homogeneity in the decisions and by that de-coupling choice and employee. Models are already being incorporated into new machinery in the form of developed software to track the processes. The software is designed to aid the production workers by for example constantly monitoring the yield and to raise an alarm if the yield deviates towards decreasing numbers. Software is used for forecasting, both for crops, as mentioned previously, but also smolt production; how many smolts are needed to meet the demand expected by the forecasting models.

According to Shmueli (2010), forecasting and prediction deviates based on their input. Forecasting is temporal and uses the past to deliver a set of conceivable outcomes in the future, whereas prediction models provide one estimate of a specific outcome at a given time point. Based on this definition the models presented in both Paper I and Paper III predicts the outcome, whether it being yield or texture, based on a set of variables. The proposed models can provide the secondary processing industry with additional decision parameters or even a complete decision tool when purchasing salmon from the slaughterhouses. But the actual implementation of the models is still required.

6.2 TEXTURE AND YIELD – DOES IT CHANGE WITH FAT AND PROTEIN CONTENT?

Yield and texture are intercorrelated parameters in Atlantic salmon from aquaculture that are also affected by other factors, like fat and protein content. The literature study presented in Chapter 4 condenses some of the available literature regarding variations of fat and protein content, and was a part of the second work package. The aim was to investigate raw material variation and to what extent this could influence texture and yield.

In general, the fat contents presented in this review ranged from 8.0% to 19.9%. The large variation seen in the data presented in Table 2 does not seem to be contributed to the feed alone, but needs to be considered together with feed ratio and intensity. The referred data strongly suggests seasonal variation when taking into account the weight of the sampled fish. Nevertheless, the proposed variation could also be ascribed to other factors than season as for example age at sea transfer might affect the growth rate. However, Espe *et al.* (2003) concluded that the variation in fat content might just as well be a result of season and not smolt type. Protein content was found to range between 17 and 23%, however the studies presented in Table 3 lead to the conclusion that protein content is not subject to seasonal variation. Moreover, it was found that the feed composition might have some influence on the final protein but to what extent is still not clear. Additional research is still needed to clarify how different protein sources and amounts affect the protein composition of the fish meat, as protein composition strongly affects texture.

Nonetheless, the filleting industry experiences that higher fat content in the salmon makes processing more difficult. Sorting the salmon according to fat content would ease the prediction of processability, as fat content and yield is linked (Mørkøre *et al.* 2001), making it possible to obtain the highest possible yield from different groups. For example, in the production of fillets or portions the producer will request a lower fat content in order to optimize the yield. Additionally, in a study by Vieira *et al.* (2007) it was shown that trait selection for low fat in breeding programmes could also affect the texture of the flesh. The study showed that flesh lipid was positively correlated with fork length (the length measured from the most anterior part of the head to the deepest point of the notch in the tail fin), and that selection for low muscle fat could result in individuals with high fibre numbers. As high fibre numbers are correlated with high fibre density, and thus increased firmness of the muscle (Johnston *et al.* 2000), selection for low fat could lead to increased firmness. Based on the data foundation large variations of fat content were seen. Hence, the idea of sorting the salmon according to fat content in the future may be one way to increase the outcome from different production types, with fillets/portions (both fresh and frozen) dominating the market (Sjømatrådet Innsikt, 2015).

In Chapter 4 it was established that feed composition might affect the final fat content, while evidence for its influence on protein content was not as clear. Proteins within the muscle can be divided into sarcoplasmic, myofibrillar (myosin, action and actomyosin), or as connective tissue (primarily collagen) (Lee *et al.* 2010). In rainbow trout four sarcoplasmic proteins, as well as two structural proteins (actin and myosin), were correlated to firmness (Godiksen *et al.* 2009). Increased tenderness has also been observed to correlate to degradation of myofibrillar proteins (Skaara & Regenstein, 1990). Espe *et al.* (2004) posed the theory that collagen content and gaping was related, while Moreno *et al.* (2012) found no correlation between soluble collagen and texture in Atlantic salmon. Additionally, collagen plays an important role in the incidence of gaping, especially with respect to the ratio between the connective tissues and muscle. Montero *et al.* (2003) observed that farmed Atlantic salmon had a firmer texture compared to wild caught salmon, which could be explained by higher collagen content and lower incidences of protein aggregation in the farmed fish due to the higher fat content. Bahuaud *et al.* (2010) showed that the breakdown of proteins in the connective tissue and muscle fibres would soften the fillet after slaughter. Firmer fillets had a tendency for higher activity of protease inhibitors, thus reducing the protein degradation. No studies concerning seasonal variations in yield during processing of raw Atlantic salmon were found, but as yield is tightly correlated to the ease of processing (Michie, 2001) the seasonality of gaping may also cause periods of lower yield when incidences of gaping are high. Accordingly, it may be reasonable to assume some level of seasonality of the yield as well.

The yield from processing of Atlantic salmon relies heavily, both indirectly and directly, on the composition of the fish muscle. Further studies elucidating how variations in fat and protein content may affect process yield, texture and gaping, should include effects of rearing site (geographical aspect), company (in relation to production practices and diet), and season in order to fully comprehend how these parameters are connected. Such knowledge can aid the primary and secondary processing industry with selecting the raw material from the preferred combination of geography, company and season that results in the desired fat and protein content, and by that utilize the Atlantic salmon in the most optimal manner.

6.3 TEMPERATURE DEPENDENCE OF QUALITY PARAMETERS

The literature review presented in Chapter 4 tries to establish to what extent season may influence different quality parameters like fat and protein, and how these parameters may influence yield and texture. Texture, and for that matter gaping, were at the beginning of this PhD project regarded as two of the main parameters responsible for quality downgrading of Atlantic salmon products (Michie, 2001) and thus received the most attention in the duration of the project. Even though several studies have reported a variation in fat content with season, it has for long been generally accepted that protein content is stable throughout the year with a repeating citing of the study by Shearer *et al.* (1994). However, a thorough discussion on how even small outswings in fat and protein content can influence yield, texture and gaping, was found to be missing. Therefore, the aim with the investigation of the literature was to move one step further to actively be using these parameters to increase the quality and value of salmon products, and in a sustainable manner. This was done by reviewing the current literature for experiments analysing fat and protein content in Atlantic salmon, determine whether the parameters were influenced by other events than season for example feed composition, and by trying to correlate changes in for example protein content to changes in yield and texture. Despite no direct recommendations for the industry can be put forward, it became clear that further studies investigating the variation in quality parameters of the raw material should include the effect of region, and in relation to this, temperature and company.

The study presented in Paper III, examining the interactions between several parameters including fat and protein content in connection with region, company and sea temperature, was made based on the knowledge gap identified as part of the literature study in Chapter 4. A significant negative correlation between sea temperature at the rearing region and protein content was observed ($p<0.0001$, $df=154$). These results strongly oppose the findings in the study by Shearer *et al.* (1994). The higher temperatures were typically observed during autumn hence it was this time of year where protein levels were low. One feasible explanation could be that the salmon were sexually maturing, however, there might be other explanations. In a study by Fader *et al.* (1994), seasonal variation in heat shock proteins was seen. Heat shock proteins have been presented in relation to the study behind Paper III, and are proteins related to the stress response to for example higher temperatures. The authors suggest that a constant level of these proteins is present in the fish in order to be able to adapt to fluctuating temperatures. In the study, lower levels were observed in both autumn and winter (Fader *et al.* 1994), which could be explained by a lower protein metabolism during winter. It is thus hypothesized that this change in protein metabolism also could give rise to the lower protein content observed in the study presented in Paper II.

The evidence to support the second hypothesis of the current thesis stating that a "*Variation in quality parameters like fat and protein may influence yield negatively due to processing difficulties*", is not indisputable. The literature suggests that especially a variation in fat content might influence the yield negatively (Mørkøre *et al.* 2001). Moreover, based on the included literature in Chapter 4, it was concluded that variation in protein content was neither a result of seasonal variation, nor as a response to feed composition. While this is valid for previous studies, the findings of this PhD project strongly suggest that protein content is influenced by sea temperature as emphasised in Paper II. In spite of the evidence that fat and protein content vary in response to several external factors including feeding intensity and sea temperature in the case of fat content, and sea temperature in the case of protein content, the final link to the effect of these variations on the yield during processing, needs to be established. From information provided by Skagerak Salmon A/S it is known that too high a fat content is not a desired parameter due to processing difficulties. Yet, no maximum level of fat content has been defined for the production. The effect of varying levels of protein content on processing can only be speculated, as no evidence in the literature connecting protein content and possible processing difficulties has been found. Data on yield from 35 salmon collected during the process analysis presented in Paper I, and the fat and protein content of these samples, are presented in Figure 9.

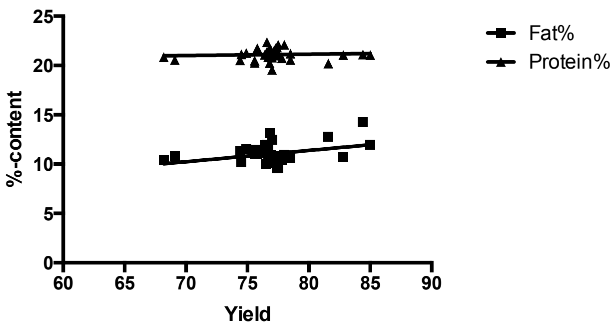

Figure 9 Yield after filleting in relation to the fat and protein content, respectively, for the sampled salmon (n=35).

Figure 9 shows the protein and fat content given in percentages in relation to the calculated yield after filleting. The slope for protein content does not significantly differ from zero and thus protein content and yield does not seem to correlate. Contrary to this, fat content and yield show some correlation, as the slope does not significantly differ from zero ($p=0.0344$), albeit the correlation is vague. Yet, the figure shows that a higher fat content is connected to higher yield. This might be due to the overall shape and size of the salmon and not only the fat content. Moreover, none of the analysed salmon samples had fat contents higher than 15%, which lies within what is regarded as an acceptable fat content (Gjerde *et al.* 2007), and thus it is not possible to conclude anything with regards to a large variation in fat content and its effect on the subsequent yield. Conclusively, the hypothesis is only partially accepted and further research is needed to establish to what degree protein content may influence processing and define a maximum level of fat content.

6.4 PROCESS ANALYSIS AND ITS USE

The initial work of the present project was aimed at optimizing resource parameters (see Figure 2) via process analysis by taking advantage of the unique possibility of access to a production line. Process analysis can be described as a structured approach in which yield is sought maximized by identifying undesirable mass loss or areas in the production that allow for adjustment prior to processing (Somsen *et al.* 2004). Although different studies have investigated the production of different products using some form of process analysis, it has not been possible to uncover any studies that considered the production of fillets from farmed salmon. Somsen *et al.* (2004) identified areas in a poultry processing company where optimization in yield could take place by calculating the yield efficiency of the transformation process. Saltini *et al.* (2013) reviewed the possibilities on optimizing the chocolate production by including farming practices into the operational decision-making process with clear benefits for the industry. These two examples combine some of the ideas that are proposed during the present thesis on investigating how yield can be increased by looking at each unit operation and the process line as a whole, and how increasing the information flow from farmer to further processing can benefit the whole industry.

Paper I examines the use of process analysis to optimize yield during the secondary processing of salmon. During the work the weight of 60 salmon were traced through the production steps in order to map the yield but maybe more importantly, the loss. The weight analysis at the different unit operations along the process line revealed that a weight difference between the right and left fillet was introduced. Several possible causes were investigated, including the heading procedure. On the images of the heads it was observed that all heads had more meat/muscle on the left side in comparison to the right side. This could imply that the observed weight difference between the fillets was introduced during the heading. If there were meat on the left side of the head after heading, but not on the right side, the left side fillet would consequently weigh less than the right side fillet. In 78% of the cases the right side fillet were heavier than the left side fillet. A paired t-test of the weight of the left and right fillets after filleting showed there was a significant difference between the two weights ($p<0.0001$, $df=59$). The mean of differences was just around 23 g with a 95% confidence interval from 14.9 g to 31.3 g. Even though the gain is only 0.5% for each fish assuming an average weight of 4.5 kg, it adds up in a 12.000 tonnes production like the one at Skagerak Salmon A/S. Consequently, a total of 61 tonnes of additional meat can be gained per year with an economical value of around 2 million Danish kroner. However, in order to recover the meat from the heads, the equipment will need a redesign as a minimum. In the study presented in Paper I, it was observed that the positioning of the salmon during heading might explain the mass loss. When the salmon is placed on their left side in the heading machine, the right side of the fish is stretched, whereas the left side becomes more compressed. This difference in positioning may cause a lopsided cut and meat is then lost.

The observations made during the process analysis, and subsequent data analysis confirms the hypothesis that by process analysis it is possible to identify certain raw material parameters that, if taken into account, can lead to a yield increase (hypothesis 1 presented in Figure 2). In the example presented in Paper I, we speculate that the dimensions of the salmon influence how the head is cut. In this context, it must be stressed that process analysis is a unique analysis, which is not generic and thus only valid for the process line that has been analysed. The focus points might be the same but the analysis will need to be repeated.

It is suggested in Paper I that the heading equipment might be re-designed. The economical incitement for the equipment producer is present considering an economical gain of around 2 million Danish kroner at a 12000 tonnes production. However, if the equipment is not updated/re-designed now, it should be considered at least when the planning for an update is on its way.

The findings from the study presented in Paper I further supports the overall hypothesis presented earlier in the discussion. In the traditional value chain the equipment producer might not gain the increased knowledge gathered during the production of fillets, as it would simply not be passed on. However, in a re-structured value chain, with a combined knowledge base, this information will be shared, and optimization is made possible on several levels in the value chain.

6.5 TEXTURE: THE RELEVANCE OF NEW KNOWLEDGE

The work presented in Paper III includes, besides the before-mentioned prediction model, an identification of a total of eight proteins, which were found to correlate with texture. Although the work did not reveal the actual cause of textural changes, it has contributed to the understanding of how soft texture might develop. From the correlation loadings for X and Y of the prediction model it was possible to identify two groupings of the proteins: one associated with high peak force and one associated with low peak force. However, it is the combined response in the protein profile that says something with respect to the overall perception of texture.

The plasma protein serum albumin was correlated with texture, a correlation that could be attributed to the loss of tissue strength due to the presence of blood, as found by Jacobsen *et al.* (2015). Dipeptidyl peptidase 3 (DPP3) could be relevant for texture in salmon muscle, as a study by Sentandreu & Toldrá (2007) denoted the relevance of DPP family for the proteolytic activity in porcine muscle and the resulting texture of the muscle. However, to the best of my knowledge, DPP3 has not been mentioned previously in relation to the texture of Atlantic salmon. The relevance of this enzyme for the texture of fish is supported by the findings from the work behind Paper IV along with previous observations in other animals (Sentandreu & Toldrá, 2007) and should thus be investigated further.

Unsurprisingly, several stress proteins were identified amongst the proteins significant for the prediction model. The heat shock proteins are excreted as a response to cellular stress including anaesthesia, handling and crowding (Roberts *et al.* 2010) and would thus be expected as the salmon were all farmed. However, no direct evidence for the influence of heat shock proteins on texture has been found for fish, although the connection has been established for beef (Guillemin *et al.* 2012; Morzel *et al.* 2008). In Chapter 3 the connection between stress and early onset of pre-rigor was reviewed in relation to texture. These connections might provide a linkage between heat shock proteins and texture, and these proteins can then be regarded as marker proteins for the stress situation of the salmon. Of course, much more research is needed, but if heat shock proteins can truly be regarded as a biological marker for the processing history around the time of harvest and slaughter of farmed salmon, the possibility to include this as a decision variable might be present. Such a variable would provide the value chain with an additional incitement for gentle harvest and slaughter if the life history could be documented and proven.

The protein family known as annexins were also among the identified proteins. In Paper III, the connection with actin is highlighted for the significance of these proteins for the prediction model. The exact explanation for the reason why the annexins were significant for the model remains to be solved. However, Skugor *et al.* (2011) observed that the annexins were downregulated when salmon were fed a soybean-based diet compared to the control diet, whereas Kortner *et al.* (2012) found the annexins to be upregulated when the salmon were fed a diet based on pea protein concentrate.

Lastly, a small protein fraction was revealed as an unidentified protein from trout. The protein fraction proved to have an 82% convergence with several domain entries of the protein titin (also known as connectin), which is responsible for the passive elasticity of the muscle (Labeit & Kolmerer, 1995). Titin can be regarded as a structural protein, and is, like the annexins, also influenced by diet (Tacchi *et al.* 2012). In this study, the authors found that Atlantic salmon fed a plant-based diet proved to have a higher expression of titin together with other structural proteins. In 1996, Kubota *et al.* observed that connectin was degraded during chilled storage of fish muscle, however, they could not link this degradation to the softening of the muscle. However, in another study it was observed that the amount of connectin decreased with time and that this coincided with an observed loss in elasticity of the tested muscle (Takahashi & Saito, 1979)

The studies by Kortner *et al.* (2012), Skugor *et al.* (2011) and Tacchi *et al.* (2012) illustrates the need for a better understanding of how diet may influence the state and health of Atlantic salmon and other farmed animals. It is simply not enough to develop feed that provides good growth rate; the welfare of the fish needs to be taken into account as well.

Seven of the eight identified proteins i.e. all proteins except the fraction possibly titin, were associated with high values of peak force according to the correlation loadings of X and Y as seen in Figure 8. This grouping challenges some of the proposed theories in this thesis. For example, the loss of tissue strength due to the presence of blood and thus serum albumin can be disputed, just as the possible involvement of DPP3 in the anorexic behaviour of fish might not be the most plausible explanation. Contrary to this, neither the identified heat shock protein nor the annexins have been linked to texture of salmon previously, yet the correlation plot clearly shows that these proteins are significant for a high value of peak force. The presence of the protein fraction of titin in the group of proteins correlated to low peak force (Figure 8) is not surprising as it would mean that the large molecule titin has been broken to smaller bits and thus altering the structural integrity of the muscle fibre.

All in all, it is believed that the findings presented in Paper III, and summarised here, are interesting for a more in-depth understanding of the development of soft texture.

7 CONCLUSION

The overall hypothesis that has been guiding the work of the current PhD project was that:

> Increased information transfer between and over the links in the value chain can strengthen the chain as a unity with increased outcome on several levels, including yield, profit and quality, as a result.

Although not directly investigated the hypothesis seems feasible when taking into account the results presented in the present PhD project. For example, it was found that information in the form of raw material characteristics could be used to predict the yield after filleting. This kind of data can be recorded already during slaughter, or even prior to slaughter, and thus be used to obtain an estimate of the yield even before the salmon has entered the processing facility. Hence, by providing the filleting company with these variables, slaughter yield for a certain batch can be estimated thus enabling better planning of the production by ordering (and assigning) the right batch to the right product category. However, the hypothesis will only be valid if 1) the data actually follows the fish or in this case come before the placement of an order, and 2) the slaughterhouse and secondary processing site are physically separated.

In the thesis objectives presented in Chapter 1, three research hypotheses, which are repeated in Figure 10, were presented.

Hypothesis 1
Using process analysis raw material parameters can be taken into account in the planning of the processes to subsequently enable a yield increase.

Hypothesis 2
Variation in quality parameters like fat and protein may influence yield negatively due to processing difficulties.

Hypothesis 3
Texture can be measured as a response in protein expression.

Figure 10 Extract of research hypotheses from Figure 1.

The first hypothesis is partly accepted. The results from the current project presented in Paper I showed that raw material characteristics could be used to predict the yield.

However, in order to enable a yield increase the new knowledge regarding the incoming raw materials will have to be used actively to direct raw materials to the best suited products. This part of the hypothesis has not been tested. Either way, the economical gain will only be at the processing step, and there will be no feedback to the upper parts of the value chain. This means that, in the existing value chain, there will be no economical incitement at farm level to increase their cost by performing an additional measurement, and this disconfirms the hypothesis. Yet, passing on the information gathered, at for example slaughter level, will enable the processors, based on the prediction of yield, to optimize order management, matching orders with customer requests, storage time, and time from harvest to consumer, all of which may increase the price of the products and reduce losses.

The second hypothesis was investigated through the literature and, based on this, it can only be partially accepted. Evidence suggests that the fat content influence processing yield while no studies have been found establishing this connection between protein content and processing yield. Additionally, only a vague correlation between fat content and yield, and none for protein content, was established based on the data from the process analysis.

The third hypothesis is accepted. The results showed that it is possible to measure the peak force via the protein response of 16 proteins. In the extended version of the hypothesis it was speculated that products could be allocated to the product category best suited for salmon of a certain texture, and by that increase the yield. However, there is no possibility of testing this particular hypothesis in the existing value chain because the method to detect texture prior to processing is not yet developed. Nevertheless, based on the literature review in combination with the results from the current project, it can be theorized that an allocation of raw material based on texture, can indeed increase yield but not in the foreseeable future.

The major results from the work conducted during the current PhD project are:

- A model enabling prediction of yield after filleting based on simple measurable parameters performed pre-harvest.
- A model enabling prediction of peak force based on the protein profile of 16 proteins.
- By analysis of the headed salmon it was observed that 78% of the salmon had a larger right side fillet compared to the left side. This difference amounted to 23 g, or 61 tonnes of additional meat a year with a value of 2 million Danish kroner. The heading procedure was identified as the one responsible for the weight difference of the fillets with a potential for increasing the recovery of high value meat i.e. fillet.

- A significant negative correlation between sea temperature at the rearing region and protein content was observed. To the best of my knowledge, no study has reported this previously, and this observation thus segregates from the commonly accepted statement that protein content is a stable parameter in farmed salmon muscle.
- A significant positive correlation between sea temperature at the rearing region and fat content was also observed. This result further supports the already accepted fact that salmon increase their feed intake as temperature rises and thus they accumulate more fat.
- Identification of 8 out of 16 proteins with significance to the measured peak force. Serum albumin, dipeptidyl peptidase 3, heat shock protein 70, annexins, and a protein fragment believed to be titin, were identified. It is contemplated that the identification of these proteins and their significance for the measured texture, will contribute to the further understanding of texture.

7.1 OUTLOOK TOWARDS FURTHER OPTIMIZATION

Some further studies are recommended based on the results of the present thesis:

- Proteome analysis of the frozen samples from the seasonal experiment. This will allow for identification of even more proteins related to texture, and add to the understanding of the factors influencing texture.
- Repetition of the seasonal experiment. Additional knowledge regarding time of sea transfer, harvest, and slaughter together with time from slaughter to processing is important for understanding inter-correlated parameters influencing raw material characteristics. Moreover, knowledge regarding feed composition is also desired.
- Coupling of protein and fat content to the observed yield after processing in order to establish the effect of these parameters on the final yield.
- New categorisation of textural faults. Based on observations made during the experimental work of the present PhD project, it was discovered that three types of textural faults are seen, contrary to the two types (soft texture and gaping) most often reported.

One of the most easily exploitable outcomes of the current project has been the identification of additional meat on the left side of the salmon head. This is knowledge that directly benefits the industry, as there is now an economical incitement in optimizing the production equipment. If the equipment is optimized the benefit of increased outcome would also affect other processing companies. However, analysis of the production lines at other processing companies needs to be completed in order to adapt the machinery to the line in question.

Another aspect of optimizing product equipment involves gaping. Gaping can occur both as the result of intrinsic changes in the composition of the muscle but also as a result of the processing, an extrinsic factor. The result of the two types of gaping is the same: slits along the muscle that, in the worst case, will cause a complete rejection of the fillet with a resulting loss in profit. However, in order to optimize the design of the production equipment the differences between these two types must be investigated. Therefore, future research should encompass an investigation of these two types of gaping and include whether they arise due to different factors. In practice this may prove difficult to analyse, as it requires the whole fish to be filleted to evaluate the extent of gaping. However, if such a method can be found it will be interesting to estimate whether extrinsic gaping can occur if no intrinsic gaping is present. This will provide the producers of process equipment for the industry with valuable information on how the equipment influences the final product. In order to get to this point the underlying causes of intrinsic gaping must be fully understood. One approach could be to investigate the differences between fish species where gaping is detected after filleting and compare them with species where no gaping is detected.

Although the filleting industry needs increased profit in order to maintain jobs in Denmark, it is not desirable to push the prices up due to the resulting change in customer segment. Raw material exploitation may therefore be the only way to increase the profit without actually increasing product price. Another step could be reductions in operational costs but this only constitutes 30% of the total cost of producing farmed salmon.

7.1.1 New categorisation of textural faults

In the previous section further research on textural categorisation was recommended. This recommendation has its grounds on observations made during the experimental part of the project. It seems relevant to include it in the present thesis, albeit not in the form of results because, due to time management, it was not achievable to perform any further experiments to investigate the phenomenon further.

During the experimental work the most prominent differences I have experienced when handling the salmon are textural, and it is relatively easy to distinguish a firm fillet from a soft and mushy one. However, what at first sight seems to be a firm fillet can be revealed as a soft one when cutting into the meat. At first, the fillets appear to be firm on the surface and during the general handling, but once the knife slices through the muscle, the tissue falls apart and behave more like a gel. After thorough discussion with some industry contacts it was found relevant to divide textural faults into at least three categories:

- Gaping
- Soft
- Gel-like behaviour

To the best of my knowledge no studies have clearly categorised textural faults in this manner. Gaping and soft texture are already widely known and highly studied textural issues, and are two characteristics, which have been covered both in the literature review of the present PhD thesis and in the papers made in connection with the project. The third category of textural faults has not been covered in this PhD project, primarily because the inkling arose late in the project and is thus not included in any of the papers or in the literature review of the present thesis. However, seen in connection with the work made during the PhD project it is an observation that has to be accounted for.

Fish myofibrillar proteins have strong gelling abilities when subjected to thermal treatment (Lan *et al.* 1995). Most of what has been uncovered in the literature on this topic has been investigated in minced muscle (Lefevre *et al.* 2007; Pérez-Mateos *et al.* 2004; Stone & Stanley, 1992) and is typically connected to the production of surimi products. However, it has not been possible to identify any studies, which have investigated gelling of the whole fish muscle. A plausible hypothesis could involve a temperature collapse at some point in the value chain, which could have initiated the setting of gel. There are several incidences from harvest to final product where temperature abuse can occur, and in a study, as much as a 12 °C difference was found inside a truck (Moureh & Flick, 2004). Hence it cannot be rejected that temperature abuse could cause the observed gel setting. Another theory could include transglutaminase (TGase), which has been shown to induce setting of actomyosin (Ni *et al.* 1999), albeit salmon muscle shows only low activity of this enzyme making it less prone to setting of the gel, at least in surimi based products (Wan *et al.* 1995). Nonetheless, these theories are only speculations based on an extract of the available literature, and thus no conclusions or partial solutions can be provided.

7.2 CLOSING REMARK

In the future, the aquaculture industry needs to adapt to the constantly changing world. Successful adaption requires that information start flowing between the parties in the value chain. The data are there, we just need to start looking at it. Once we look, it is in our human nature to react to them. Once we react, we are in motion and things will start changing. However, the food industry is a rigid structure and a closed culture. Care has to be taken when suggesting changes in the operation for them not to be taken as a personal offence to the line operator.

Theory might be good but food is real. Food is biological complex matrices, which does not always act as expected. Therefore, it is of the uttermost importance to understand and keep gathering information regarding the raw materials, the processing, and the consumer requests for the three entities to join together. It is not enough to speed up the production line if the raw material cannot handle the additional force exerted on it, and the resulting product is unacceptable to the consumer.

The diversity of the food industry and its consumers needs to be exploited. Different combinations of raw materials and consumers need to be matched to fully utilize the raw material in question. This does not only account for salmon. The industry needs to think big, do big, and gather big for this to happen, and to join forces in the aim of sustainably optimizing the food industry.

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9 APPENDIX

The appendix includes the three scientific research papers submitted in relation to the current PhD project.

I. In Paper I, process analysis of the production line at Skagerak Salmon A/S is presented. With the process line at our disposal, each individual fish could be traced through each processing step and weighed in order to identify how much loss were generated during the production of fillets from whole, gutted salmon.

II. In Paper II the focus was to investigate the raw material variation based on the knowledge gap identified during the literature review made sidelong the research based activities. 156 fillets gathered over 11 months from March 2014 to November 2015 formed the sampling foundation.

III. Paper III aimed at investigating correlations between protein profile and measured peak force of each sample of salmon. Moreover, mass spectrometry was used to identify the proteins significant for the textural response.

9.1 PAPER I

Ørnholt-Johansson, G., Gudjónsdóttir, M., Nielsen, M.E., Skytte, J.L. & Frosch, S. (2016). Analysis of the production of salmon fillet – prediction of production yield.

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Analysis of the production of salmon fillet – prediction of production yield

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Abstract

The aim was to investigate the influence of raw material variation in Atlantic salmon from aquaculture on filleting yield, and to develop a decision tool for choosing the appropriate raw material for optimized yield. This was achieved by tracking salmon on an individual level (n=60) through a primary production site. The majority of the salmon exhibited a heavier right fillet compared to the left fillet after filleting. No explicit explanation was found for this observation although the heading procedure was shown to have a large impact. A Partial Least Square model was built to predict the yield after filleting. The model was based on six pre-processing variables and allowed an acceptable prediction of the filleting yield with a root mean square error cross validation of 0.68. The presented model can estimate the slaughter yield for a certain batch before ordering from the slaughterhouse. This may facilitate optimal planning of the production of salmon fillets by ordering and assigning the right batch to the right product category to obtain an optimal yield and quality.

Keywords: Production analysis; Prediction; Atlantic salmon; Yield; Multivariate data analysis; PLS

1. Introduction

Due to the growing population in the World, an increase in food demand of around 70% by 2050 is foreseen (Searchinger *et al*. 2013). This provides the food industry with a strong incitement to increase product yield in a cost-effective manner (Somsen *et al*. 2004). Food products are highly complex biological matrices with a combination of chemical and physical factors, which all together define the product characteristics (Rahman, 2005). The inherent variation in these factors, such as fat, protein and size, results in a natural raw material variation that influences the processing of the product. Moreover, the most valuable part of the salmon is the fillet hence increasing the overall exploitation of the salmon meat with focus on optimizing the yield of the fillets is desirable (Powell *et al*. 2008).

A structured approach to increase production yield may identify undesirable mass loss or areas in the production that allow for adjustment prior to processing (Somsen *et al.* 2004). Somsen *et al.* (2004) implemented a production yield analysis (PYA) method to identify areas in a poultry processing company where optimization in yield could take place by calculating the yield efficiency of the transformation process. Ineffective operating machinery and fine-tuning of machinery were just two of the actions that were identified. In contrast to PYA, which is focused on process steps and where they can be improved, process analytical technology (PAT) is aimed at monitoring the product throughout the production. To ensure the desired quality of the final product, PAT has long been used in the pharmaceutical industry and the methods have also been adapted to the food industry (Chew & Sharratt, 2010; Pomerantsev & Rodionova, 2012; van den Berg *et al*. 2013). PAT focuses on control using real-time monitoring that allows for modifications during production in case the indicators of the desired quality do not fulfil specified requirements (van den Berg *et al*. 2013). Instead of only applying post-production quality testing, it is beneficial to investigate the raw material properties and process variables during the production. This allows for adaption of the processing parameters in real time, which ensures the selected quality traits for the final product (Pomerantsev & Rodionova, 2012). The two methods clearly have specific advantages when applied separately. Yet, a combination of them will provide the food producer with a valuable tool to first analyse the production, considering both process and biological variation of the raw material, and secondly, couple these findings to identify the processability of the product.

The processing of Atlantic salmon (*Salmo salar*) from aquaculture into fillets was used as case in this study. Aquaculture production of Atlantic salmon consists of a rearing period (24 to 36 months), including harvesting, slaughtering and gutting, all handling and transportation, before entering the primary processing. The primary processing encompasses the production of fillets or portions, either fresh or frozen (Melberg & Davidrajuh, 2009). This study comprises an analysis of the production using PYA in order to identify areas where PAT can be applied in a future production situation. The hypothesis is that, by combining the ideas behind PYA and PAT, the characteristics of the incoming raw materials can be considered when planning, and also monitoring, the processes to subsequently enable a yield increase.

The aim of this study was therefore to investigate if comprehensive collection and analysis of data from processing companies could be utilized to increase the production yield in the salmon industry. To secure comprehensive data and traceability, each salmon entering the processing plant were followed on an individual level through the process. Thus, possible influences of biological variation in the raw material on the subsequent production yield could be revealed.

2. Material and methods

2.1 Sampling

Atlantic salmon (*Salmo salar*) (n=60) from three different slaughterhouses (1, 2 and 3) in Norway was used for the experiment. The salmon were all in the weight class from 4-5 kg and classified as SUPERIOR[a] with respect to their quality. In January 2015, the salmon were harvested, iced and transported by truck to the production facilities of the participating company in the northern part of Denmark.

[a] The quality grade SUPERIOR represents salmon with no considerable defects such as damaged skin and significant loss of scales. They must be void of bruises, damaged belly or musculature (Regulation (EU) No 1151/2012).

2.2 Experimental design

All salmon were tagged in the mouth with an individually numbered pit tag. This was done to ensure tracking of the fish during processing and to later distinguish the heads. Images of all salmon were taken to enable objective evaluation of the belly cut. The salmon were held by the gills, hanging straight down, and a RedGreenBlue (RGB) image was taken with a digital camera. The weight (W), length (L) and thickness (T) across the dorsal fin of each fish were recorded. The processing line used for the study was from BAADER Food Processing Machinery (Nordischer Maschinenbau Rud Baader GmbH+Co KG, Lübeck, Germany). The gutted salmon were headed using the U-Cut heading machine for salmon (BAADER 434 S), filleted (P1) on a high speed filleting machine (BAADER 581), auto-trimmed (P2) on a high speed trimming machine (BAADER 988) and finally manually trimmed (P3) by well trained staff at the processing company. The salmon were placed consecutively on the production line for heading. Heads and tails were cut and the heads were collected for weighing and further analysis. The salmon were filleted mechanically and then collected, numbered and weighed after each processing step P1-P3.

2.2 Data acquisition

The heads were packed on ice in polystyrene boxes and transported to the Technical University of Denmark (DTU) in order to investigate the head cut. Each head was weighed on a Kern FCB scale (Kern & Sohn CmbH) with a weighing range of 8 kg and a readability of 0.1 g. The heads were placed upside down in a beaker and a photo was taken with a digital camera in a specially designed white painted box (size 1150 x 760 x 800 mm) with 20 m LED light bands (5000K, 390 Lumens, ClimaCare.dk) placed in a spiral along the sides (longitudinal direction) with approximately 10-15 cm between each winding in order to create a diffuse light. Images of the heads were investigated by a panel of four with respect to the presence of additional meat on either left or right side. Figure 1a presents an example of one of the head cuts where the presence of additional meat on the left side, marked by a circle, was unmistakable. The images of the belly cut were quantitatively analysed and ranked based on how big an arch the cut displayed. The ranking was made as presented in Figure 1b.

Figure 1

Based on the measured values of weight (g), length (cm) and thickness (cm) a range of variables were calculated, and their definitions are presented in Table 1.

Table 1

The groupings of variables were chosen based on their use as normal evaluation criteria, their availability (simple to measure), and because they hypothetically could have an influence on the final yield.

Yield was calculated as the weight of the two fillets divided by the weight of the whole gutted salmon and multiplied by 100%.

2.3 Statistics

Data were statistically analysed using the Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA) software for Mac. A paired t-test was used to test whether there was a significant size difference between the left and right fillets. The significance level was set to $P<0.05$. The influence of the gutted weight, length, thickness, degree of belly cut and K factor on the size difference between the left and right fillet were tested using ANOVA in the open-source software for statistical calculations, R (R Foundation for Statistical Computing, Vienna, Austria).

2.4 Multivariate data analysis

To establish the relationship between the main variables related to physical appearance and percentagewise yield, Partial Least Squares regression analysis (PLS) (Wold, 1975) was used to build a model for the prediction of yield. All models were built with the measured variables as the X matrix and the calculated yield as the Y vector. All data were auto scaled with 1/standard deviation. Outliers were detected and removed based on influence, Hotelling T^2 statistics and Q-residuals. Variables were excluded based on lowest regression coefficients and weighted regression coefficients. The models were calibrated using a full cross-validation, and evaluated based on the calibration root-mean-square error (RMSEC), and the cross-validation root-mean-square error (RMSECV). Principal Component Analysis (PCA) (Hotelling, 1933) was used for explorative data analysis and visualization of correlations between variables. The software Unscrambler X (Camo ASA, Oslo, Norway) was used for the multivariate data analysis.

3. Results and discussion

3.1 Yield

In this study, the weight after each processing step was followed for 60 salmon. This allows for knowledge on how processing influences each single fish and possibly identifying parameters relating the yield to the physical appearance of the salmon such as length, weight and thickness over the dorsal fin, or with calculated variables, such as the shape ratio, W/LT and K factor. Moreover, comparisons of belly cuts can aid in understanding how the slaughtering may affect the subsequent processing steps. Figure 2 presents the mass flow of the production with the calculated yield, the mean total weight, the mean weight of the left and right fillet, and the calculated loss after each processing step.

Figure 2

Figure 2 illustrates the reduction in yield (including standard deviations) after each process step from an average of 76.7%±6.5% after mechanical filleting (P1), to 67.5%±7.2% after auto-trimming (P2), and further down to 51.9%±11.3% after manual trimming (P3). The trimming recipe determines how much is trimmed from the fillet and will therefore influence the resulting weight reduction. In this case study, approximately 50% of the gutted salmon could be sold as fillet. In comparison, Rørå *et al.* (1998) reported the yield of the untrimmed and trimmed fillets with skin to be 77.6% and 67.3%, respectively. Nevertheless, Rørå *et al.* (2001) put the yield of farmed fish species in the range of 40-70%. Hence, taken into consideration that the salmon in this study underwent deep skinning, a final fillet yield of 50% is regarded as consistent to what has been found by other researchers.

The weight loss during filleting was 23.3% on average. This comprises the removal of the skeletal frame as well as the head and tail. The auto-trimming loss accounted for 12.0% while during the manual trimming and deep skinning 23.1% was removed. In total the trimming loss amounts to 32.4%. In comparison, Rørå *et al.* (1998) reported a filleting loss of 22.5% by mechanical filleting, and a trimming loss of 13.2%. However, in their study the fillets were trimmed manually and the skin was not removed, which can explain the differences between the reported trimming losses of the two studies.

3.2 Weight difference of fillets

According to Figure 2 the mean weights and standard deviations of the fillets after P1 were 1710 g (±147.1 g) for the left side and 1733 g (±150.2 g) for the right side. A paired t-test showed that the observed difference was significant with a P value < 0.0001. After P2 the mean weights (and standard deviations) of the left fillet was 1505 g (±124.5 g) and the right fillet 1524 g (±128.3 g) and the paired t-test showed a significant difference with P = 0.0006. After the last trimming and skinning (P3) the mean weights and standard deviations of the left and right fillet were 1176 g (±112.9 g) and 1213 g (±108.5), respectively, with P = 0.0085. The P values increase after each processing step meaning that the fillets become more alike after each trimming. Hence the automatic trimming procedure trim the larger fillet more for the two fillets to become more alike, which in the worst case may result in over-trimming and thus increased loss.

Two data subsets were created for each of the three processing steps (P1-P3) in order to ensure that the weight differences between left and right fillet were significantly different from zero. One set containing the differences where the left fillet was larger than the right fillet, and another set for vice versa. A one-sample t-test was performed for each of the six data subsets, to test null-hypothesis that the means were equal to zero. The results are summarized in Table 2 with standard deviations (SD), number of samples in each group (n) and P values.

Table 2.

From Table 2 it can be seen that for nearly all data subsets the null-hypothesis can be rejected (P<0.05). For one subset (P2, left > right) the null-hypothesis cannot be rejected, which can be explained by the large standard deviation, that arises from a single data point being notably different from the others. This analysis suggests that the inspected fillet weight differences are significantly different from zero.

To ensure that the weight differences between all left and right fillets were not separated by a small margin, all fillets were divided into three groups: One group where the left fillets were larger than the right fillet by a certain margin, one group where the right fillets were larger than the left fillet by a certain margin, and finally a group were the left and right fillet differences were smaller than a certain margin. Two different margins were selected corresponding to the lower and upper bound of a 95% confidence interval calculated for the absolute mean difference between all left and right fillet weights. This was chosen in order to encompass every possible mean difference based on the available data.

Table 3.

The number of samples in each of the three groups for all processing steps (P1-P3) is summarized in Table 3. The table shows a clear tendency of the right fillet being larger than the left. Even when considering the greater margin at the initial processing step, more than a third of the right fillets are larger than the left fillets.

In the present study, yield was calculated as (weight of left fillet + weight of right fillet)/gutted weight*100%, in contrast to other studies where yield has been calculated as (2*fillet weight)/gutted weight*100% (Rørå *et al.* 1998; Skjervold *et al*. 2001). In this study, it was shown that the weights of the two fillets differed significantly, and thus do the calculations here result in a more realistic and precise measure of yield compared to previous studies. Seen in the light of process analysis it is of paramount importance that the foundation for optimization is built on actual amounts in order to set up realistic goals for future production processes.

To identify at which step(s) during processing the weight difference was introduced the weight data were further examined. After P1, the right fillet was generally heavier than the left fillet except in 13 instances where the opposite was seen. After P2, 11 of the 13 incidences after P1, where the left fillet was heavier than the right fillet, was repeated. Additionally, two different salmons displayed a heavier left fillet summing up to a total of 13 incidences where left side fillet > right side fillet. After P3, 14 occurrences of the left fillets being larger than the right fillets were noted whereof nine of them were new, compared to the previous steps. Hence the weight differences after each process step did not necessarily coincide and the difference between the fillets after P2 and P3 seemed to be of less importance. Yet, it was the mechanical filleting that revealed the initial weight difference and the cause of this difference must therefore be a process prior to or during the mechanical filleting.

To trace back and investigate possible causes of the observed difference in weight between the right and left side fillet the belly cut and heading procedures were given a closer look.

Prior to the experiment it was hypothesized that the belly cut from the slaughtering process might influence the yield after filleting as an uneven cut would favour either the left or right side fillet, thus explaining the observed weight difference. Visual inspection of the belly cut in relation to the weight difference did not reveal any correlation. Nevertheless, the result of an ANOVA showed that the belly cut was the only significant variable related to the weight difference between the left and right fillet when performing the ANOVA on weight, length, thickness, degree of belly cut and K factor. This shows that extensive data acquisition and subsequent analysis can reveal correlations that are not caught by the human eye.

The heading procedure was examined by investigating the images of the head cuts. It was observed that all heads had more meat/muscle on their left side compared to the right side. Hence, if this procedure were the only processing step causing the observed weight difference then we would expect that all the salmon would display a heavier right side fillet. More meat on the left side of the head should mean less meat on the left fillet and consequently a heavier right fillet. Although this was generally the case, a comparison of the weights revealed that 22% of the samples still exhibited a heavier left fillet compared to the corresponding right fillet. Consequently, the heading procedure cannot solely be responsible for the observed weight differences.

Factor analysis of how the measured and calculated variables (presented in Table 1) interact and influence the weight difference after each process step was performed. It showed that the weight difference after P2 solely depended on the weight difference after P1, and the weight difference after P3 did not correlate to any of the variables. These findings were expected since P2 and P3 both are influenced by predefined recipes, such as choice of trimming based on customer orders, and human factors during the manual trimming. The weight difference after P1, however, was most likely a result of the raw cut that separates the fillets from the skeletal frame. Consequently, it is only up to this processing step where prediction of yield is truly meaningful.

3.3 Prediction of yield

From the previous analyses presented in this study, indications were found that some parameters measured prior to processing influenced the yield after mechanical filleting. Building a prediction model for the yield after mechanical filleting, based on a combination of specific measurable pre-processing parameters, can provide an estimate of the yield even before the salmon has entered the processing facility. By providing the filleting company with these variables the yield after mechanical filleting for a certain batch can be estimated thus enabling better planning of the production by ordering (and assigning) the right batch to the right product category. This may assist the processing companies in obtaining the highest possible outcome from the incoming raw materials.

Several prediction models were built to predict the percentage yield after mechanical filleting based on the variables measured in this study. Initially, a model was built without excluding any variables and only by removing outliers. A total of 16 outliers were detected and removed (this will be discussed further in section 3.5) and both the RMSEC and RMSECV values of 0.47 and 0.60, respectively, validated the model as being rather good. However, the model comprised all measured and calculated variables thus obscuring the outcome, which should contain variables that can be measured prior to processing in order to be truly applicable in the industry for predictive purposes. Hence the model was used as the basis for building three successive models, which were further analysed. These models are presented in Table 4.

Table 4.

A PLS model (PLS1_1) was built on the seven variables listed in Table 4 remaining after a variable reduction. In total, 15 samples with outlying behaviour were removed from the dataset, which resulted in a RMSEC of 0.40 and a RMSECV of 0.43 for a five-factor model. Even though PLS1_1 showed very good prospect it was chosen to exclude the head weight from the variable selection, since ideally the variables included in the model should all be measurable prior to processing. Omitting the head weight and including all samples in the PLS1_2 model resulted in a total of 14 outliers, a RMSEC of 0.63, and a RMSECV of 0.68 for a two-factor model.

The K factor is already measured at farm level by random sampling to determine the optimal time for harvesting, and again before and after slaughtering to direct products into the optimal product flow. The K factor comprises measurements of weight and length, both of which are used to construct some of the other variables. The thickness over the dorsal fin is the only necessary variable that is currently not registered. Therefore it was interesting to investigate the effect of excluding variables that contain the thickness as it results in a model that can be incorporated based on variables already measured in the production. PLS1_3was built on the complete data set and the K factor, length and weight. Leaving out the stand alone variable length from the model gave the best result and resulted in a total of 12 outliers, a RMSEC of 0.67, and a RMSECV of 0.71 for a two-factor model. Even though PLS1_3 gives a reasonable error of prediction, it is not the best model of the three presented in Table 4, and will thus not be investigated further.

Figure 3 depicts a score plot (a) and a correlation loading plot (b) of Factor-2 versus Factor-1 from the PLS1_2 model. Figure 3a depicts the scores of the samples. The samples are clustered depending on which slaughterhouse (1, 2, or 3) supplied them.

Figure 3

Figure 3b show how the variables (shape ratio, length, W/LT, K factor, thickness and weight) correlate, as highly positive correlated variables have similar weights and will thus appear close together. Together the plots describe certain characteristics of the salmon depending on the supplying slaughterhouse. Salmon from slaughterhouse 1 overall were longer and had a higher shape ratio than samples from slaughterhouse 3. Samples from slaughterhouse 2 were characterised by being heavier in weight, thicker measured over the dorsal fin, and having a higher K factor compared to the two other slaughterhouses. The salmon from slaughterhouse 3 distinguished themselves by having lower values for all variables compared to the two other slaughterhouses. Although, all three groups overlap, the clustering of samples from slaughterhouse 2 and 3, respectively, is well defined. On the other hand, samples from slaughterhouse 1 span the whole plot with samples displaying the largest variation in both weight and W/LT index. This means that the variation in the raw material batch when buying salmon from either slaughterhouse 2 or 3 are more homogeneous and thereby easier for the production to handle while the width in batch variation of salmon from slaughterhouse 1 is bigger.

With PLS1_2 it is possible to predict the yield after filleting from only few measurable variables with a RMSECV of 0.68. The equation for this prediction model is given by the intercept and the beta coefficients together with the respective X loadings. The equation for PLS1_2 can be written as

$$\mathit{Yield(\%)}=52.95+0.293*W+0.114*L+0.241*T+0.216*W/LT+0.257*K\,\mathit{factor}-0.121*\mathit{shape\ ratio}$$

with W being the fish weight in grams, L the fish length in cm, and T the thickness over the dorsal fin in cm. The K-factor and shape ratio are both without units. The beta coefficients are all weighted, meaning that they describe how much they change when the predicted value changes one standard deviation. All beta coefficients (except Length) were significantly different from 0 with P values < 0.0001. Length showed to be just on the limit with P = 0.0731.

By defining a common knowledge base for the salmon industry the processing companies can request that more parameters are measured prior to slaughtering, in this case the thickness. Such requests for particular parameters can be fed a model to determine the predicted yield of individual batches. Such a model can be incorporated as a decision support tool in the acquisition phase of the salmon allowing the processing company to define their demands when ordering raw materials from the farms. If knowledge transfer between the parties in the value chain should be facilitated the economical incitement to perform additional measurements must be present. In relation to the present study, we found that the thickness over the dorsal fin will provide the production companies with valuable information in the decision-making process. Ordering of raw materials that match the consumer requests for a specific trimming will ultimately reduce the loss of otherwise good meat and increase the profit of the filleting company. On the other hand, this additional information must also result in an increased price of raw material for the farm, as it is here the extra work is required. Therefore, further investigations must include the cost of adding an extra measurement at farm level in order to make a detailed prediction of the yield possible.

3.5 Further Analysis of Deviating Samples

We have demonstrated by PLS how the yield of the majority of the data (corresponding to 80%) could be predicted with acceptable accuracy based on the available data. Hence these samples were assumed to be within a normal range with respect to the measured variables. With the aim of defining the processability of salmon the remaining 20% of the samples were further examined. This was achieved by investigating the differences of the 13 deviating samples, shared between the PLS1_2 model and the PCA model, to explore why the yield% of these specific salmons could not be predicted.

No explanation was found with respect to origin of slaughterhouse or weight difference between the left and right fillets. Seven of the 13 deviation-duplicates originated from slaughterhouse 2, four were supplied by slaughterhouse 1, and two had come from slaughterhouse 3. Ten of the 13 samples exhibited a heavier right fillet than left fillet. This is almost the same proportion, 75%, as in the full dataset with 78%.

In order to determine which variables could explain the variance in the deviation dataset, all variables were included in the analysis. Exploring the dataset with respect to all variables showed that fewer variables were needed to explain the variance. The performed PCA on the 13 deviating samples, and after variable reduction, resulted in three distinct PCs, which together contained 100% of the total variance. Figure 4 presents a bi-plot of the results with PC-1 vs. PC-2. The samples are circled to illustrate the clustering of the samples.

Figure 4

The bi-plot in Figure 4 reveals two groups of salmon in the deviation based dataset based on the PCA model. The first group, marked with the left circle, characterised samples with a straight belly cut (rank 0). The second group, marked with the right circle, represents samples that display an angling of the belly cut to the left (rank 1 and 2). Figure 4 illustrates how the samples cluster in relation to the loadings; samples to the right were salmon with higher values of length and W/LT ratio compared to the cluster to the left. The left cluster, however, is dominated by higher values of yield (P1) compared to the sample cluster to the right. Although the difference in weight of the fillets cannot be fully explained by the belly cut, the angling of the cut on the deviating samples seems to be correlated to the yield. The variance among the deviating samples can be explained with fewer variables compared to the variance in the full dataset. However, both the length and the W/LT ratio were negatively correlated to the yield and thus may be two variables that should be investigated further. Knowledge of which factors that relate to the yield may be used in a forward-looking way to optimize production and define new requirements in the industry. Yet, the processing companies alone cannot achieve this. The information flow in the value chain must be adapted to be able to handle requests from the primary processing, or even further down the value chain. Despite the development within traceability systems, the norm today is that no or only little information follows the fish, except what is required by law, and hence will not be passed on to the next step in the value chain (Frosch *et al*. 2008). This makes it difficult to optimize along the value chain, as information is not shared between and over the processing links. Changing the information flow from the traditional linear flow to a circular flow will enable all parties to share knowledge regarding the raw materials. This can facilitate knowledge transfer between the links of the value chain, both upstream and downstream, by directing the information to the part of the value chain that has an influence on the specific share. Hence a question regarding measurements of new parameters should be directed from the processing company to the farm, as it is here the salmon are measured prior to determination of optimal harvest time.

Even if prediction of yield is made possible in the future the economic gain might not be enough to lift the cost of the measurement. Another way to increase the outcome from the production companies is to look at how to remove the additional meat from the heads. In this study we found that all the salmon had more meat on the left side of the head after heading. This may be explained by the positioning of the salmon during heading where the fish is placed on the left side and as a result is resting on the surface when the cut is made. From the observations made in the production the presence of additional meat on the head was always the case. Therefore, it is not believed that resetting the equipment will recover the meat. More likely, it is the design of the machine in which the salmon is placed flat on the left side that is responsible for a crooked head cut with meat left on the head as a consequence. When the salmon is lying flat in the heading machine the right side of the fish is stretched whereas the left side becomes more compressed. This difference in positioning may cause a lopsided cut and meat is lost. Even if the additional meat only amounts to 30-40 grams per fish (~ 1%) it adds up and for a 12000 tonnes production, 73.5 tonnes extra salmon meat can be gained, amounting to 300.000 €/year. Because of this, in addition to understanding how raw material variation influence the yield, further analyses of productions and machinery must be made. In this context it is important to stress that not all processing lines are identical and thus present results may not be applicable to all companies.

4. Conclusions

The production analysis conducted in this study focused on the three main processes: filleting, auto-trimming, and manual trimming. It was found that 78% of the salmon exhibited a weight difference between the fillets favouring the right side. Even though the heading procedure could explain part of the observed weight difference it does not explain it all as the belly cut also seems to influence the observed weight difference. Furthermore, the study revealed six variables; shape ratio, length, W/LT, thickness, weight and K factor, which together enabled an acceptable prediction of the filleting yield with a RMSECV of 0.68. Although the data set was small, and thus did not allow for testing of the predictive ability of the model on new data, the RMSECV show that it is possible to establish a relevant prediction model. The final prediction model was built on data from salmon of 4-5 kg harvested in January. Therefore, it must be investigated if different size groupings, seasonal differences and/or other variables influence the predictability of the yield. The beta coefficients in the model will change according to the size grouping and thus the model might need some adjustments with regards to raw materials from other seasons and/or origin.

Comprehensive data collection and analysis may at first seem a cumbersome method, yet the presented model could be used to give an estimate of the yield of a specific salmon batch before ordering the raw materials from the slaughterhouse. This will give the production company an advantage with respect to maintaining a healthy business. Additionally, the salmon farmer can follow the rearing of the fish more intensively with spot checks in the net pens, and by that find the optimal time of harvest based on the prediction model presented in this study.

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Figure 1

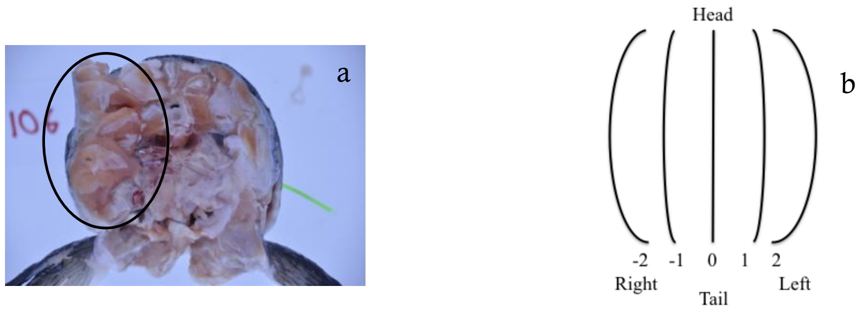

Figure 1 Evaluation of heads and belly cut. Figure 1a depicts the presence of additional meat on the left side of the head marked by a circle. Figure 1b show a schematic drawing of the angle of the belly cut. Cuts angling to the right are denoted -2 and -1, straight cuts are 0 and cuts angling to the left 1 and 2.

Figure 2

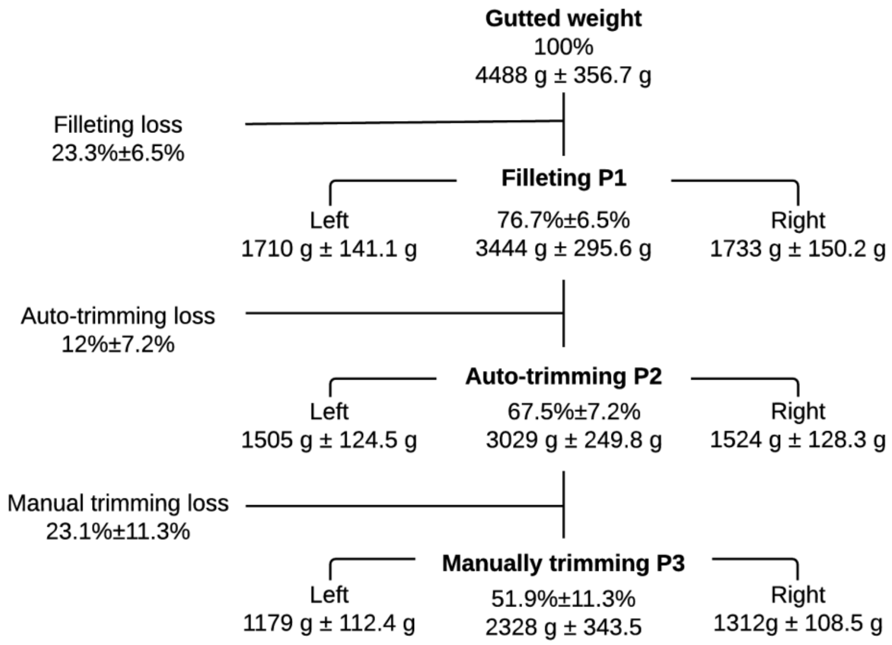

Figure 2 Mass flow of the production of salmon fillets. Presentation of mean weight, percentage yields and loss after each processing step together with the mean weight of the left and right fillets (n=60).

Figure 3

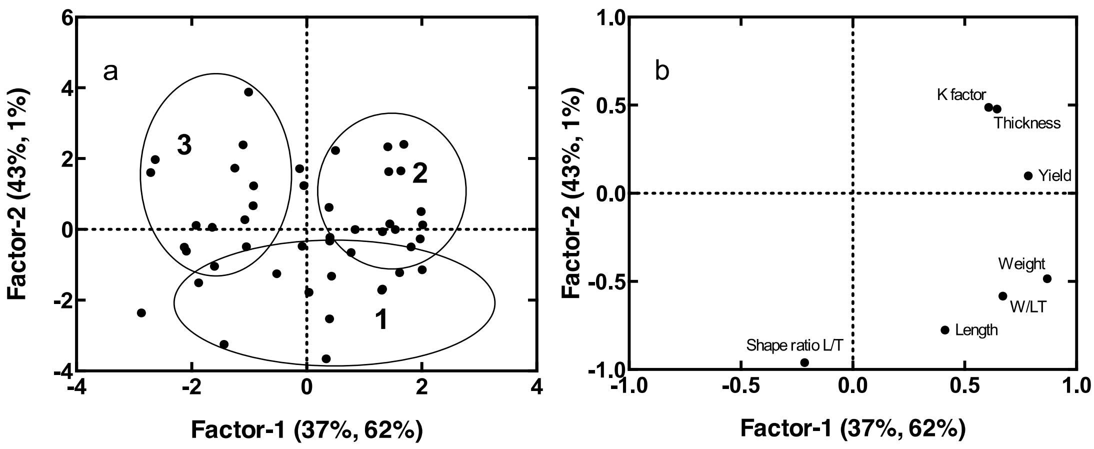

Figure 3 Partial Least Squares (PLS) regression. Plots showing the final model PLS1_2 with six variables related to the physical appearance of the salmon prior to filleting. The scores plot (a) shows the clustering of the samples according to slaughterhouse (1, 2 or 3) highlighted with circles. The correlation loading plot (b) show how the variables correlate. Both plots show the maximum variation of the dataset after outliers have been removed.

Figure 4

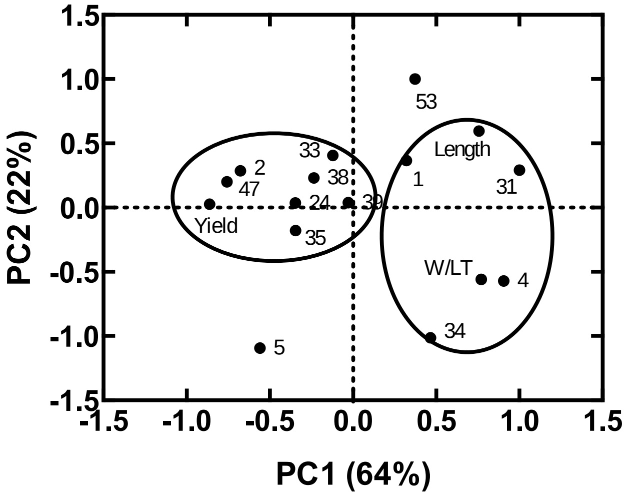

Figure 4 Principal Component Analysis (PCA) of outlier samples. Bi-plot of outlier samples together with the variables (yield, length and W/LT). The plot shows two sample clusters related to the loadings. The two clusters are highlighted with circles, the left being samples with a straight belly cut and the right being samples with an angled belly cut. The plot shows the maximum variation of the dataset. PC-1 accounts for 64% of the variation in the dataset. PC-2 accounts for 22% of the variation.

Figure captions

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Table 1

Table 1 Variable definition. Table presenting the calculated variables together with their definitions with W being the weight, L the length and T the thickness of each fish.

Calculated variables	Definition
Shape ratio (L/T)	Length-to-thickness ratio
W/L^2	Weight divided by the squared length
L^3/WT	The cubed length divided by the weight and length
W/LT	Weight divided by length and thickness
K factor (W/L^3)	Weight divided by the cubed length

Table 2

Table 2 Weight differences. Presentation of the results from a one-sample t-test on the cases where right> left and right<left for each process step (P1-P3). The results are provided as weight difference (g) together with standard deviation (SD), number of samples (n) and P values.

	P1	P2	P3
Weight difference (g) right > left	36.2 (SD=20.3, n=47) P value = 4.8511e-16	31.7 (SD=15.7, n=47) P value = 5.6827e-18	73.4 (SD=58.2, n=43) P value = 2.3965e-10
Weight difference (g) right < left	23.8 (SD=19.7, n=13) P value = 9.2100e-04	30.0 (SD=57.1, n=13) P value = 0.0821	87.8 (SD=75.4, n=14) P value = 7.7666e-04

Table 3

Table 3 Number of cases where the difference between left and right fillet exceeds a certain margin. For each processing step (P1-P3), each fish is divided into one of three groups, depending on whether the difference between left and right fillet exceeds a certain margin or not. The margins correspond to the bounds of a 95% confidence interval calculated on the absolute mean differences between all fillets.

	P1		P2		P3	
Margin, M	28.2g	38.8g	23.8g	38.9g	60g	93.5g
No. of fish where left fillet is larger right by M	4	2	3	1	7	5
No. of fish where the difference between left and right fillet are smaller than M	25	36	26	42	32	38
No. of fillets where left << right by M	31	22	31	17	18	14

Table 4

Table 4 Prediction models. The table presents three PLS models and the resulting Root Mean Square Error of Calibration (RMSEC), Root Mean Square Error Cross Validated (RMSECV), number of factors, and the number of outliers.

Model	Variables	RMSEC %yield	RMSECV %yield	# Factors	Outliers
PLS1_1	Shape ratio Length, L Head weight W/LT Thickness, T K factor Weight, W	0.40	0.43	5	15
PLS1_2	Shape ratio Length, L W/LT Thickness, T K factor Weight, W	0.63	0.68	2	14
PLS1_3	K factor Weight, W	0.67	0.71	2	12

Table captions

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Table 2 Weight differences between left and right side fillet. Presentation of the results from a one-sample t-test on the cases where right side fillet > left side fillet and right side fillet <left side fillet for each process step (P1-P3). The results are provided as weight difference (g) together with standard deviation (SD), number of samples (n) and P values.

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Paper II

Ørnholt-Johansson, G., Jørgensen, B.M. & Frosch, S. (2016). Variation in some quality attributes of Atlantic salmon fillets from aquaculture related to geographic origin and water temperature.

Research paper, submitted to Aquaculture.

Variation in some quality attributes of Atlantic salmon fillets from aquaculture related to geographic origin and water temperature

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Abstract

It is well know that factors like fat content and texture affect the yield when making products from Atlantic salmon (*Salmo salar* L.). The relation between these factors and other quality attributes like water holding capacity and protein content, however, has received limited attention. To enable an efficient use of the information gathered in the different links of the value chain, a deeper knowledge of the correlations between the various quality attributes and between these and factors like the geographical origin of the salmon, the company and the water temperature of the fish farm is needed. In the present study a multivariate approach was taken to investigate the variation in some quality parameters (fat, protein, texture, water holding capacity, weight) amongst salmon samples (n=156) from Norwegian aquaculture in order to establish which parameters were accounting for most of the variation seen in relation to the geographical origin and thereby of the sea temperature at harvest. The protein content of farmed Atlantic salmon sampled at different times of year varied substantially and was significantly correlated to the sea temperature. Fat and water content were also negatively correlated yet the correlation coefficient was numerical lower than what is usually seen in fatty fish, which could be a consequence of the varying protein content. A variation in both texture and water holding capacity was observed between the rearing companies, with uniaxial

compression peak force increasing when going from north to south. The present study adds to the existing knowledge regarding texture differences and contributes with new knowledge about the proximate composition of Atlantic salmon from aquaculture. Moreover we show that analysing different parameters holistically may reveal a new dimension in the information regarding differences between companies and regions in relation to the final quality of the filleted salmon.

Keywords: fat, protein, texture, water holding capacity, multivariate data analysis

Abbreviations

PCA *Principal Component Analysis*

PLS *Partial Least Squares*

1. Introduction

The increasing demand for Atlantic salmon (*Salmo salar* L.) calls for an optimized production throughout the whole value chain and especially in the processing step. The result of processing is largely influenced by the quality of the incoming raw material, and factors like too high fat content, soft texture and/or gaping are undesired traits due to a decrease in yield (Einen *et al.* 1999; Michie, 2001; Rørå *et al*. 1998) and inferior appearance (Ashton *et al*. 2010).

Production practices during rearing and harvesting have been found to be important aspects in relation to the processability of salmon (Johnston, 2001). However, no previous studies on salmon from aquaculture have examined the effect of production practices (reflected in products from different rearing companies) in combination with geographical location of rearing site (region) in order to investigate the combined effect of these two on quality parameters like composition and texture.

Several studies have investigated the variation in fat and protein content in relation to season, feed composition and feeding intensity, in which the effect of varying levels of fat and/or protein content on yield, texture and gaping was studied (Hemre & Sandnes, 2008; Johnsen *et al.* 2011; Johnsen *et al.* 2013; Mørkøre *et al.* 2001; Nordgarden *et al.* 2003). But in order for the industry to actively use the information gathered in the different links of the value chain, a deeper understanding of the correlations between the different quality attributes and variables like region, company and water temperature is needed. In the present study, a multivariate approach was taken to explore the raw material variation amongst salmon samples from aquaculture situated in different regions in Norway in order to establish which parameters were accounting for most of the variation in the muscle in relation to region and water temperature. This kind of information will benefit the salmon processing industry by revealing valuable connections between the raw material characteristics and the desired quality parameters of the final products.

2. Material and methods

2.1 Sampling

A total of 156 salmon were collected at 30 different times spread over the period from March 2014 to November 2015. The fish were all supplied by commercial aquacultures in Norway, and were harvested, slaughtered and gutted at the respective slaughterhouses, packed on ice in expanded polystyrene boxes and transported by truck to the collaborating processing company in the north of Denmark, at which filleting took place. In the present study, salmon was supplied by eight different companies (anonymised as C1 to C8) located in the regions of Finmark (11 samples), Troms (49 samples), Nordland (43 samples), Sør-Trøndelag (33 samples), Møre og Romsdal (4 samples), Sogn og Fjordane (6 samples) and Hordaland (10 samples). According to Figure 1, Finmark (F), Troms (T) and Nordland (N) comprise the northern regions, Sør-Trøndelag (ST) the middle of Norway, and Møre og Romsdal (M), Sogn og Fjordane (SF) and Hordland (H) the southern regions. Several of the companies supplied fish from more than one region. Due to the different geographical distances to the slaughterhouses, the time from slaughter to secondary processing varied, but in all cases filleting took place not later than 6 days post-mortem.

Figure 1

Prior to filleting the whole gutted salmon were washed and headed using a U-Cut heading machine for salmon (BAADER 434 S, BAADER DANMARK A/S, Glostrup, Denmark). Filleting took place on a high-speed filleting machine (BAADER 581, BAADER DANMARK A/S, Glostrup, Denmark). The fillets were all from salmon classified as SUPERIOR and randomly selected by the staff at the processing site immediately after filleting. Thus no trimming had been exerted in order to influence the fillets minimally with respect to texture. Fillets were transported on ice, in expanded polystyrene boxes, to the laboratory at DTU Food, Denmark, on the day of filleting, and analysed upon arrival. Between four and six fillets were supplied at each sampling time. The weight of each fillet was recorded prior to the removal of pin bones, which was done by hand. As the weight of the whole gutted salmon was not known, the fish weight (designated Mcal) was calculated as 2*fillet weight/0.77. This was based on the results from pre-experiments on fish from the same company showing that average percentagewise yield was 77% for the filleting process (data not shown).

2.2 Temperature data

Temperature data from the respective rearing regions at each respective time point were collected online and supplied as an average value for each region per month (AKVAFAKTA, 2016). Analysis of the temperature for each region and between years was performed in order to establish whether data from 2014 and 2015 could be combined.

Firstly, the temperature distribution for the relevant regions was investigated. The data showed that there exists a temperature variation within the year (seasonal), as would be expected. When comparing 2014 with 2015 also an annual variation in temperature seems to exist. The observed annual difference in temperature was tested for significance using a one-way ANOVA with multiple comparisons. The analysis showed that, only July showed significant difference in temperature between 2014 and 2015, respectively. However, no fish sampling was conducted in July in the present study why these results support the combination of data from both 2014 and 2015.

The temperature levels in the northern regions display lower temperatures than in the southern regions. Therefore, the average annual temperature for 2014 and 2015 was compared between each region using a paired t-test. The mean sea temperatures for each region in 2014 and 2015, respectively, are provided in Table 1 together the mean difference, 95% confidence interval (CI) and level of significance given as p-value.

Table 1. Mean seawater temperature for 2014 and 2015, respectively, for each region that salmon was supplied from. The mean difference and 95% confidence interval (CI) of the difference is listed. The summary indicates level of significance given by the p-value and with ns indicating non-significant differences between temperatures in 2014 and 2015.

	Mean temperature 2014	**Mean temperature 2015**	**Mean difference**	**95% CI of difference**	**Summary (p-value)**
Finmark	6.5	6.5	-0.062	-0.6 to 0.5	ns
Troms	7.2	7.1	0.073	-0.5 to 0.6	ns
Nordland	8.1	8.2	-0.031	-0.6 to 0.5	ns
Sør-Trøndelag	9.5	9.3	0.193	-0.3 to 0.7	ns
Møre og Romsdal	10.3	9.6	0.658	0.1 to 1.2	≤ 0.01
Sogn og Fjordane	10.8	9.8	0.956	0.4 to 1.5	≤ 0.0001
Hordaland	11.2	10.0	1.158	0.6 to 1.7	≤ 0.0001

The results from the paired t-test presented in Table 1 showed no significant difference between the years in the northern regions (Finmark, Troms, Nordland and Sør-Trøndelag). Contrary, the results show significant differences between 2014 and 2015 in the average sea temperatures of Møre and Romsdal, Sogn and Fjordane, and Hordaland, indicated by the p-values. However, only 20 (out of 156) samples originated from the southern regions.

Conclusively, because of the small number of samples from the south, and the fact that there was no significant difference between 2014 and 2015 except for July, it is regarded as acceptable to combine data from 2014 and 2015 into one data set.

2.3 Texture

The texture of each fillet was measured using a TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, England), equipped with a 30 kg load cell and using a flat-ended cylinder with a diameter of 35 mm. Single compression with a penetration depth of 10%

of the total fillet height was chosen after testing several scenarios, as this solution affected the muscle fibres the least by not breaking them. The textural measurements were performed above the lateral line of each fillet, and texture was recorded as peak force (measured in Newton) automatically by use of the software Texture Exponent 32 (Stable Micro Systems, Surrey, England). The first measurement was performed 2 cm from the neck cut, and then consecutively with 5 cm between each measurement point down the length of the fillet. According to a preliminary experiment the best measurements were obtained from point 3 and 4 (approx. 12 cm and 17 cm from head cut, respectively). The results from these points were therefore used for further analysis in the current study.

2.4 Chemical analyses

Fat content was determined gravimetrically in duplicates according to the method described by Bligh & Dyer but with reduced solvent use (Baron *et al.* 2007).

A calibration curve produced by Kjelldahl with N*6.25 was fed to a SPRINT protein analyser (CEM Corporation, Matthews, USA), which was then used to quantify the total protein content by a built-in-colorimeter.

Analysis for water holding capacity (WHC) was determined on minced salmon muscle as described by Eide *et al*. (1982) by centrifugation at 4000 rpm, at 10 °C for 5 minutes. The WHC is expressed as the percentage of water preserved in the mince after centrifugation relative to the total water content. The analyses were run in quadruplicate, and the calculated mean was used for further analysis.

Water content was determined based on the weight loss during drying at 105 °C overnight.

2.5 Data analysis

Data were statistically analysed using the Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA) software for Mac, using a significance level (α) of 0.05. The software

Unscrambler X (Camo ASA, Oslo, Norway) was used for the multivariate data analysis. Principal Component Analysis (PCA) (Hotelling, 1933) was used for explorative data analysis and visualization of correlations between variables. Data were mean centred and scaled with 1/standard deviation (over samples). A total of 16 outliers were detected and removed based on influence, Hotelling T^2 statistics and Q-residuals. It was primarily an abnormal and non-consistent relation between fat content and textural score that defined the outliers. The models were evaluated based on the degree to which each of the principal components explained the variance in the data set.

To establish the relationship between the different parameters and temperature, Partial Least Squares regression analysis (PLS) (Wold, 1975) was used to build a model for the prediction of temperature. The models were built with the quality parameters as the X matrix and the temperature as the y vector. The models were calibrated using a full cross-validation and evaluated based on the coefficient of determination (R^2), the root mean square error of calibration (RMSEC), and the root-mean-square error of prediction estimated by cross-validation (RMSECV).

3. Results and discussion

3.1 Exploratory data analysis

The relationship between the five parameters (fat, protein, WHC, peak force, weight) measured in relation to this study was explored using a multivariate approach. Table 2 shows the proximate composition of the sampled salmon.

Table 2. Proximate composition of the sampled salmon showing the range, mean and number of samples (n). Mcal designate the calculated weight of the salmon, and the textural score is listed as peak force (PF) given in Newton (N).

	Mean	**Min**	**Max**	**n**
Fat (%)	12.9	6.7	18.3	156
Protein (%)	21.9	19.3	24.1	156
WHC (%)	87.7	80.3	96.2	149
Water (%)	65.8	59.9	71.9	155

Peak force (N)	1.5	0.5	3.0	156
Mcal (g)	4585	2869	6310	156

The fat content measured in present study ranged between 6.7% and 18.3%, which is comparable to data reported by Gjerde *et al.* (2007), who gave a normal fat range for farmed salmon between 6% and 22%. The protein content showed to range between 19.3% and 24.1%, which is a little higher than what has been reported by Shearer *et al.* (1994) who observed an average protein content of 18.5% for salmon from 125 g up until they reached maturity. Although the measurement of WHC is sensitive towards the method used, the range observed in the present study (80-96%) was regarded as reasonable when comparing them to earlier reported values (Aursand *et al.* 2010; Gómez-Guillén *et al.* 2000). In agreement with Aursand *et al.* (2010) the water content of the present study was found to lie between 60 and 72%.

Most of the textural methods employed in other studies investigate the breaking strength of the muscle measured by 60% compression as a measure for firmness (Bahuaud *et al.* 2010; Einen & Thomassen, 1998; Kiessling *et al.* 2004). However, due to the analyses performed in relation to this study it was not desired to break the muscle, and thus 10% compression was chosen albeit this choice complicates comparisons. The range in peak force was from 0.5N to 3.0N, with 95% of the samples displaying peak forces between 1.2N and 1.7N.

The data was first analysed explorative using a PCA to examine how much of the variation in the dataset each of the five parameters could explain. The first principal component (PC-1) explained 40% of the variation and was mainly related to the fat content, WHC and the calculated weight (Mcal). The second principal component (PC-2) explained 22% of the total variation and related to the protein content, whereas the third principal component (PC-3) described the variation in peak force (PF) with 19% of the variation. The correlation loadings for the PCA of the first and second principal component can be seen in Figure 2a and the first and third principal component in Figure 2b.

Figure 2.

Based on the correlation loadings presented in Figure 2a, a strong relationship between fat and protein content on the first and second principal component, respectively, was observed for the samples.

In order to establish whether any relationship between the temperature and one or more of the included variables existed, the ability to predict the temperature based on these parameters was investigated. In section 2.2 it was established that the temperature profile was not significantly different between 2014 and 2015 in the months where the samples were taken and therefore data from both years were analysed together. Even though the temperature history during rearing at a specific company can be presumed to affect some of the measured quality parameters, the data from the present study do not allow such an analysis, as samples from many regions were included.

PLS regression was used to build the model and the resulting correlation loadings of both the X and the Y matrix can be seen in Figure 3 for a one-factor model.

Figure 3.

The predictive ability of the model was not satisfactory with a root mean square error of prediction of 2.2 for a one-factor model. However, the PLS regression showed that there was a one-factor correlation between protein content, fat content and temperature. The correlation loadings in Figure 3 showed that WHC and peak force (PF) do not contribute to this correlation. The calculated weight only contributed marginally to the second factor, and was thus not included in further analyses.

3.2 Univariate data analysis

Analysing the connections between parameters univariately revealed a significant negative correlation ($r=-0.52$, $p<0.0001$, $df=154$) between the protein content of the salmon and sea temperature. To the best of our knowledge such a correlation has not been mentioned previously in relation to the proximate composition of farmed Atlantic salmon albeit Aksnes *et al.* (1986) reported reduced levels of protein and fat content in September, which coincides with the highest temperatures reported for the current study. Amongst the analysed data a significant positive correlation ($r=0.43$, $p<0.0001$, $df=154$) was observed between fat content and temperature. These findings support earlier observations stating that fat content is temperature dependent (Elliott, 1982). Immature salmon has been observed to increase their feed intake as water temperature rises (Kadri *et al.* 1997). Additionally, the correlation observed in the current data set infer that lower levels of fat is seen at lower temperatures, which can be explained by a reduced metabolic demand with decreasing temperature as the activity level of the salmon is reduced (Hiscock *et al.* 2002).

Fat and water content were also negatively correlated ($r=-0.62$, $p<0.0001$, $df=154$), which is not surprising. However, the correlation coefficient was considerably numerical lower than what is usually seen in fatty fish like herring (Oehlenschläger & Rehbin, 2009). The lower correlation coefficient between fat and water content could be a consequence of the non-constant level in protein content.

The relationships between peak force and temperature as well as WHC and temperature are not significant. Other associations including these parameters can thus be investigated independently from temperature and this will be presented in the next section.

3.3 Variation in parameters in relation to company or region

The original data included region and company thus connections between these variables and texture or WHC was investigated. The resulting column diagrams are presented in Figure 4.

Figure 4.

A significant effect of sample origin on both the texture parameter and the water holding capacity was found. Also, there was a weak but significant linear trend between peak force and geography defined by the different regions with F, T and N defining the north, ST the middle, and M, SF and H the south of Norway. This means that peak force increases numerically when going from the north to the south.
Observations during this study led to the hypothesis that with 10% compression the development of softness might result in an increasing peak force. Storage time has been shown to correlate with degradation of muscle texture making the muscle softer (Espe *et al.* 2004). Hence the hypothesis was tested in a different experiment where blocks of salmon were stored at 2 °C for 14 days and it was observed that peak force increased over time. One explanation can be that the measurements were performed at the same location thus compressing the height of the sample over time. A more compressed sample would exert a higher resistance towards the probe and thus resulting in a higher peak force. Yet, the analysis showed that a compressed height could not explain the increase in peak force over time alone. Although no explanations have been found for these observations the hypothesis is that soft fillets gives rise to higher peak force when measured by 10% compression. Mørkøre & Rørvik (2001) found that texture was influenced by fast growth, and regional differences in texture has been observed by the industry with soft texture being more prevalent in the northern part of Norway than in the southern part during autumn. This could be due to the intensive growth period stimulated by an increased photoperiod (longer days) during the summer in the northern

regions. The observations from the industry support our hypothesis regarding the value for peak force, yet cannot be fully supported by the results of the current study. The variation in texture between the rearing companies (Figure 4a), the effect of region, as well as the inherent biological variation amongst the samples, might explain all of the observed variation, however, more research is needed in order to investigate the interactions between these and their combined effect on texture. Other factors influencing texture like feed composition has previously been discussed include other references.

In the data, there was no correlation between WHC and geography or between WHC and temperature. WHC has previously been associated with the textural properties of raw Atlantic salmon (Jonsson *et al.* 2001), and has been found to be positively correlated to the breaking strength (fracturability) of both salmon and cod fillets (Hultmann & Rustad, 2002). Denaturation and oxidation of proteins will reduce the WHC (Lund *et al.* 2011) and decreasing WHC increases hardness and decreases elasticity when looking at textural properties of the meat (Gallart-Jornet *et al.* 2007). However, no connection between texture and WHC was observed in the current study. Additionally, no significant correlations between peak force and fat content, protein content or dry matter, respectively, could be established. Moreover, neither protein nor fat content was observed to differ between regions or companies.

4. Conclusion

This study shows that the protein content of farmed Atlantic salmon sampled at different times of year was significantly correlated to the sea temperature of the regions at which rearing has taken place. Protein levels were found to be lowest when temperature was at it highest. It is suggested that sexual maturation might be the cause of the lower protein content. Additionally, the positive correlation between fat content and temperature reported by others was confirmed in the present study.

Despite other studies reporting a relationship between WHC and texture in salmon this could not be confirmed in the present study. However, a clear difference in both WHC and texture was observed for some companies. This should be seen in context to the geographical tendency observed amongst the texture data. All in all, this study contributes with new knowledge about the proximate composition of Atlantic salmon from aquaculture, its variation due to temperature and/or geographical region, and the correlation between some quality-related parameters. The results from this study also support that analysing different parameters holistically may reveal a new dimension in the use of information regarding differences between companies and regions in relation to the final quality of the filleted salmon. Further studies should include data on feed composition, time of sea transfer for the smolts, method of killing, and time from slaughter to secondary production.

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Table(s)

Table 1. Mean seawater temperature for 2014 and 2015, respectively, for each region that salmon was supplied from. The mean difference and 95% confidence interval (CI) of the difference is listed. The summary indicates level of significance given by the p-value and with ns indicating non-significant differences between temperatures in 2014 and 2015.

	Mean temperature 2014	**Mean temperature 2015**	**Mean difference**	**95% CI of difference**	**Summary (p-value)**
Finmark	6.5	6.5	-0.062	-0.6 to 0.5	ns
Troms	7.2	7.1	0.073	-0.5 to 0.6	ns
Nordland	8.1	8.2	-0.031	-0.6 to 0.5	ns
Sør-Trøndelag	9.5	9.3	0.193	-0.3 to 0.7	ns
Møre og Romsdal	10.3	9.6	0.658	0.1 to 1.2	$\leq$ 0.01
Sogn og Fjordane	10.8	9.8	0.956	0.4 to 1.5	$\leq$ 0.0001
Hordaland	11.2	10.0	1.158	0.6 to 1.7	$\leq$ 0.0001

Table 2. Proximate composition of the sampled salmon showing the range, mean and number of samples (n). Mcal designate the calculated weight of the salmon, and the textural score is listed as peak force (PF) given in Newton (N).

	Mean	**Min**	**Max**	**n**
Fat (%)	12.9	6.7	18.3	156
Protein (%)	21.9	19.3	24.1	156
WHC (%)	87.7	80.3	96.2	149
Water (%)	65.8	59.9	71.9	155
Peak force (N)	1.5	0.5	3.0	156
Mcal (g)	4585	2869	6310	156

Figure captions

Figure 1. **Regions.** Illustration showing the different regions of Norway. In the present study, salmon was supplied by companies located in the regions of Finmark (11 samples), Troms (49 samples), Nordland (43 samples), Sør-Trøndelag (33 samples), Møre og Romsdal (4 samples), Sogn og Fjordane (6 samples) and Hordaland (10 samples). Map from the University of Agder (2011).

Figure 2. **Principal Component Analysis (PCA)**. Correlation loadings for the PCA showing a) the first and second principal components, and b) the first and third principal component.

Figure 3. **Partial Least Squares regression**. Correlation loadings for the X and Y matrices showing a strong relationship between protein and fat content for a one-factor model. WHC=water holding capacity, PF=peak force, Mcal=calculated weight.

Figure 4. **Texture and water holding capacity**. Column diagrams with error bars representing the 95% confidence interval for a) peak force in relation to company, b) peak force relative to region, c) water holding capacity according to company, and d) water holding capacity for each region.

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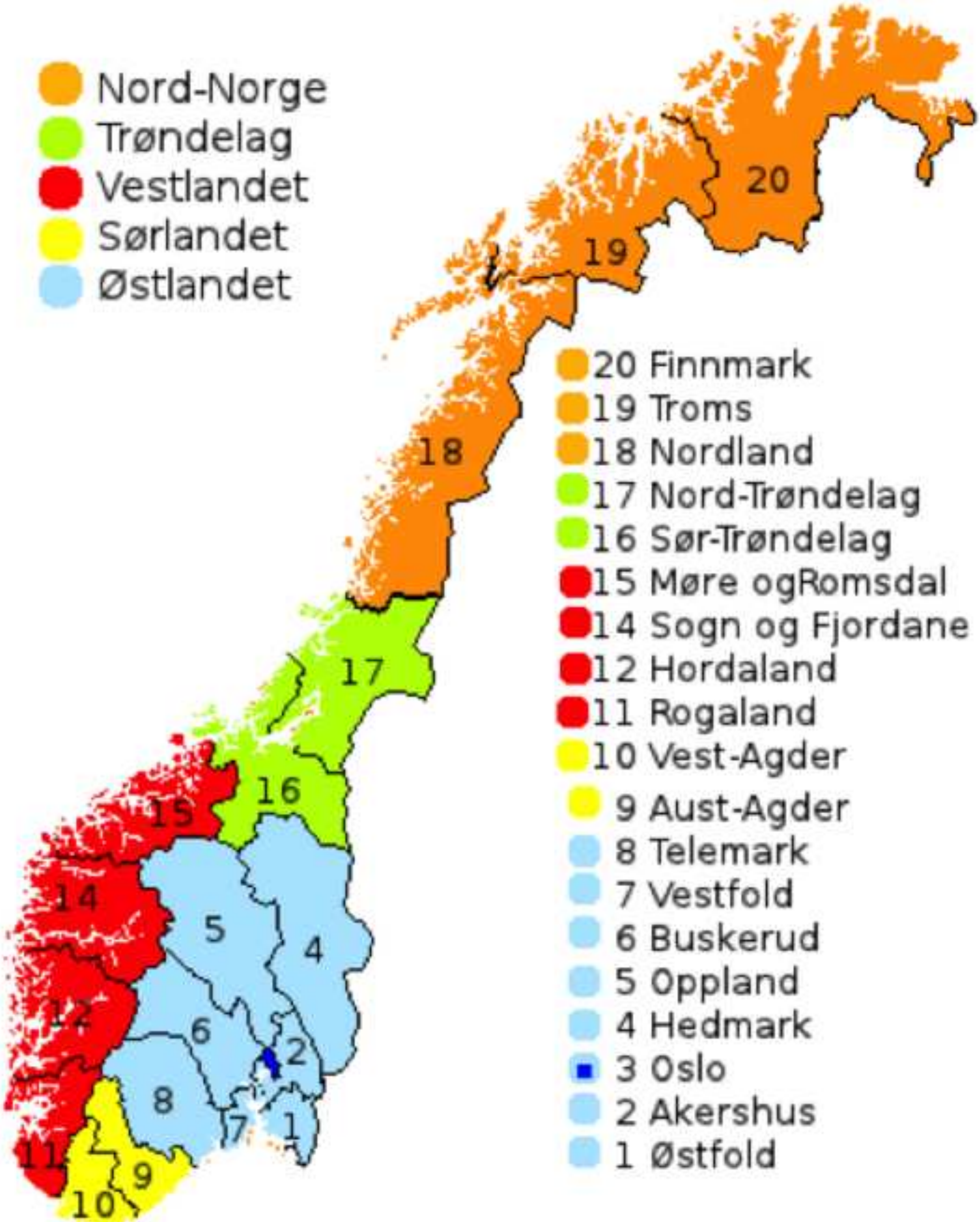

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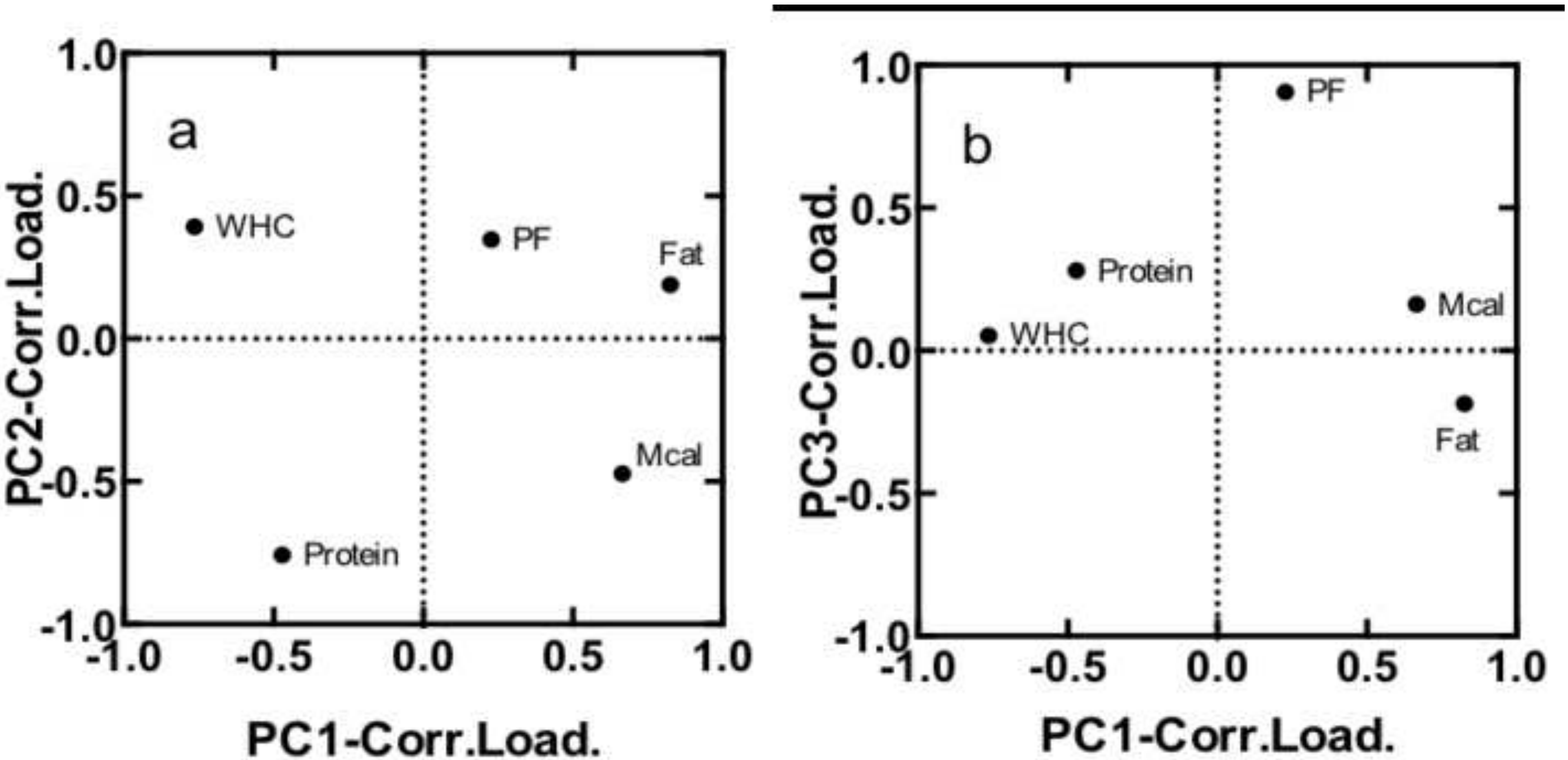

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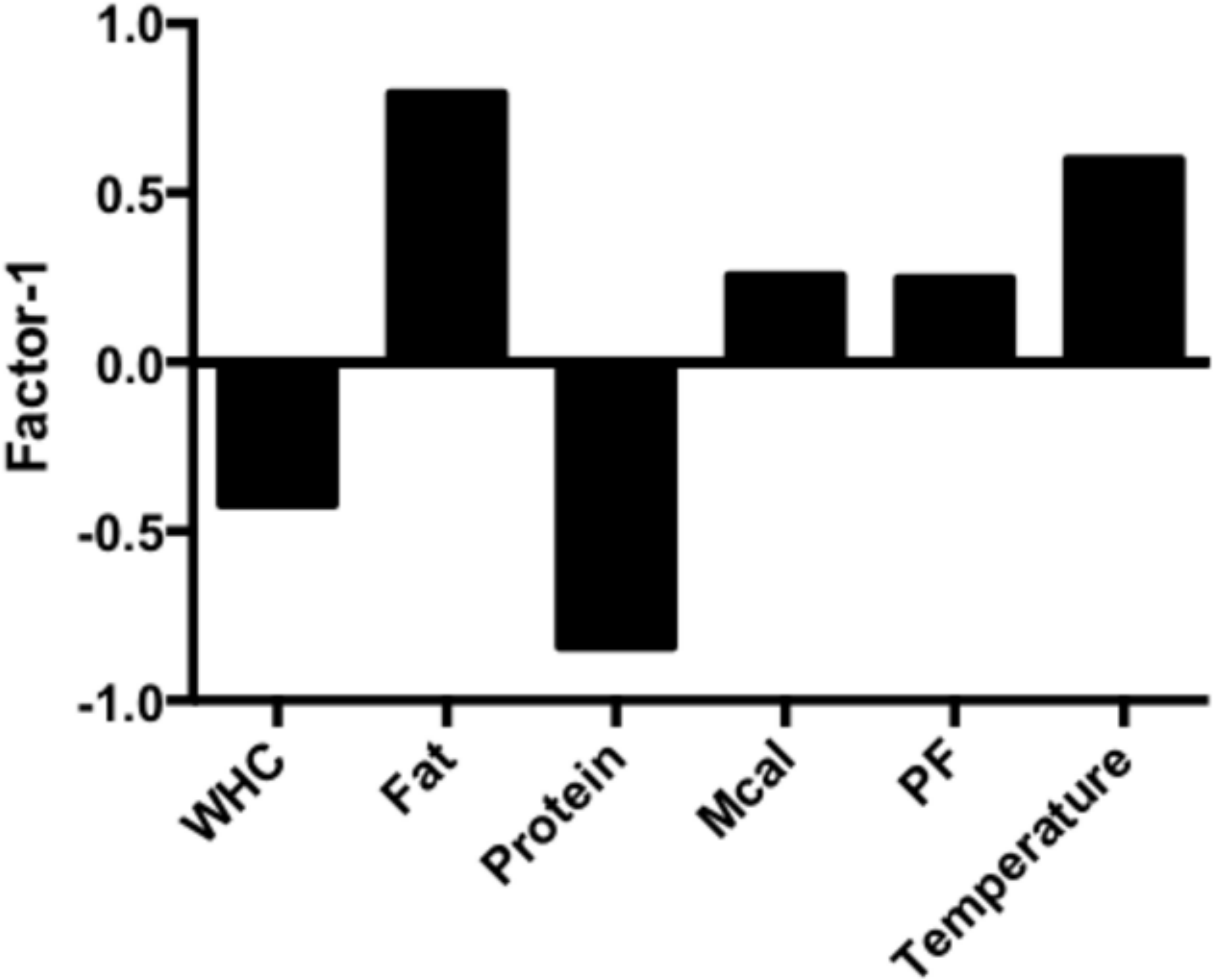

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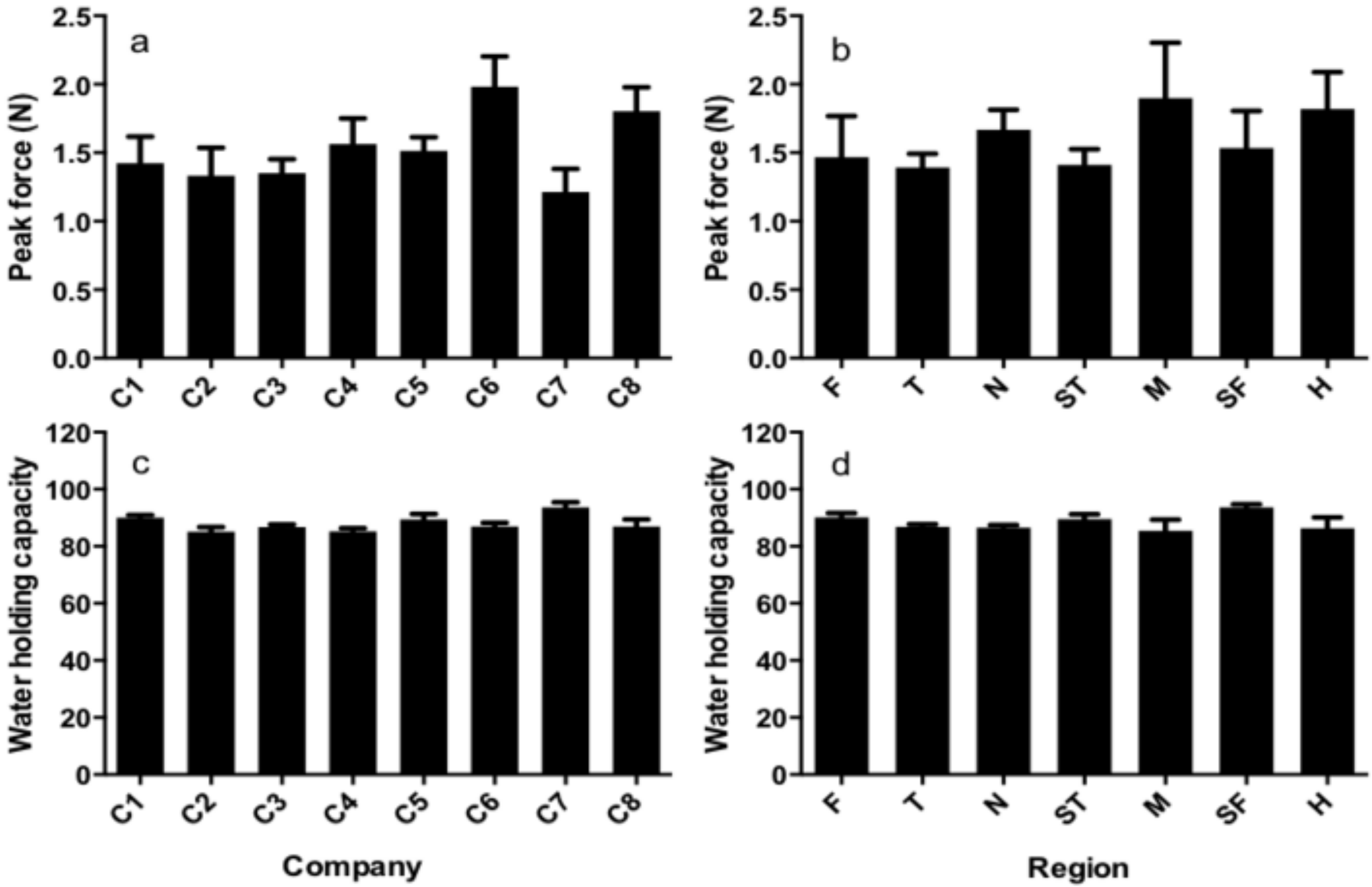

9.2 PAPER III

Ørnholt-Johansson, G., Frosch, S., Gudjónsdóttir, M., Wulff, T. & Jessen, F. Muscle protein profiles used for prediction of texture of farmed salmon (*Salmo salar* L.) fillets.

Research paper, submitted to Journal of Agricultural and Food Chemistry

Muscle protein profiles used for prediction of texture of farmed salmon (*Salmo salar* L.)

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ABSTRACT

Soft texture is undesired in Atlantic salmon as it leads to downgrading and reduced yield, yet it is a factor where the cause is not fully understood. This lack of understanding highlights the need to identify the causes of soft texture, and develop solutions by which the processing industry can improve the yield. Changes in muscle protein profiles can occur both pre- and post-harvest and constitutes an overall characterization of the muscle properties including texture. The aim of this study was to investigate this relationship between specific muscle proteins and the texture of the salmon fillet. Samples for 2D-gel based proteome analysis were taken from the fillet above the lateral line, at the same position as where texture had been measured. The resulting protein profiles were analyzed using multivariate data analysis. Sixteen proteins were found to correlate to the measured texture, showing that it is possible to predict peak force based on a small subset of proteins. Additionally, eight of the 16 proteins were identified by tandem mass spectrometry including serum albumin, dipeptidyl peptidase 3, heat shock protein 70, annexins, and a protein presumed to be a titin fragment. It is contemplated that the identification of these proteins and their significance for the measured texture will contribute to the further understanding of the Atlantic salmon muscle texture.

Keywords: texture, prediction model, PLS, 2DE, proteome, tandem mass spectrometry, *Salmo salar*

INTRODUCTION

Aquaculture accounts for around 70% of the produced salmon and is still the fastest growing food production system in the world[1]. Despite of this growth, as well as efficient farming methods, more focus should be directed towards increasing productivity in order to satisfy the still increasing demand. This can be accomplished by focusing on optimizing the production during the primary and secondary processing rather than just increasing farming volumes[2].

The quality of the raw material has a strong influence on processing where traits like soft texture and gaping are undesired as they lead to downgrading of the products, as well as a decrease in yield[3,4]. Muscle texture is a complex parameter, which is affected by both ante- and post-mortem changes[5]. Salmon caught at different seasons have been reported to differ in hardness[7], and different studies have also found different effects of seasonality, which could be explained by water temperature variation between seasons and years[6,7]. Stress during harvesting and method of slaughtering are also known to negatively affect the texture[8-10], and it has been shown that poor cleaning of the abdominal cavity after slaughtering results in increased occurrence of gaping and loss of firmness[11]. Following slaughter, factors like storage temperature and handling also influence the texture[12,13]. All of these factors can change the muscle protein profile (the muscle proteome) and this proteome constitutes an overall characterization of the properties of the muscle, including texture[14]. The protein profile gets altered when fish is subjected to for example ante-mortem stress[15] or feed changes[16], because of changed gene expressions, by protein post-mortem changes in the muscle[17,18], protein degradation, or changes in protein solubility. The relation between the protein profile and texture is rather complex, as demonstrated by the combined results from several previous investigations. In salmon softening after slaughter has been found to relate to changes in the connective tissue[12,19], and degradation of muscle proteins[20]. Other studies of salmon have found a specific role of collagen on texture[12,21,22], and one study has shown that glycogen content is related to soft texture[23]. Martinez *et al.* [24] investigated the differences in skeletal muscle proteins expressions in normal and soft textured salmon fillets, but could not find any systematic differences between the two groups of fish. The literature reports many attempts to establish the cause of soft texture and gaping; still, no exact conclusions have been drawn. This lack of understanding highlights the need to i) identify the causes of these two significant problems, and ii) develop solutions that will lead to improved quality assessment of texture for the processing industry.

One way to secure the highest quality is to develop a method to categorize salmon so that the fish can be allocated to products according to texture. The fish muscle proteome is hypothesized to include information describing the texture and therefore applicable for measurement or prediction of texture. The aim of this study was therefore to investigate the relationship between specific muscle proteins and texture in farmed salmon fillets to obtain a better understanding of muscle texture at the molecular level.

MATERIALS AND METHODS

Fish and sample handling. Atlantic salmon (*Salmo salar*) fillets were sampled on three occasions (n=24) during the fall of 2014 (Sample designations: OH1, OH2 and OH3 in Table 1) from the industrial production line of Factory A (Hirtshals, Denmark), and at one occasion (n=7) in 2012 (Sample designation U in Table 1) from Factory B (Hirtshals, Denmark). The samples were picked randomly by the chief of production at the factories. All fillets were trimmed according to the same recipe. Discarded salmon fillets, representing soft fillets, and approved fillets, representing firm fillets, were taken from the production line, packed on ice in a polystyrene box, and transported by truck to the National Food Institute, Technical University of Denmark (DTU Food), where the boxes were placed at 0 °C overnight. The next day four circular samples for texture analysis were cut from each fillet just above the lateral line, starting 9.5 cm from the head, and with 2 cm between the outer perimeters of each sample. Average height of the samples was 3 cm. The circular metal cutter had an inner diameter of 49.5 mm. After cutting each sample was placed in a petri dish and stored on ice for maximum 1 hour until texture was measured.

Textural measurements. The texture of each circular sample was measured using the TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, England), equipped with a 30 kg load cell, using a flat-ended cylinder with a diameter of 35 mm. Single compression with a penetration depth of 15% of the total fillet height was chosen after testing several scenarios, as this solution affected the muscle fibers the least by not breaking them. The samples were transferred to a smaller glass petri dish in order to fixate the samples on the texture analyzer instrument. Texture was registered as peak force (measured in Newton), which was recorded automatically by the software TextureExpert32 (Stable Micro Systems, Surrey, England).

2DE and Image Processing. Just after texture analysis had been performed muscle samples were cut out from the middle of the circular samples, frozen immediately and stored at -80 °C until further analysis. Proteins were extracted and analyzed by 2DE as described by Wulff *et al.*[25] using a linear immobiline pH gradient from 4 to 7 in the first dimension and 12% SDS-PAGE in the second dimension. The gels were stained by colloidal Coomassie Brilliant Blue[26] and digitized using a CCD camera (Camilla, Raytest, Straubenhardt, Germany). The software Progenesis SameSpots (version 4.5, Nonlinear Dynamics, Newcastle, UK) was used for image analysis[25]. Spots were aligned and quantified in all gels and resulted in a total of 433 spots, which were present on all gels. Spot lists (data vectors) were constructed for the individual gels, each element being a relative spot volume (using a gel-to-gel normalization in Progenesis SameSpots based on a logarithmic abundance ratio of the spot volumes). The 433 spot lists were combined into a data matrix, each row being the spot list from a single gel and each column being data for a single spot. This matrix was used for further data analysis.

Multivariate data analysis. To establish the relationship between the spots and the measured texture, Partial Least Squares regression analysis (PLS)[27] was used to build a model for the prediction of texture based on the protein spots. All multivariate models were built with the spot volumes as the X matrix and the texture measured as peak force as the Y vector and jackknifing was used to identify variables (spots) with significant ($p<0.05$) regression coefficient. Spots were treated with different combinations of group scalings[28] conducted using MATLAB v.8.4.0 (The MathWorks, MA, USA) prior to exporting the data to Unscrambler X (Camo ASA, Oslo, Norway) for multivariate data analysis. The groups and combinations used for the group scalings are listed in Table 1. Initially variables (spots) were excluded if strong significance were present for either the group analyzed, or for the position on the salmon where the sample had been taken. This approach would avoid including spots that showed significance possibly due to different rearing strategies, or due to differences in protein composition between the sample locations in the data set. Furthermore, variables were excluded based on lowest regression coefficients and weighted regression coefficients, as they did not show significance for the models. The models were validated using a full cross-validation and evaluated based on the coefficient of determination (R^2) and the root mean square error of cross validation (RMSECV). This was followed by validation of the models using randomized matrices together with different combinations of validation samples. Due to the small size of the dataset, validation was performed using an extraction of 4 to 5 samples from the original dataset, which were excluded before performing the model building. Hence to test the robustness of the models samples were extracted and the model was build and validated followed by a new extraction of samples in which the last two samples in one validation set were the first two in the next validation set and so forth.

Identification of proteins by NanoUPLC-MS^E. Proteins were identified as described by Bonde *et al.*[29] with a few modifications. After trypsin digestion the samples were trapped on a precolumn (Symmetry C18 5μm, 180μm x 20mm, Waters, Manchester UK) and washed for 4 min before being loaded on the nanoACQUITY ™ BEH130 C18 1.7 μm, 75 μm x 250 mm analytical reversed-phase column (Waters, Manchester UK). The reverse phase elution profile included mobile phase A [0.1 formic acid in water] and mobile phase B [0.1% formic acid in acetonitrile], during which B was increased from 5-40% over 30 min with a flow rate of 250 nL.min-1 and a column temperature of 35°C. In order to minimize carry over each sample were divided by a blank sample run using a 25 min wash method.

A Synapt G2 (Waters, Manchester, UK) Quadrupole time-of-flight (Q-TOF) mass spectrometer using electrospray ionization (ESI) with a NanoLock-spray source was used for data acquisition. The mass spectrometer was operated in positive and resolution mode with continuum spectra being acquired. Continuous calibration of data was performed using Leucine encephalin as lock mass. Data were acquired using data-independent acquisition (MS^E) during which the mass spectrometer alternated between low- and high-energy modes using a scan time of 0.8 s for each mode over a 50-2000 Da interval. In the low-energy mass spectrometry (MS) mode, data was collected at constant collision energy of 4 eV. In the elevated-energy MS mode, the collision energy was increased from 15 to 40 eV.

Protein identification was obtained in the ProteinLynxGlobalServer software v2.5.3 (Water corporation) using raw data files and the in-build MSE search function against two Uniprot databases (http://www.uniprot.org/taxonomy/8030 and http://www.uniprot.org/taxonomy/8015). The search parameters were trypsin as an enzyme, carboxamidomethyl on cysteine as a fixed modification, and oxidation of methioninen as a partial modification while allowing one missed cleavage.

RESULTS

Texture and 2DE gels. Texture measurements of the samples gave peak forces ranging between 0.45 N and 4.84 N. An unpaired t-test including all samples showed no difference with regards to peak force between samples belonging to the two texture categories (soft or firm), which were pre-defined by the filleting companies and thus based on their subjective categorization of quality. This could indicate that the assessment of filleted salmon performed by assessors at the companies was not strictly focused on soft or firm texture, but might have been influenced by other quality parameters that the assessors were not able to exclude from their evaluation.

2DE gels were produced from the biopsy samples of the muscle and the result of one of these gels can be seen in Figure 1.

In order to establish connections between proteins and the texture parameter, Pearson correlations between the spot volumes of all protein spots and the peak force for all samples were performed. The analysis revealed 15 proteins that showed significance for the peak force. These proteins were further analyzed for their use in a PLS model, and the resulting correlation loadings can be seen in Figure 2.

PLS analysis. To reduce the number of spots found on the gels by another approach a combination of different group scalings[28] (as presented in Table 1) and multivariate analysis in the form of PLS was used. Spot patterns, which characterized the differences between sampling time and sample position, were removed in order to investigate the underlying differences in protein profiles between the samples. This procedure reduced the spot selection from 433 to 291 protein spots. The further reduction of spots and selection of significant spots were performed using an iterative method similar to the one described by Jacobsen *et al.*[30]. Spots with significant regression coefficient from the PLS on the remaining 291 spots, with different combinations of samples, for example soft samples alone and firm samples alone, were pooled if significant ($p<0.05$) in more than one occasion. This resulted in a new data matrix comprising all samples and 45 proteins. Validation revealed that some of the spots could not be used as predictor variables due to high prediction errors, yielding higher values for texture than the reference value. Two samples were removed from the data set due to very low peak force, which could not be handled by the initial model building, and the process was repeated. This third iteration was performed as described above but without the two excluded samples. The resulting matrix comprised 300 protein spots and several validation runs were performed as previously described and the significant proteins pooled. In the end 30 proteins were selected. Of these, 16 proteins could predict the peak force acceptably with a RMSECV of 0.56 N for a one-factor model. Furthermore, as little as five of the 16 proteins could be used for a model resulting in the same level of acceptability (RMSECV of 0.56 N for a one-factor model). Validation of the two models of 5 (M5) and 16 (M16) protein spots were done iteratively in Unscrambler. Additionally, the models prediction ability was double-checked in MATLAB with 100 runs of double three fold cross validation, which showed similar results. Figure 3 shows the correlation loadings and explained variance, described by the reference value for peak force versus the value predicted by the model, of the M5 and M16, respectively.

Figure 3a and Figure 3c depict two groupings of proteins for both M16 and M5: one that correlates positively to the peak force, and one that correlates negatively to peak force. Figure 3b show that M16 tends to predict higher values for the peak force when the peak force exceeds 3 N, whereas the values are more evenly distributed around the regression line for M5.

Protein identification. Of the spots defining M16, 13 were anticipated to be of a sufficient abundance for extraction from the gels and identification by tandem mass spectrometry (MS/MS). Figure 4 show the location of all 16 spots on the gel. The 16 proteins used to build the PLS models are presented in Table 2 together with a description where possible. Eight of the 13 protein spots selected for identification were positively identified and the identifications were based on protein sequences from *S. salar* (7 spots) and *Oncorhynchus mykiss* (1 spot), the closely related Rainbow trout. Identification of the remaining five of the selected spots was unsuccessful with no significant hits by the database searches.

DISCUSSION

PLS regression. This study measured the texture of fillets of Atlantic salmon, and tried to relate this measure to the protein profile by the use of PLS regression analysis. The initial approach using Pearson correlation revealed 15 proteins that were significantly correlated to the texture. However, the resulting PLS model of these proteins was not satisfactory as the RMSECV was 0.79 N for a two-factor model, which should be seen in context of the observed values for peak force ranging between 0.45 N and 4.84 N. Moreover, Figure 2 shows no clear grouping of the proteins with regards to peak force. Hence, even though the selected proteins were strongly correlated to the peak force they were regarded as not suitable for categorizing salmon according to texture. Consequently a different approach for identifying proteins that could predict the texture was chosen. The resulting models, M5 and M16, were both accepted as they showed good predictability of the peak force with a RMSECV of 0.56 N for a one-factor model. The validations were performed iteratively using different validation sets in both Unscrambler and Matlab with both procedures yielding similar results. Even though the predictive error is rather large compared to the measured range in the peak force it still provides a reasonable estimate for the peak force, and more importantly, the textural category as presented in Figure 3.

The model building was accomplished using an iterative approach by which the data set was reduced. This method was used to minimize the inclusion of false positives in the final models, meaning that proteins showing to be significantly different between two or more groups of samples were eliminated from the data set. This was done to reduce the differences in the protein profiles amongst the groups, as it was not the main focus of the investigation. In this way, the actual difference in the protein profiles of the muscle samples relating to texture in general were sought and not biased by protein differences, which could be due to sampling date or sample position on the fillet.

An overlap in protein spots was observed between the resulting models for the two selection methods: Pearson correlation and PLS, as spot number 185, 588 and 629 were found in both models.

Identified proteins. One thing is to build a good prediction model another is to understand the mechanisms behind the information fed to such a model. For that purpose, MS/MS was used to identify the proteins, sampled from the spots on the gel that were found to be correlated to the measured texture according to the multivariate analysis. The present work has encompassed the correlation of a set of proteins to the measured texture by prediction of peak force based on the protein profile. The actual development of softness has thus not been investigated in this study. From the correlation loadings plot in Figure 3a, showing the grouping of proteins from M16, it is possible to categorize a group of proteins, which correlated highly to a high peak force, and a group of proteins that correlated to a low peak force. Eight of the 16 proteins included in M16 were positively identified (Table 2).

Serum albumin as found in spot 185 is located at 87.4 kDa on the gel seen in Figure 4. The protein is the main protein in plasma[31] and the presence of serum albumin is not surprising as there is blood present in the muscle. A study by Jacobsen *et al.*[11] showed that the presence of blood in the abdominal cavity of slaughtered salmon had a significant effect on the gaping score and firmness and the authors suggested that this was due to the proteolytic activity of blood. Hence presence of blood in the muscle could explain why serum albumin was found to have a significant correlation to the texture in the current study. This shows the importance of proper bleeding of the fish during slaughter.

Additionally, spot number 185, and thus serum albumin, was found to be significant for texture in both the model built with the Pearson correlations and the model based on the PLS analysis (M16). Hence blood in the muscle can be deduced to have a significant influence on the texture.

Spot 189 located at 87.4 kDa on Figure 4 was identified as dipeptidyl peptidase 3 (DPP3). From UniprotKB this protease is reported to catalyze the hydrolysis of N-terminal dipeptides from a polypeptide chain through metal binding of zinc ions. Not much literature has been found covering the functionality of DPP3 in salmon, but around 70% of the amino acid sequence is shared between humans and salmon (BLAST, Basic Local Alignment Search Tool, algorithm for comparing sequence information). Prajapati & Chauhan[32] reviewed the function of DPP3 in humans and found that it correlated with protein maturation and defense from oxidative stress, to mention some. These authors place DPP3 in a family of metallopeptidases also including dipeptidyl peptidase 4. In a study of the DPP family in porcine muscle the proteolytic activity in post-mortem muscle was suggested to be involved in texture[33]. This study indicated the relevance of the DPP family in the early proteolytic activities in the porcine muscle.

DPP3 has not, to the best of our knowledge, been mentioned previously in relation to the texture of Atlantic salmon. Nevertheless, the present finding that DPP3 is of significance for the PLS model suggests relevance of this enzyme for the texture of Atlantic salmon, which is supported by the previous observation in other animals[33].

Spot numbers 215 and 231 at 78.8 kDa and 74.8 kDa, respectively, were both identified as heat shock protein 70 (HSP70), although in two different isoforms. Heat shock proteins (HSP) are a family of proteins produced in the cell as a response to cellular stress[34], and are therefore also referred to as stress proteins. HSP70 is a chaperone that aids the polypeptides emerging from the ribosome from folding prematurely, and is thus a key player in protein maturation together with the other HSPs[34]. Stressors of the cell include toxins, microbial, viral, and heat, to mention some, and in farmed Atlantic salmon it has been found that anesthesia, handling and crowding have an effect on the HSP70 levels[35]. Accordingly, the presence of stress proteins like HSP70 is not surprising as the salmon included in this experiment were all farmed and thus subjected to these specific stressors during netting and transfer to the slaughterhouse, as well as during slaughter. Additionally, stress is known to influence the texture of salmon[36]. Several proteins in the heat shock family have been linked to texture, either negatively as HSP70, or positively as the smaller HSP proteins. The latter prevent the formation of protein aggregates, which otherwise would affect the tenderness of the meat[37]. A study by Guillemin *et al.*[38] also showed that the ratio between the smaller HSPs and HSP70 explained the variation in tenderness in beef. However, no evidence for a direct connection between HSP70 and the texture of salmon has been found previously.

Besides containing HSP70, spot number 231 also contained annexin. Additionally, annexin was also identified in spot number 228 (74.8 kDa) and 535 (38.2 kDa). Annexins is a protein family binding calcium and phospholipids[39]. Annexins take part in the repair of the cellular membrane, both via detection of the actual damage as well as in the repair itself[39]. There are several genes coding for the annexins and in this study we have identified two different annexins encoded by either ANXA4 (spot 535) or ANXA6 (spot 228). Moss & Morgan[40] reviewed the functions of the different annexins and coupled them with protein interactions. ANXA4 interacts with lectins and a glycoprotein, whereas ANXA6 interact with ras GTPase activating protein and actin, to mention some[40]. It is the connection with actin that might primarily explain the relationship between the annexins and texture, albeit no previous studies support this theory.

Rab GDP dissociation inhibitor (Rab-GDI) was identified in spot 353 located at 56.3 kDa on the gel in Figure 4. Rab-GDIs are involved in regulating formation, targeting and fusion of vesicles involved in membrane trafficking, which is essential in the endocytic and secretory pathways in eukaryotic cells[41]. No obvious connection between membrane trafficking and texture could be observed in (neither) the present study (nor in the literature).

The last protein identified was found in spot 664 at 19.0 kDa, albeit the Uniprot knowledge database only revealed it as an unidentified protein from Rainbow trout. However, by searching the structural biology knowledgebase (PSI) associated with Uniprot an 82% similarity between several domain entries of the protein titin and of the uncharacterized protein was found. Titin is the third most abundant protein in the muscle after actin and myosin, and it connects the Z-line to the M-line in the sarcomere[42]. Moreover, titin functions as a molecular spring that is responsible for the passive elasticity of the muscle[42]. In bovine meat the post-mortem disruption of titin is related to tenderness[43]. In fish no direct connection between titin and texture has been found, although it has been observed that cathepesin L could hydrolyze titin in salmon[44]. Traditionally, the significance of cathepsins and calpains in relation to texture of salmon is reported in the literature[24,45-47] with main focus on these endopeptidases.

It was expected that collagen would have been part of the proteins selected for the final model due the amount of literature that states the connection between collagen and texture[12,21-23]. However, since all proteins were not successfully identified it is not possible to conclude whether collagen is indeed amongst the 16 proteins. Moreover, it is possible that the selection process in which proteins spots were eliminated from the data set could have eliminated collagen. This would be the case if the differences in collagen content amongst the groups could be attributed to for example sampling time.

Although the present study did not disclose the actual cause of different textures, it has contributed with knowledge on proteins and protein fragments correlating with peak force as measured in this work. Whether there are causal relations between some of these proteins and peak force will be investigated further. From the prediction models shown in Figure 3 it was possible to estimate the peak force of a sample, based on the combination of the included protein volumes, and thus provide a prediction of the texture category of a salmon. Thus, fast measurement of these specific proteins could constitute a method for objective evaluation in industry of salmon texture profiles in order to divide batches in two or more texture categories.

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FIGURE CAPTIONS

Figure 1. Scanned 2DE gel of the proteins representing the proteome of a salmon designated U-firm according to Table 1 (*S. salar*). Size and pI range are indicated on vertical and horizontal axes, respectively. The 15 spots showing significance for the peak force and included in the PLS model shown in Figure 2 are marked.

Figure 2. Correlation loadings for Factor-1 (27%, 29%) and Factor-2 (8%, 5%) from a PLS on 15 different proteins spots and peak force (PF). The numbers indicate the spot numbers.
The predictive ability of the model was not satisfactory with a RMSECV of 0.79 and R^2=0.47 for a two-factor model, and thus a different approach was chosen.

Figure 3. Correlation loadings and explained variance (reference PF vs. predicted PF) of M16 in subplot a (Factor-1 (30%, 80%) and Factor-2 (8%, 6%)) and b, respectively, and M5 in subplot c (Factor-1 (45%, 77%) and Factor-2 (10%, 1%)) and d, respectively. RMSECV for both plot b and d are 0.56 for a one-factor model.

Figure 4. Scanned 2DE gel with the 16 spots that comprise the prediction models (M16) marked. Spot 431, 629 and 724 all had too low spot volumes for MS/MS identification. Therefore only the remaining 13 spots were sought identified.

Table 1. Presentation of the different groups used for the group scalings. Each group comprised 3-4 fillets.

Group scaling no.	Group IDs	No of groups
1	OH1, OH2, OH3, U-soft, U-firm	5
2	OH and U	2
3	OH1, OH2, OH3 and U	4
4	U-soft and U-firm	2
5	OH1-3 both soft and firm	6
6	OH1 – soft and firm	2
7	OH2 – soft and firm	2
8	OH3 – soft and firm	2

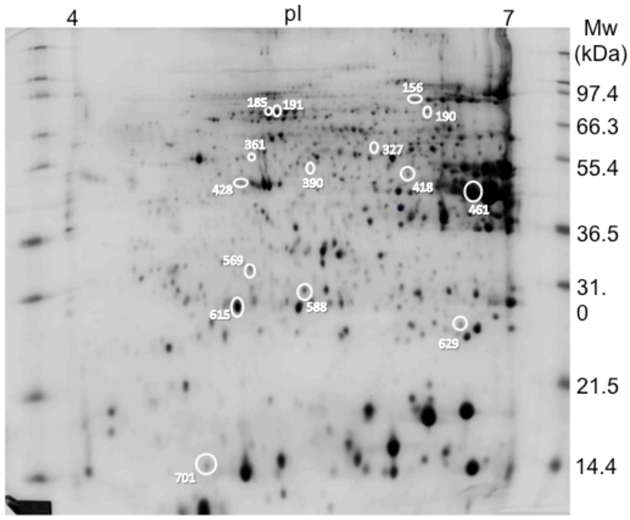
OH represents samples supplied by Factory A at sample time 1, 2 and 3, respectively.
U represents samples from Factory B.
Soft and firm corresponds to the predefined quality grouping made by the companies.

Table 2. Detailed list of the protein spots for which identification was sought by tandem mass spectrometry. Three spots (431, 629, and 724) were not selected for protein identification, as their spot volume was too low. Not identified designates that the database search of MS data gave no significant hits.

Spot no.	Acession no.[a]	Description	Species	Mw-e[b] kDa	Mw-t[c] kDa	pI-e[b]	pI-t[c]	Total score[d]	Sequence coverage[e]
185	Q03156	Serum albumin	*Salmo salar*	87.4	67	5.4	5.3	333.5	11.3
189	B5X435	Dipeptidyl peptidase 3	*Salmo salar*	87.4	81.6	5.3	4,9	94.3	6.5
215	B5DG30	Heat shock protein 70 in two isoforms	*Salmo salar*	78.8	70.8	5.4	5.2	8372.6	35.8
	B5X4Z3							5810.2	10.4
228	C0H996	Annexin in two isoforms	*Salmo salar*	74.8	74.8	5.3	4.7	179.1	12.5
	B5RI22							137.3	8.9
231	B9ELQ1	Heat shock protein 70	*Salmo salar*	74.8	70.8	5.3	5	620.8	20.3
	C0HAK5	and						581.9	8.5
	B5RI22	Annexin	*Salmo salar*					236.3	9.5
	C0H996							232.3	12.1
353	B5X1S6	Rab GDP dissociation inhibitor	*Salmo salar*	56.3	50.7	5.4	5.1	70.5	3.8
431		Not selected for MS[a]		51.0		4.7			
483		Not identified[b]		44.6		6.3			
535	B5XEI6	Annexin	*Salmo salar*	38.2	35.2	5.4	5	318.0	21.3
588		Not identified[b]		30.9		5.6			
629		Not selected for MS[a]		26.6		6.7			
655		Not identified[b]		20.2		6.4			
664	A0A060 WLG4	Uncharacterized	*O. Mykiss*	19.0	232.7	4.5	7.4	25.7	0.4
675		Not identified[b]		16.8		5.1			
714		Not identified[b]		18.0		4.2			
724		Not selected for MS[a]		39.5		5.9			

[a] *Accession numbers are given according to Swiss-Prot.*
[b] *Experimental Mw or pI*
[c] *Teoretical Mw or pI*
[d] *Protein identification was obtained using the search engine within the PLGS software against all entries in UniprotKB of Salmo Salar or Salmonidae. Reported is the protein given the highest score.*
[e]*Percentage coverage of the full length sequence.*

Figure 1.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pI
7
Mw
(kDa)
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36.5
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361
327
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629
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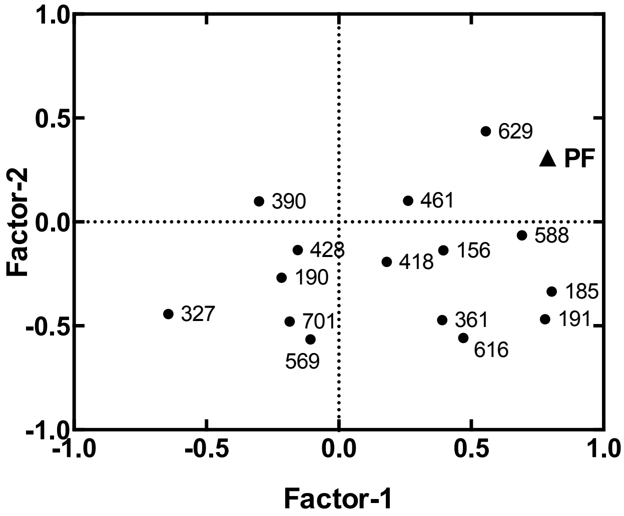
Figure 2.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701
361
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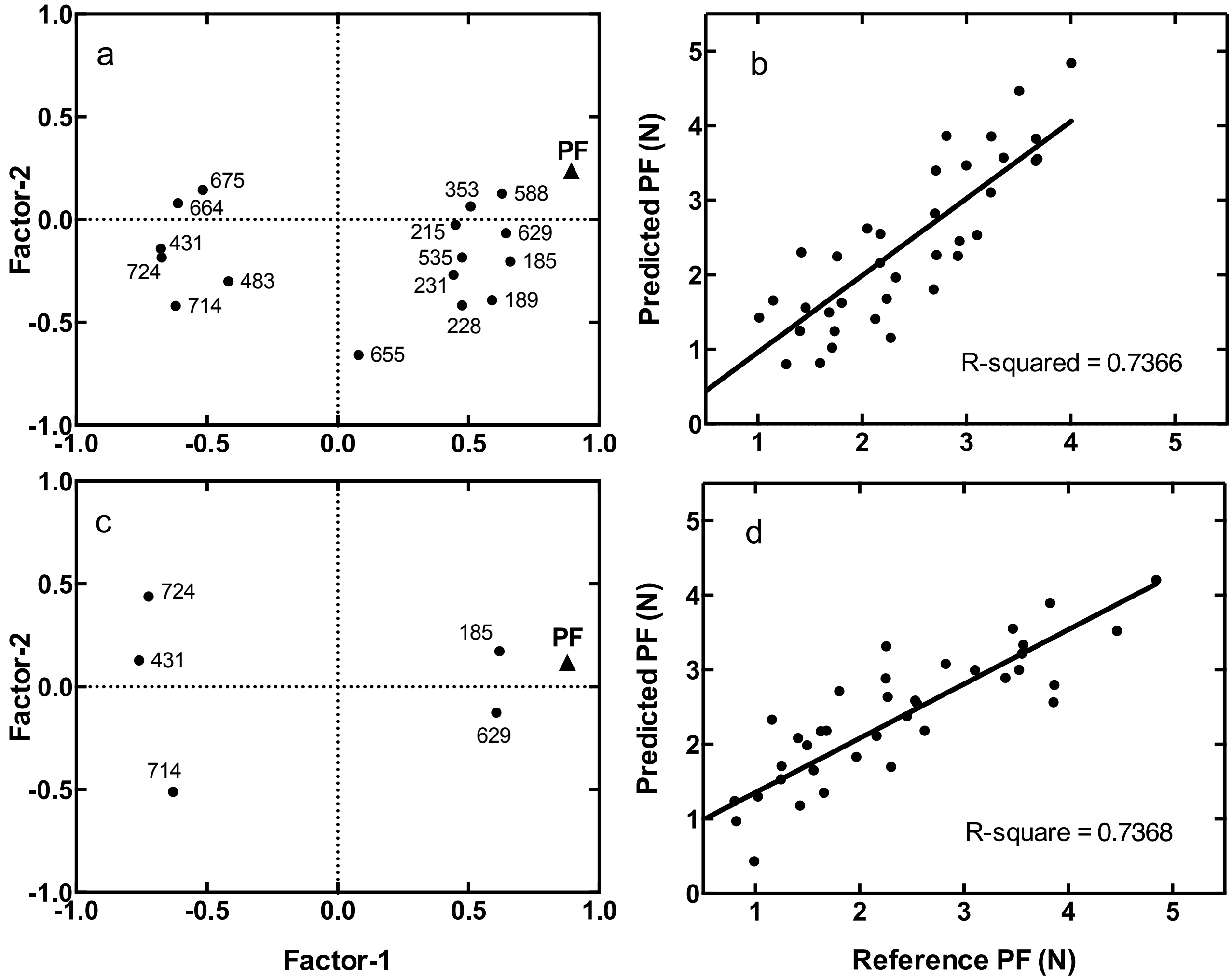
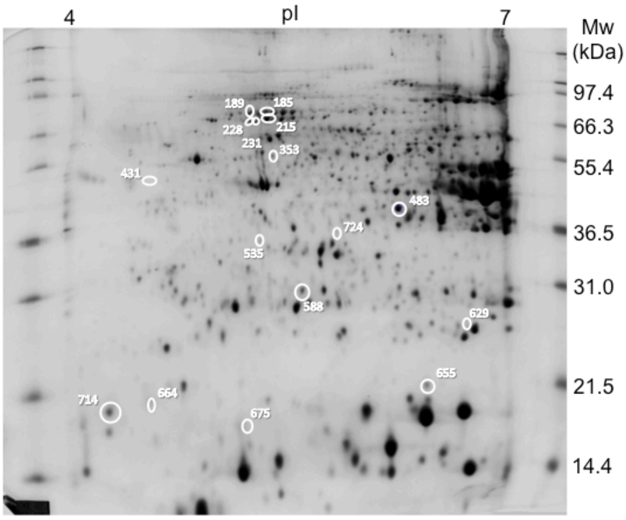
569
616

Figure 3.



a
b
c
d
Factor-1
Factor-2
PF
675
664
431
724
714
483
353
588
215
629
535
185
231
189
228
655
Predicted PF (N)
Reference PF (N)
R-squared = 0.7366
R-square = 0.7368

Figure 4.


4
pI
7
Mw
(kDa)
97.4
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